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Molecular and Structural Insights into COVID-19 Pandemic

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ABSTRACT

The outbreak of a novel coronavirus (SARS-CoV2) associated with acute respiratory disease called COVID-19 marked the introduction of the third spillover of an animal CoV to humans in the last 2 decades. The SARS-CoV2 genome analysis with various bioinformatics tools revealed that it belongs to beta CoVs genera, with highly similar genome as bat coronavirus and receptor binding domain (RBD) of spike glycoprotein as Malayan pangolin coronavirus. Based on its genetic proximity, SARS-CoV2 is likely to be originated from bat derived CoV and transmitted to humans via an unknown intermediate mammalian host, probably Malayan pangolin. Further spike protein S1/S2 cleavage site of SARS-CoV2 has acquired polybasic furin cleavage site which is absent in bat and pangolin suggesting natural selection either in an animal host before zoonotic transfer or in humans following zoonotic transfer. In the current review, we recapitulate a preliminary opinion about the disease, origin and life cycle of SARS-CoV2, roles of virus proteins in pathogenesis, commonalities and differences between different corona viruses. We have also highlighted the evidences regarding the potential drugs and vaccine candidates with their modes of action to cope with this viral outbreak. Our review provides comprehensive up-dated information on molecular aspects of the SARS-CoV2.

Keywords Angiotensin-Converting Enzyme 2; Spike glycoprotein; TMPRESS2; Furin; Malayan pangolin

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1 INTRODUCTION

Corona viruses (CoVs) are the positive stranded RNA viruses which taxonomically come under family Coronaviridae and subfamily Coronavirinae. These enveloped viruses are spherical, oval or pleomorphic in shape and their diameter ranges between 60-140 nm [1]. The subfamily Coronavirinae consists of 4 genera namely alpha coronavirus, beta coronavirus, gamma coronavirus and delta coronavirus [2]. The CoVs are not new to human being and most of them produce mild respiratory diseases in human and infects domesticated animals from decades [3]. But since the beginning of 21st century, they emerged as a big threat to human population and warrant immediate and researchful remedies. There were six CoVs known, out of which severe acute respiratory syndrome CoV (SARS-CoV) and Middle East respiratory syndrome CoV (MERS-CoV) outbreak took a wide toll of human life in 2002 and 2012 respectively. In 2002, SARS-CoV was emerged in China and infected 8422 persons leading to the death of 916 individuals. Later MERS-CoV appeared in Arabian countries and infected around 1800 humans. Very recent, in 2019 seventh CoV caused large scale epidemic affecting almost all countries across the globes. Being close relative of SARS-CoV the novel coronavirus named as SARS-CoV2 (details discussed later in the article). As compare to SARS-CoV and MERS-CoV, SARS-CoV2 is spreading faster and number of deaths are multifold higher [4]. Till the mid of May 2020 SARS-CoV2 has infected more than 4.5 million people worldwide (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports).

1.1 A novel human coronavirus

In December 2019, patients with pneumonia like symptom were reported from several local health facilities in Wuhan city of China. The cause was unknown and most of the patients were

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from sea/wet food market in Wuhan, China. The pathogen was confirmed as virus by PCR, qPCR and sequenced by next generation sequencing. The virus was considered as novel because its genome was not completely matching with any previously sequenced virus genome. Also the clinical symptoms were distinguishable from that of other know viral infections. Hence virus was named as 2019-nCov where 'n' stands for 'novel' [4] and the disease caused by this virus was named as COVID-19. On the basis of highest conserved protein encoding open reading frame (ORF) 1a/1b sequence, the new virus clustered with SARS-CoV under genus beta coronavirus. Thus, the name was changed to SARS-CoV2 by International Committee on Taxonomy of viruses [5].

2 GENOME SEQUENCING

Genome sequencing of the SARS-CoV2 started at the early stage of the outbreak at Wuhan. The bronchoalveolar lavage fluid samples were collected from the initial patients. The quantitative PCR assays with pan CoV primers, including RNA dependent RNA polymerase (RdRp) primers were utilized as the first stage for confirmation of CoV as causative pathogen. Whole genome sequencing carried out using next generation sequencing platforms- Illumina sequencer and nanopore sequencing technology. Zhu et al and Zhou et al reported the early genome sequences of SARS-CoV2 with approximate size 29,891 bp. The sequences are submitted to GISAID with accession number: EPI_ISL_402119; EPI_ISL_402120; EPI_ISL_402121; EPI_ISL_402124 and EPI_ISL_402127-402130 [4,6].

After these initial submissions of SARS-CoV2 genome sequences, multiple entries from different parts of world were appeared in GISAID. The numbers are increasing with the spread of virus. By the cutoff date of this article more than 17,000 genome sequences were submitted to

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GISAID. With more submissions of sequencing data strain variations, mutations and their impact on pathogenicity can be study to control this pandemic.

3 GENOME STRUCTURE

The CoVs including SARS-CoV2 are enveloped viruses with genome size around 30 kb and possesses protruding spikes for interaction with host cells [7]. The SARS-CoV2 contains a positive sense single stranded RNA genome covered by an enveloped structure. As mentioned, the shape of SARS-CoV2 is either pleomorphic or spherical and is characterized by club shaped (or crown like) projections of surface glycoproteins [8]. The genomic RNA encodes 9860 amino acids. The GC content is very low i.e. 38%. SARS-CoV2 genomic RNA consists of 5′-cap and 3′-poly-A tail structure. This positive sense RNA is used as template for translation in host. The number of open reading frames (ORF) genomic RNA possess varies across the CoVs but minimum six ORFs are reported. First ORF is the longest and occupies almost two third portion of the genome encoding polyprotein 1a/b (ppa1a/b). From ppa1a/b, 16 nonstructural proteins (NSP) are synthesized. The NSP forms replication-transcription complex including two proteases papain-like protease (NSP3) and main protease (M^{pro}) which is also known as $3CL^{pro}$ (NSP5) and one RNA dependent RNA polymerase (NSP12). Remaining portion of the genome encodes four vital structural proteins- spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins along with accessory proteins (Figure 1a) [9,10]. The comparisons between SARS-CoV2 and the related SARS-CoV reveal about 380 amino acid substitutions. Of these, nsp2 and nsp3 proteins showed 61 and 102 amino acid substitutions respectively. Moreover, 27 amino acid substitutions are also located in spike proteins, whereas no substitutions occurred in nsp7, nsp13, envelope protein, matrix protein and accessory proteins p6 and 8b. The examinations of the amino acid

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substitutions in different proteins could enlighten how these differences affect the virulence and pathogenesis of SARS-CoV2 [11]. The various components of viral genome are discussed below in detail.

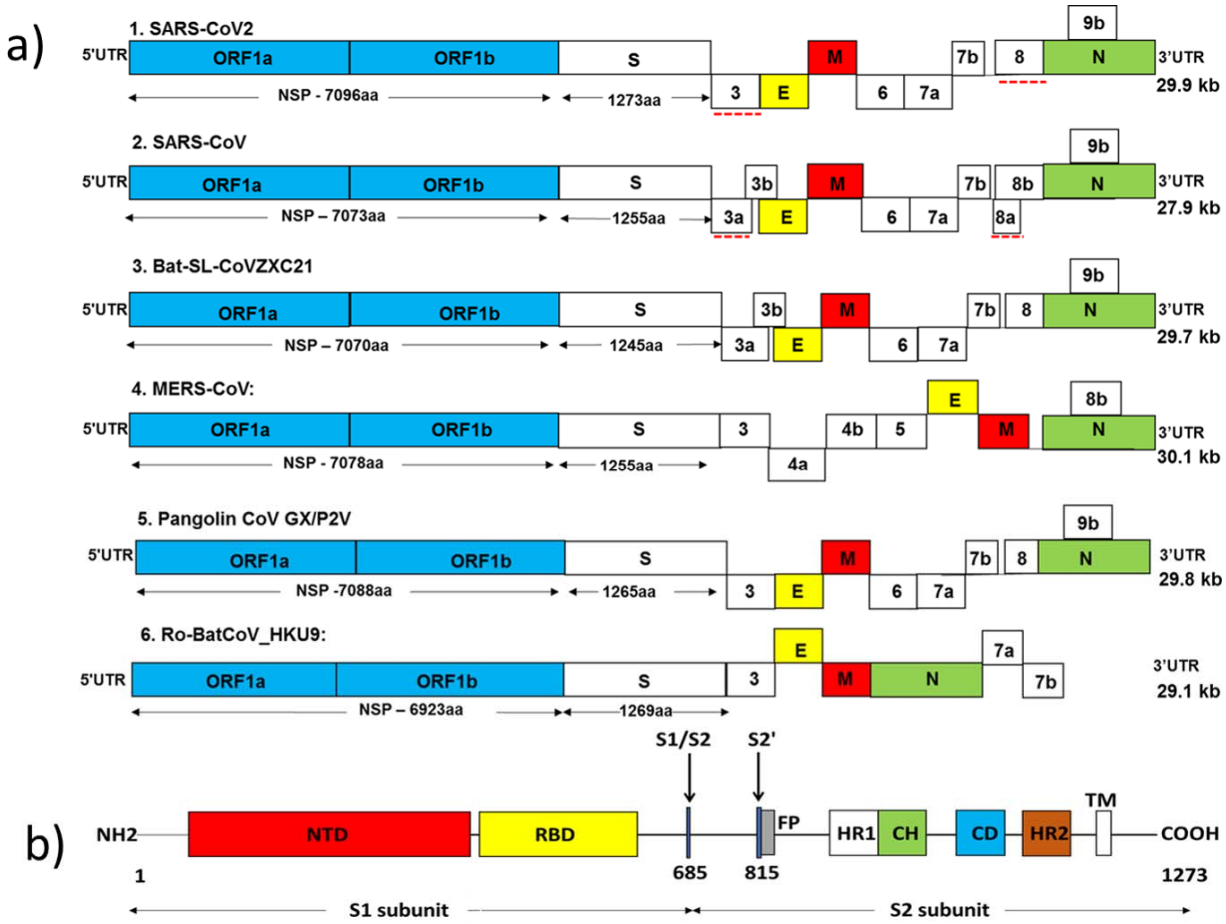

Figure 1

a) Viral genome structure and comparison with other CoVs:

Coronaviruses contain a positive sense, ssRNA genome varying from 27-32kb in size. The genome comprises of 5' and 3' untranslated region (UTR), open reading frame (ORF) 1a/b (blue boxes) comprising two third of the genome and encodes the polyprotein pp1ab and pp1a which is further cleaved into 16 nonstructural proteins (NSPs) involved in viral RNA replication and transcription. The structural proteins are encoded by 4 structural genes present at 3' terminus

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including Spike (S), Envelope (E), Membrane (M) and Nucleocapsid (N) genes which are common features to all CoVs. Among these, the S protein plays an important role in virus attachment and entry; E protein facilitates assembly and morphogenesis of virions within cell; M protein functions in regeneration of virions in the cell and N protein plays a role during virion assembly through its interactions with viral genome and M protein. In addition, the accessory genes interspaced between the structural genes encodes for accessory proteins which varies in different CoVs in terms of number and are dispensable for virus replication. The comparison of coding regions of SARS-CoV2 with different CoVs showed a similar genome organization to SARS-CoV, Bat SL-CoVZXC21 and Pangolin CoV GX/P2V. There is no remarkable difference in the ORF1 of different CoVs but it encodes for NSPs of variable lengths and there is a distinction in the accessory genes. The red dotted line shows the notable variation between SARS-CoV2 and SARS-CoV.

b) Schematic representation of SARS-CoV2 S protein

The spike protein consists of S1 and S2 regions. The S1 region contains a N Terminal Domain-NTD (red box) and a C-domain or receptor binding domain- RBD (yellow box) responsible for recognition and binding to the host cell receptor (ACE2). The S2 subunit responsible for membrane fusion, contains the fusion peptide-FP (grey box), heptad repeat 1 - HR1 (white box), central helix-CH (green), connector domain-CD (blue box), heptad repeat 2 - HR2 (brown box) and transmembrane domain-TM. Cleavage sites at S1/S2 boundary (R685) and S2' (R815) are indicated with black arrows.

3.1 Accessory proteins

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Accessory protein coding genes are present in between the structural genes but dominantly clustered at 3' end of the genome. They are thought to be replaceable but they must have some essential role in virus life cycle as they have retained their position in the genome very well across the CoVs. Specific functions of some accessory proteins are experimentally reported and their possible role to counter attack host immune response is getting wide acceptance. Most of the CoVs contains eight accessory proteins but some accessory proteins are expressed selectively in few CoVs only [12]. There are at least six accessory protein encoding ORFs annotated in SARS-CoV2 including 3, 6, 7a, 7b, 8b and 9b [13]. The SARS-CoV and SARS-CoV2 shows variations in accessory proteins (Figure 1a). For example, 8a protein is absent in SARS-CoV2 and 8b is 37 amino acid longer as compare to SARS-CoV [1,11]. The effect of these variations on the SARS-CoV2 infectivity and pathogenicity haven't been established. Previous studies in other CoVs have identified the roles of accessories protein. For example 3a and 7a are known to have functions like ion-channel activity, up regulation of host inflammation regulators like NF-κβ and induction of host cell apoptosis [14]. Thus further study may provide a connection between variations in accessory proteins and high degree of virulence shown by SARS-CoV2. It may also highlight ability of CoV to cross the species barriers [15].

The hypothesis that whether amino acid substitutions in spike proteins have the potential for generating SARS-CoV2 with super infection ability is still worthy of future investigations.

3.2 Viral structural proteins

3.2.1 S protein

CoVs make entry in the host cell by engaging their S protein with host receptors. The S proteins are class 1 transmembrane proteins which protrude extensively from the virus envelope. These

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trimeric proteins are composed of three regions viz. ectodomain region, transmembrane region and intracellular domain. Recently cryo-electron microscopy revealed the structure of S protein suggesting it can make hinge-like movement resulting into transitions between 'up' and 'down' confirmations [16]. The intracellular domain shows a short intracellular tail. The ectodomain region has S1 and S2 subdomains. The S1 domain of spike protein acts as a major surface antigen. It contains two subunits, N terminal domain (NTD) and C terminal domain (CTD) [17]. The S1-CTD acts as a RBD. The RBD interacts with the 18 residues of ACE-2 [18]. RBDs are shielded by glycosylation which is commonly observed in viral glycoproteins including S proteins from SARS-CoV and HIV-1. But glycosylation percentage of SARS-CoV2 S protein is low as compare to HIV-1 S protein [19]. The S2 domain is a membrane fusion subunit. It contains the fusion peptide-FP, heptad repeat 1-HR1, central helix-CH, connector domain-CD, heptad repeat 2- HR2 and transmembrane domain-TM. There are two cleavage sites, one at S1/S2 boundary (R685) and second at S2' (R815) (Figure 1b) [17,20]. The HRs trimerises to form a coiled-coil structure and drags virus envelope as well as the host cell bilayer to close proximity, facilitating their fusion [21]. At the boundary of S1 and S2 subunits a furin cleavage site (RRAR) is present. This site distinguishes SARS-CoV2 from SARS-CoV and other CoVs. Another remarkable feature of SARS-CoV2 is addition of proline residue at the start of furin cleavage site (Figure 2) [22,23].

Figure 2 The multiple sequence alignment of spike glycoprotein S1/S2 and S2' cleavage sites. The proprotein convertase (PPC) or furin motif RRAR with leading proline insertion is unique to SARS-CoV2 (PRRA insertion highlighted in red box) although NL63 and MERS have proline without additional basic residues. Such polybasic cleavage site is absent in other

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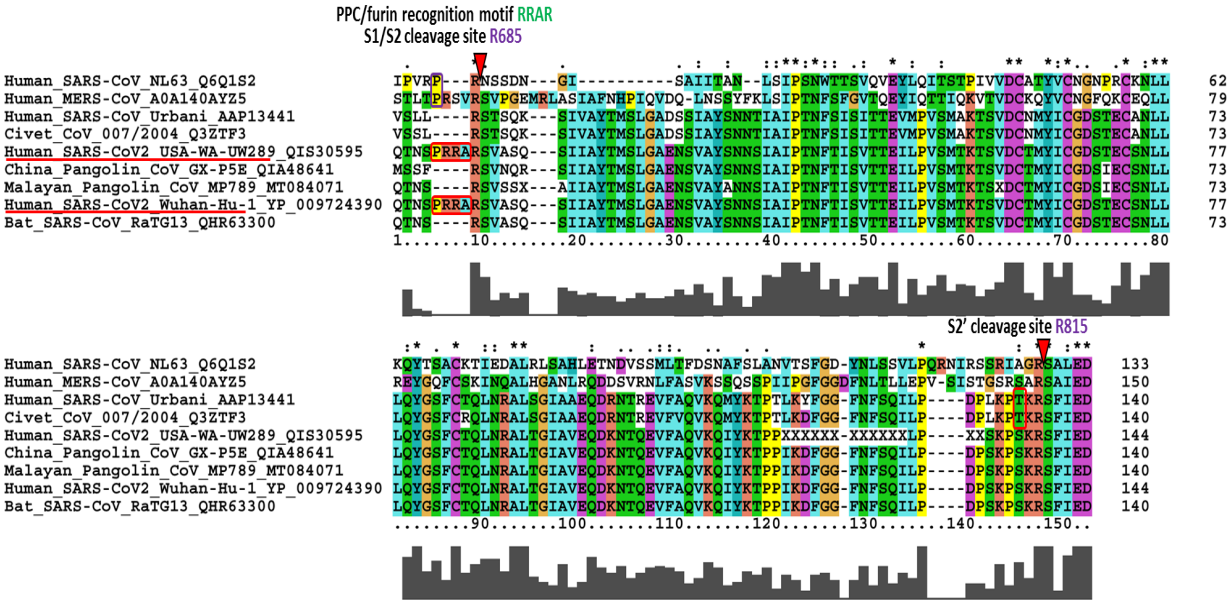
betaCoVs including bat, chinese as well as malayan pangolin and even previous human SARS-CoV. The S2' cleavage site at R815 is conserved across all the sequences analysed however, SARS-CoV2, bat and pangolin has KP**S**KR and civet and hSARS-CoV has KP**T**KR.



This inserted proline creates a turn which is predicted to result into O-linked glycosylation at positions S673, T678 and S686. O-linked glycan may contribute to strong shielding of SARS-CoV2 epitopes [24].

Amino acid sequence of S protein of SARS-CoV2 is 76% identical with SARS-CoV while it shows more identity i.e. 97% with bat CoV RaTG13. Interestingly, identity between SARS-CoV and SARS-CoV2 decreases in the RBD region (Figure 3). Only 74% identical RBD possibly explains why they binds to two different receptors on the host cells [25]. In case of SARS-CoV, it has been observed that mutations in RBD can occur to adopt with host cells during passage in cell culture [2,26]. Thus theoretically it is possible that SARS-CoV2 gained the mutations in RBD as an adaptation during cross-species transmission. Mutations in RBD not only enhance the structural stability of S protein but it can also weaken the binding of the antibody raised against

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the strain [27]. After the initial interaction between the S1 domain and the host receptor Angiotensin- converting enzyme 2 (ACE2), S2 segment mediate membrane fusion of the host and the viral membrane that allow the CoV RNA genome to enter inside the host cells. [18]. Steps involved in virus entry are discussed in later section 'Entry, multiplication and release mechanism of SARS-CoV2'.

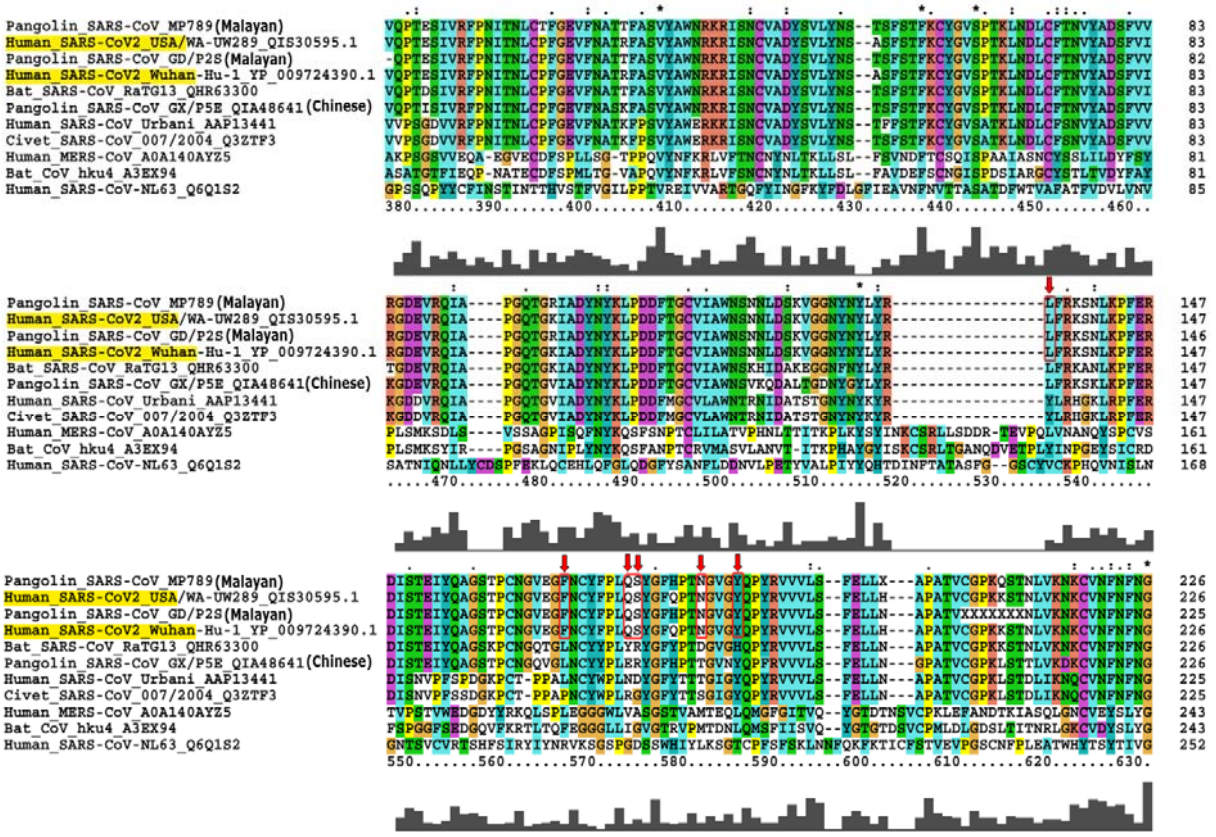

Figure 3 Multiple sequence alignment of SARS-CoV2 receptor binding domain of spike glycoprotein (S) build using Clustal X2. The contact amino acid residues of RBD that interacts with ACE2 receptor are marked with red boxes. All six amino acid residues exactly matches with Malayan pangolin CoV strains MP789 (Liu et. al. 2019; NCBI acc no: MT084071) and GD/P2S (Lam et. al. 2020; GISAID acc no: EPI_ISL_410544), both the samples originated from the Guangdong Wildlife Rescue Centre. These Malayan Pangolins were rescued by Anti-smuggling

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Customs Bureau in March 2019. This suggests that ancestral strain of SARS-CoV2 might have infected Malayan pangolins.

3.2.2 E protein

The E protein is a small polypeptide, ranging from 8.4 to 12 kDa. It comprises of the two distinct domains; the hydrophobic transmembrane domain and the charged cytoplasmic tail. E protein is the most conserved proteins across the studied CoVs, hence displays common characteristic features and functions among them. For instance, SARS-CoV E protein is identical to SARS-CoV2 except four variations (which are not expected to affect any feature or function of E protein). Thus features shown by SARS-CoV including ion channel activity are also thought to be exhibited by SARS-CoV2 E protein [28,29]. E protein of CoV possesses another unique function of 'oligomerization' resulting into formation of viroporin [30]. The viroporins are capable to selectively transport ions like Ca^{2+} and participate in assembling and release of virus particles from host cells [31–33]. The CoV E protein is also known to contribute in pathogenesis. It participates in increasing the protein folding load on endoplasmic reticulum (ER). This results in incorrect protein folding emerging into condition known as unfolded-protein response (UPR). UPR may ultimately lead to apoptosis [34]. Such pathogenesis by SARS-CoV E protein is experimentally evidenced in cells infected with mutated strains rSARS-CoV and rSARS-CoVΔE, and can be explored for SARS-CoV2 as well [35]. Further, E protein participate in formation of specialized structure ER–Golgi intermediate compartment (ERGIC) facilitating release of matured virus [36].

3.2.3 M protein

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The M glycoprotein is the most abundant constituent of the CoVs. Being the major component of envelope, by interacting with S and E protein the virion envelope reconcile the characteristic shape. The M protein is multi-spanning membrane protein which is characterized by three transmembrane domains having C terminal inside and N terminal outside. The third transmembrane domain contains amphipathic region at the end. This region found to be highly conserved across Coronaviridae members. Apart from this region, other region of M protein shows variability in protein sequences, but interestingly these variations doesn't impact secondary structure of CoV M proteins [37]. When SARS-CoV2 M protein sequence was compared with that of bat CoV RaTG13 and Malayan pangolin CoV MP789, unique insertion of a Ser residue is observed at N terminal. Moreover, alignment also showed substitutions at position 70, which is predicted to be a part of transmembrane domain. It has been proposed that such mutations in N-terminal and transmembrane domain, which are exposed to the surface, may have contributed to cross-species transfer of the SARS-CoV2 [38]. Through various protein-protein interactions, M protein plays a major role in viral assembly and its internal homeostasis [18]. Transmembrane as well as endodomain of M protein participate in protein-protein interaction [39]. It has been also known that CoV M proteins can interact with RNAs which encodes information about genome packing signals [40]. These findings support their central role in assembly of the virion particles. As one of the major protein of the CoVs, it is hypothesized to be involved in regulation of replication and packing of RNA into matured virus particles [41]. It has been evidenced that M proteins can endorse two structural confirmations, compact and elongated. Compact M proteins are frequently associated with low density of S proteins as compare to elongated one [42]. Such confirmation needs to be studied in SARS-CoV2. The M protein from SARS-CoV is reported to interact with nuclear factor kappa B (NF-κB) of host cell,

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lowering the gene expression of cyclooxygenase 2 (Cox 2). Moreover, M protein may contribute to pathogenesis by hijacking NF-κB and Cox-2 mediated host inflammatory response [43]. Being highly similar with that of SARS-CoV, SARS-CoV2 M protein may have similar role in pathogenesis.

3.2.4 N protein

The N protein ranges from 43 to 50 kDa and is thought to bind genomic RNA. In all, it is divided into three conserved domains, viz. N arm, central linker (CL) and C tail. The N terminal domain (NTD) and C terminal domain (CTD) are the important structural and functional domains. The function of the NTD is the RNA binding and its major portion is occupied by positively charged amino acids. The CTD mediates dimerization of N protein by self-association and contains nuclear localization signal. The CTD takes important part in nucleocapsid protein oligomerization and N-M protein interactions. The CL region is thought to interact with M protein [44]. Amino acid sequence of SARS-CoV2 N protein is approximately 90% identical with SARS-CoV [45]. The functions of N protein include replication and transcription of viral RNA, formation and maintenance of the ribonucleoprotein (RNP) complex [18]. Moreover, it is also reported that N proteins are involved in host-virus interaction. They regulates host cell cycle including apoptosis to facilitate virus multiplication and spread [46].

4 PHYLOGENETIC ANALYSES

In order to understand genome characteristics of SARS-CoV2 structural, phylogenetic and mutational studies are being carried out intensively [47]. As discussed in genome structure section, RBD of S protein plays important role in selection of the host for pathogenesis and variations in RBD distinguishes SARS-CoV2 from other CoVs. Thus for aforesaid reasons, we

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selected S protein from different hosts and performed multiple sequence alignment (MSA). The full length spike glycoprotein sequences were retrieved from UniProtKB, Genbank and GISAID website.

MSA was performed using MUSCLE program and IQ-Tree web server was used for tree building [48]. To understand the best fit model of spike glycoprotein evolution, Modelfinder tool was employed that evaluated more than 200 models [49]. For full length spike glycoprotein, model WAG+F+I+G4 was found to be best fit. We constructed phylogenetic tree of spike glycoprotein sequences from various genera using maximum likelihood method with 1000 bootstraps. The consensus tree was visualized using FigTree software (http://tree.bio.ed.ac.uk/software/figtree/). As evident from the phylogenetic tree, spike glycoprotein of CoVs could cluster the various genera in to alpha, beta, gamma and delta coronaviruses (Figure 4). The SARS-CoV2 is being clustered with betaCoV genera and having most similar taxa as CoV from pangolin isolate GX-P5E indicating possible intermediary host for SARS-CoV2. Moreover, SARS-CoV2 is forming separate clade from other CoVs having hosts such as bat, mouse, bovine, civet, porcine and human including MERS-CoVs with significant bootstrap value suggesting convergent evolution (Figure 4).

Figure 4: Phylogenetic tree of spike glycoprotein sequences from various coronaviruses. The sequences were clustered according to generas viz. Alphacoronavirus, Betacoronavirus, Gammacoronavirus and Deltacoronavirus. The sequences were downloaded from UniprotKB and GenBank website. The MSA was build using MUSCLE tool of MEGAX software. The phylogeny was inferred using maximum likelihood method with model of substitution:

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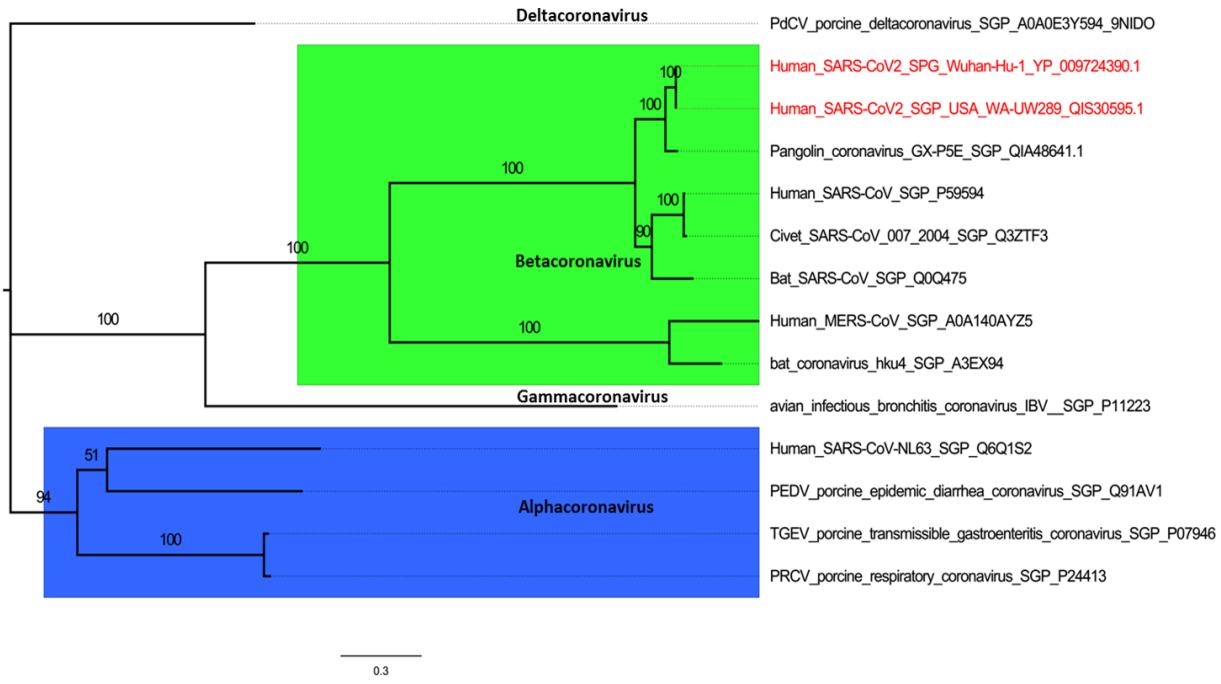
WAG+F+I+G4 and 1000 bootstrap employing IQ-Tree webserver (http://iqtree.cibiv.univie.ac.at/).



The SARS-CoV2 spike glycoprotein consists of S1 and S2 subunits. RBD approximately of 230 amino acids recognizes the host ACE2 as its receptor. Therefore, RBD is the critical determinant of virus receptor interaction and reflects host selectivity, virus tropism and infectivity [27]. The RBD of S glycoprotein is responsible for initiating the viral attachment and viral entry and any mutation to RBD may have significant impact on receptor binding. Thus it was earlier believed that the RBD should be highly conserved [27]. To investigate this hypothesis, we performed the MSA for the analysis of the mutational dynamics of RBD of SARS-CoV2 against the RBD of most closely related CoVs using Clustal X2. Based on our results of MSA and as shown in recent reports [24,49,50] we confirmed that six amino acids of RBD those are involved in interaction with ACE2 have been changed, possibly altering the host range. The SARS-CoV2 S protein may bind to ACE2 through Leu (L455), Phe (F486), Gln (Q493), Ser (S494), Asn (N501), Tyr (Y505)

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residues whereas in case of SARS-CoV Tyr (Y422), Leu (L472), Asn (N479), Asp (D480), Thr (T487) and Try (Y4911) are the interfacing positions for binding [24]. The red boxes in the Figure 3 indicate that five out of the six residues those are cruicial for interaction with human ACE2 differ between SARS-CoV2 and SARS-CoV. Interestingly all these six residues are exactly same in Malayan pangolin CoVs (MP789 and GD/P2S) and differ in Chinese pangolin CoV (GX/P5E) indicating Malayan pangolins as an intermediate host for SARS-CoV2 (Figure 3). These mutations in RBD have altered the receptor binding affinities. Sequence and structural comparisons of RBD and ACE2 suggest that SARS-CoV2 RBD is well suited for binding to ACE2 from humans and other species with high receptor homology [24].

5 ORIGIN: POSSIBILITY OF MALAYAN PANGOLIN AS AN INTERMEDIATE HOST

As mentioned earlier in introduction CoVs are subdivided into four genera. Out of these, alpha CoVs and beta CoVs infects mammals while other two can infect birds mostly. The two alpha CoVs infecting humans (hCoVs) are hCoV-NL63 and hCoV-229E; and four beta hCoVs are hCoV-OC43, hCoV-HKU1, SARS-CoV and MERS-CoV [51]. The SARS-CoV2 is the fifth beta hCoV recently added to the list. As the initial evidences of SARS-CoV2 infection were from sea/wet food market, the link between seafood and the disease was hypothesized. Later, supportive evidences were lacking to link the origin of SARS-CoV2 to seafood market since human to human spread of SARS-CoV2 was substantiated [4,52]. Meanwhile genome sequence confirmed the virus as CoV, for which bats act as a major reservoir. [53]. The genome sequence of SARS-CoV2 is found to be 96.1% identical with bat CoV (SARS-CoV-RaTG13). The CoV from Chinese pangolin (SARS-CoV-P4LGuangxi-2017) was found to be 85.3% identical. The other CoVs were found to be similar at genome level in the range of 73.8 to 78.6% with SARS-

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CoV2 [54]. High similarity between bat CoV and SARS-CoV2 indicates common ancestor for them. Previously also, bats were extensively reported as major reservoirs of CoVs [2,55,56]. Thus it is more likely that SARS-CoV2 also originated from bats. But interestingly, no bats were reported in seafood market in Wuhan from where COVID-19 emerged [52]. Hence, similar to the Himalayan palm civet and dromedary camel as intermediate hosts for SARS-CoV and MERS-CoV respectively, a prima facie 'unknown' intermediate host was considered for spreading SARS-CoV2 from bats to human.

Another approach tried to understand the origin and intermediate host for SARS-CoV2 was protein sequence alignment. Spike proteins of CoVs binds to receptors on host cells by their receptor binding domain (RBD). The ACE2 act as receptor for SARS-CoV2 [6,57]. By analyzing host receptor and viral spike proteins interaction, species which can act as host/ intermediate host can be identified. Pangolin, turtle and snakes were the species which also possess and express ACE2 receptors and hence proposed as probable intermediate host for SARS-CoV2 [52].

It has been known that viruses shows flexibility for codons according to their host genome in order to facilitate their interaction [58]. Relative synonymous codon usage (RSCU) provides possibility of viruses and their host interaction on the basis of 'codon usage bias' shown by the viruses. By using RSCU, Ji et al reported that snake served as intermediate host for SARS-CoV2 [59]. But later Zhang et al proved that the findings of the experiment were inconsistent due to small size of sequence data analyzed and inclusion of out dated database. Their further study also provided strong evidences for Malayan pangolin as an intermediate host for SARS-CoV2 through metagenomics [60]. SARS-CoV2 like virus is also identified from Malayan pangolin which shows high similarity with SARS-CoV2 at amino acid level. The RBD of S protein from Malayan pangolin CoV showed single amino acid variation when compare with SARS-CoV2 S

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protein, indicating Malayan pangolin as an intermediate host for SARS-CoV2 [10]. Similar findings were also reported by Wahaba et al. In a strong support of the Malayan pangolin as intermediate host, they reported homology between the reads from lungs samples of dead pangolin and SARS-CoV2 [61]. The RBD of Guangdong (Malayan origin) pangolin CoVs were closely related to SARS-CoV2 RBD. Including present study and previous metagenomics has consistently identified Malayan pangolin as an intermediate host for SARS-CoV2 [62].

It has been also proposed that recombination in SARS-CoV2 genome might have occurred in intermediate hosts. The genome of SARS-CoV2 is 96.1% identical with bat CoV RaTG13. However, RBD domain of both viruses shows divergence. Strikingly RBD residues of pangolin, specifically Malayan pangolin CoV and SARS-CoV2 are 98% identical. Moreover, Malayan pangolin CoV RBD possessed all six key amino acids which are also present in SARS-CoV2, whereas RaTG13 RBD could present only single key amino acid (Figure 3). These evidences advocated that recombination event between bat and pangolin CoV materialized in Malayan pangolin through which a new strain of virus might have emerged. But interestingly, insertion of polybasic furin cleavage motif (RRAR) at S1/S2 (Figure 2), which plays significant role in membrane fusion, is present only in SARS-CoV2 and absent in other two CoVs. Thus all together the study proposed that the recombination events occurred are complex and needs more detail experimentation to understand the intermediate host of SARS-CoV2 [63].

6 ENTRY, MULTIPLICATION AND RELEASE MECHANISM OF SARS-COV2

Viral infections are initiated with the binding of viral particle i.e. glycoprotein spikes on outer surface to the host surface receptor. The RBD domain of S1 region of the S protein interacts with the host receptor ACE2 [6]. The ACE2 receptor is present on the cell membranes of the multiple

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394 organs including lungs, arteries, kidney, heart and intestines. Cell types and the organs at risk of
395 SARS-CoV2 infection inside the human body can be predicted on the basis of ACE2 gene
396 expressing cells. The expression of ACE2 is enhanced by interferons which are one of the body's
397 main defenses when host detects the virus. The single-cell RNA sequencing study revealed that
398 type II alveolar cells of lungs, myocardial cells, esophagus upper and stratified epithelial cells
399 and digestive system (specifically absorptive enterocytes from ileum and colon) shows high
400 expression ACE2 RNA [64–66]. Further, high expression of ACE2 in mucosa transcriptome of
401 the oral cavity probably emphasize the entry routes of SARS-CoV2 [67].
402 The SARS-CoV2 RBD shows higher affinity to ACE2 as compare to SARS-CoV RBD. Apart
403 from the amino acid sequence variations in RBD domain of these viruses, presence of variability
404 in glycosylation pattern may also have contributed to differential affinity shown by these two
405 viruses [22]. The RBD can possess two confirmations i.e. 'up' and 'down'. However, RBD with
406 up confirmation binds more efficiently as compare to alternative conformation.
407 In addition, the entry requires S protein activation mediated by host type II transmembrane serine
408 protease 2 (TMPRSS2). Human TMPRSS2 protein is chymotrypsin family serine proteases (492
409 aa) possesses three functional domains. It has been shown that TMPRSS2 is expressed in
410 prostate, salivary gland, colon and stomach [68]. It mediates first cleavage of S protein at S1-S2
411 boundary (R685) and second cleavage at S2' (R815) sites. The S1/S2 cleavage site of SARS-
412 CoV2 S protein contains repeated basic arginine residues generating high cleavability [69].
413 Essentialness of TMPRSS2 is evidenced by many recent experiments, thus the co-expression of
414 ACE2 and TMPRESS2 protein is prerequisite for the initiation of pathogenesis. The co-
415 expression analysis for ACE2 and TMPRSS2 proteins using single cell transcriptome analysis of
416 various human cells has identified three cells types; nasal goblet epithelial cells, type II

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pneumocytes and enterocytes thus possible host cells for SARS-CoV2 [70]. Further polybasic furin cleavage site in S protein increases priming of the S protein. Recently in a meticulous study, ACE2, TMPRSS2, and FURIN are shown to co-express in human lung tissue probably due to which multiplication of SARS-CoV2 is higher in the lung [71]. After the cleavage at S2' site, the fusion peptide is inserted into the host membrane. The two HR regions in S2 domain form anti-parallel six-helix bundles (6-HB). The HR1 region of SARS-CoV2 shows mutations when compared to SARS-CoV. This variations are expected to provide stability to 6-HB [72]. The 6-HB bundle brings about the fusion of two membranes and releases viral genome in the host cell.

In many CoVs, it has been reported that 5' and 3' UTRs of viral genomic RNA possesses cis-acting elements. Host factors interact with viral RNA at these sites and participates in viral RNA synthesis [73]. There are many host factors which includes heterogeneous nuclear ribonucleoprotein A1 and Q, polypyrimidine tract-binding protein, and poly(A)-binding protein, for which experimental evidences are available to confirm their role in CoV RNA synthesis [74]. Further, viral RNA being positive stranded, is translated into polypeptide chain by using host cell machinery. A programmed frame shift in translation of ORF 1a synthesizes ppa1a and ppa1b from the 5' end of ORF. Viral proteases, main protease (M^{pro}/ $3CL^{pro}$) and papain-like protease cleave these ppa1a/b chains to generate various NSPs. These NSPs then assemble to forms replicase-transcriptase complex (RTC). Different sub genomic RNAs as well as genomic RNAs are then synthesized by the RTC complex. [13]. From the genomic RNA, intermediate negative-strand RNAs are synthesized, from which positive strands of genomic and subgenomic RNAs are generated. In the next step, translated structural and accessory proteins are then released in endoplasmic reticulum. A specialized smooth walled golgi intermediate compartment (ERGIC)

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carries these viral particles across the secretary pathways. The ERGIC is characteristic feature of CoVs [75]. For the assembling of virion like particles viral protein-protein interaction is required and mediated by M protein. One constrain in this assembling is that membrane proteins through secretory pathway reaches plasma membrane but they are required to be retained near ERGIC for efficient assembling [40]. For this purpose viral protein possesses intrinsic intracellular retention signals. One of such properly studied signal is endoplasmic reticulum retrieval signal retained by the cytoplasmic tail of S proteins [76,77]. In between, genomic RNA translated earlier, forms RNP complex by interacting with N protein and enters into ERGIC. The fully assembled virion is then release by exocytosis [53,78,79].

As SARS-CoV2 is recently emerged human virus, its molecular event including replication and transcription are proposed on the prior information from other CoVs. As the studies are progressing, our knowledge about exact molecular mechanisms in the SARS-CoV2 will get updated. The schematic representation of pathogenesis is shown in Figure 5.

7 TREATMENTS AND PREVENTION OF COVID-19 PANDEMIC: A CHALLENGE TO ACCEPT

The COVID-19 outbreak in Wuhan rapidly transformed into pandemic, infecting millions of people. Hence it is prime time now to find treatments to cure the patients and vaccines to prevent further spread of the SARS-CoV2. Next generation sequencing (NGS) techniques are well furnished now, in term of speed and accuracy of their outcomes. Due to which it was possible to sequence whole genome of the virus in a record time. Fortunately, bioinformatics today is in advance stage to analyze NGS data as compare to previous two CoV outbreaks period. Thus the academic and professional research laboratories and private pharmaceutical companies are trying

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multiple approaches based on the integration of biotechnology, pharmacogenomics and computer modeling to come up with convincing solution for SARS-CoV2. Each step of the virus life cycle (such as receptor binding, fusion of host and viral membrane), every viral protein (like RdRp) is being targeted to find the remedy on SARS-CoV2. Global efforts includes nearly 133 vaccines and more than 100 proposed treatments (including 58 antibody mediated treatments, 15 cell base therapies, 18 molecules scanned for repurpose) are at different stages of the drug development (https://milkeninstitute.org/covid-19-tracker). Figure 6 represents status and the stake of the different approaches for the treatment and vaccine development for COVID-19. Broadly measures to combat SARS-CoV2 can be classified as 1) Repurpose drugs, 2) Immunotherapy and 3) vaccines.

Figure 5: The schematic representation of the SARS-CoV2 pathogenesis.

The structural proteins of virus constitutes of trimeric spike (S) glycoprotein, membrane (M) glycoprotein, envelope (E) protein and nucleocapsid (N) structuring protein. The virus has ~30Kb single stranded positive sense RNA as genetic material. The SARS-CoV2 enters human body through naso-oral route and in response to virus, body initiates innate response by producing interferons (IFN) however, IFN activates expression of angiotensin-converting enzyme 2 (ACE2) protein which acts as receptor for virus attachment to host cell. Receptor binding domain (RBD) of S1 region of spike (S) protein interact with ACE2 leads to proteolytic cleavage at the S1-S2 boundary and S2′, R815 site mediated by type II transmembrane serine protease (TMPRSS2) inducing the viral and host cell plasma membrane fusion. The viral genomic ssRNA is translated by host machinery to produce viral polypeptide and these polypeptide undergo proteolytic cleavage by M^{pro} or $3CL^{pro}$ synthesizing pp1a and pp1ab. These

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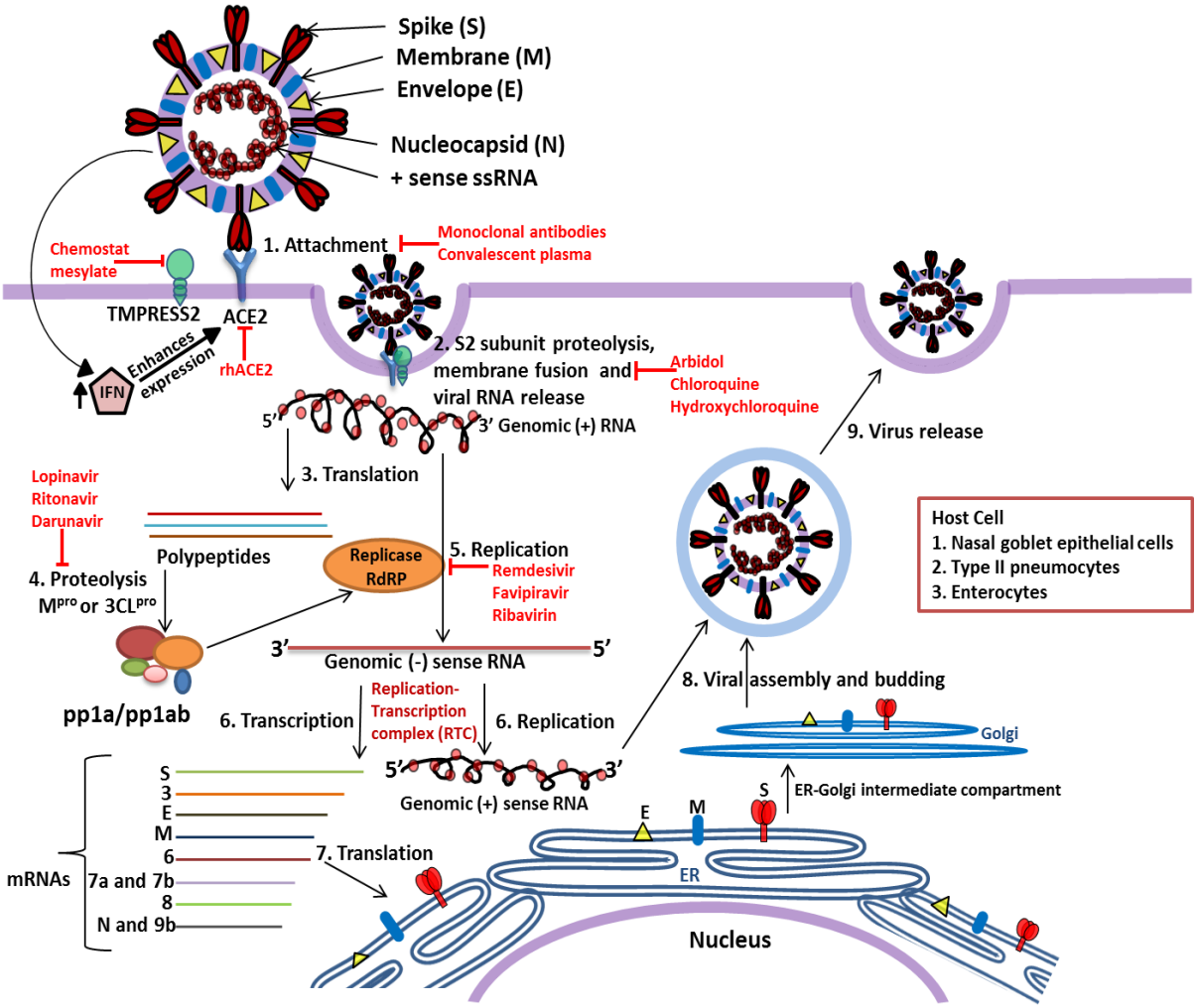
polyproteins encode Replication-Transcription complex (RTC) which continuously replicate and produces a series of subgenomic mRNAs encoding the accessory and structural proteins. The viral genomic RNA and proteins are assembled to form the viral particle and buds in the ER and Golgi. Later, the virus containing vesicles fuse with plasma membrane of host and release the viral particle out of the cell. Since the co-expression of ACE2 and TMPRESS2 protein is primary requirement of initiation of pathogenesis, single cell transcriptome analysis of various human cells identified only three cells types viz. nasal goblet epithelial cells, type II pneumocytes and enterocytes can express both the proteins and indicating possible host cells for SARS-CoV2. The antiviral molecules with target sites are highlighted in red colour.



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7.1 Repurpose drugs

Repurposing drug involves screening the existing drugs for their novel clinical application. It accelerates the drug development and reduces the cost of process. The success of this approach depends upon polypharmacology of small molecules enabling the drugs to act on multiple targets and cross talk between the different biological pathways [80]. More importantly, such drugs have already been gone through clinical trials hence considered as un-risked. In case of SARS-CoV2, drugs against malaria and HIV-1 are being tested on priority. In an affinity purification-mass spectrometry based study, 332 protein–protein interactions have been identified between SARS-CoV2 and human. From these interactions almost 66 virus proteins which can be targeted for drug designing, were identified with multiple binding sites, for which good number of existing drugs can be screened [81]. Few of these drugs are discussed below;

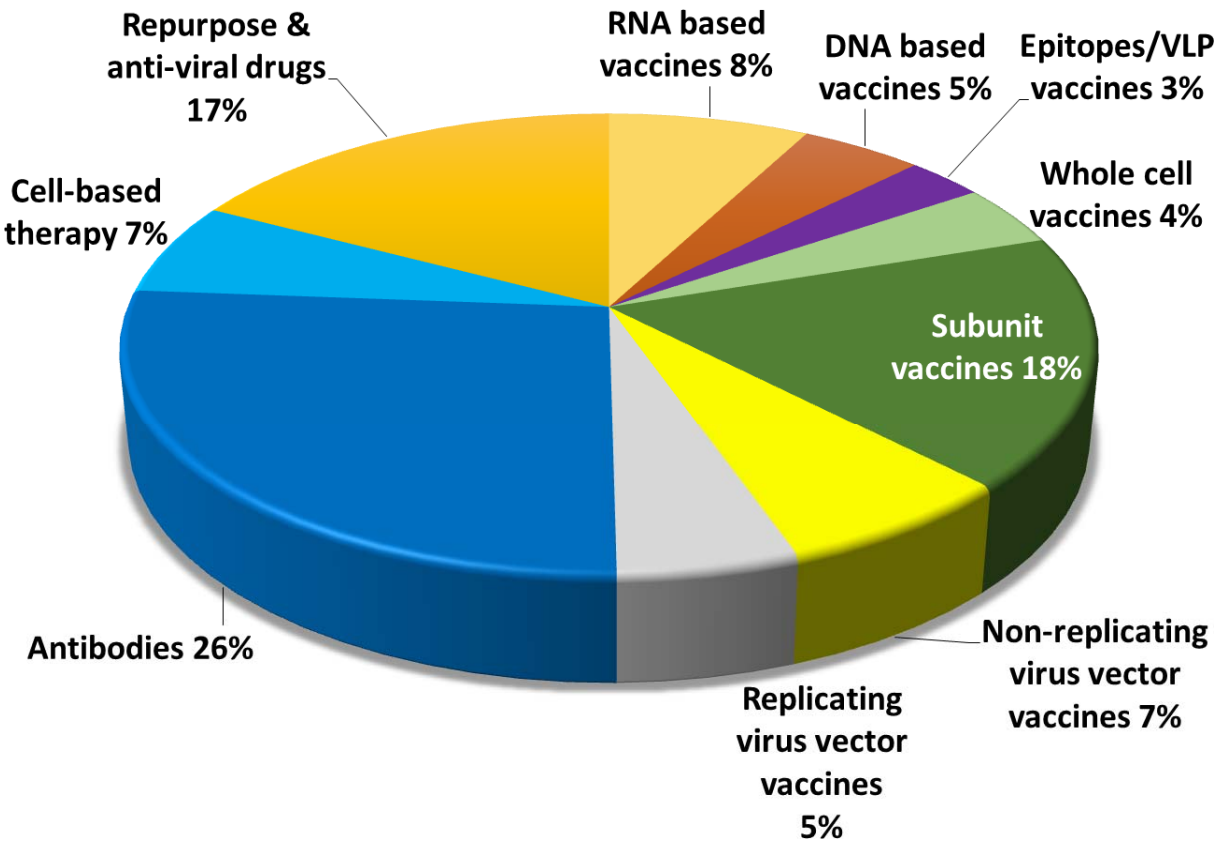

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Figure 6: Current status of treatments and vaccine development against SARS-CoV2. Researchers across the world are trying multiple approaches to configure the remedy for SARS-CoV2. The stakes of these approaches is mentioned in percent.

Remdesivir is a broad spectrum antiviral drug developed by Gilead Sciences. It is a pro-drug which metabolizes into a ribonucleotide analogue. It was originally developed to treat RNA virus Ebola. The RNA dependent RNA polymerase (RdRp) is a key component of SARS-CoV2 and other RNA viruses. Remdesivir targets RdRp and inhibits RNA replication. Being nucleotide analogue of ATP, RdRp incorporates Remdesivir into new RNA strands. This incorporation of Remdesivir in growing RNA unable the RdRp enzyme to further replicate the genomic RNA of virus [82]. In MERS-CoV, SARS-CoV and SARS-CoV2 it has been observed that Remdesivir does not terminates the RNA replication immediately after its incorporation. But three more nucleotides are seen to be added, hence classified as a delayed chain terminator [83–85]. Few side effects including liver inflammation, low blood pressure and sweating reported related to Remdesivir. Moreover, viral genome is prone to mutations and hence may affect drug activity. In case of murine hepatitis virus two mutations in the nsp12 gene which encodes RdRp developed resistance to Remdesivir [86]. Thus, albeit Remdesivir is promising drug at present, alternative or additive molecules to it should be made available. Recently, Remdesivir is approved in Japan by Japan's Ministry of Health, Labour and Welfare. The Committee for Medicinal Products for Human Use of the European Medicines Agency also has recommended use of Remdesivir to non-critical patients.

Another RdRp inhibitor, **Favipiravir** is a purine nucleoside analogue. Its incorporation results into inaccurate viral RNA synthesis [87]. It is now approved for clinical trial in Japan. Similarly

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another nucleotide analogue is **Ribavirin** which resembles guanosine and disturbs viral RNA replication (and of DNA too in DNA virus). It also interferes with RNA capping mechanism as RNA requires guanosine for the process. This molecule has shown positive results in pre-clinical trials [88]. **Penciclovir** and **Galidesivir** are another RdRp inhibitors which are also under consideration for COVID-19 treatment [84,86,89,90]

Lopinavir and **Ritonavir** are protease inhibitors. They were discovered as the anti-HIV and anti-influenza virus drugs respectively. In recent studies Lopinavir has shown considerable antiviral activity against SARS-CoV2. In case of SARS-CoV and MERS-CoV, Lopinavir alone showed antiviral effect [83]. Lopinavir targets viral protease including the main protease $3CL^{pro}$ and papain-like protease (Pl^{pro}) [82,91,92]. The application of these drugs thus halts the virus multiplication cycle immediately after translation.

Camostat is a potent serine protease inhibitor. It is approved in Japan for treating pancreatic cancer. As mentioned earlier, along with binding to ACE2 receptors, SARS-CoV2 entry in host cell requires priming of S protein by TMPRSS2 [93]. **Camostat mesylate** binds strongly with three residues (His296, Ser441 and Asp435) on TMPRSS2, thus inhibiting the priming of SARS-CoV2 S protein. Homology modeling and docking studies revealed another two molecules **Nafamostat** and **Bromhexine hydrochloride** as TMPRSS2 inhibitors [94]. Interventional clinical trials are undergoing for these drugs (https://clinicaltrials.gov/ct2/show/NCT04321096).

Chloroquine and **hydroxychloroquine** are the well-known antimalarial drugs that have demonstrated antiviral activity against SARS-CoV2 *In vitro*. The *In vitro* study has revealed that chloroquine target at the entry as well as post entry multiplication steps of SARS-CoV2. The hCoVs after binding to ACE2 receptors requires sialic-acid-containing glycoproteins and gangliosides which serve as primary attachment factors on respiratory tract [95]. Recently, large

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554 ganglioside-binding domain is identified on the NTD of SARS-CoV2 S protein. The chloroquine
555 and hydroxychloroquine binds with high affinity to sialic acid and gangliosides containing sialic
556 acid, thus reducing the attachment of virus to the host cell surface [96]. It is also proposed that
557 chloroquine and hydroxychloroquine inhibits terminal glycosylation of the ACE2 receptor which
558 disturbs binding of S protein to host receptor. Chloroquine gets protonated inside the endosome,
559 lysosomes and golgi vesicles which raises the pH of these vesicles. Chloroquine-induced
560 inhibition of endosomal acidification trap the virus in endosomes itself. Similarly, it may also
561 inhibit virion assembling in ERGIC and prevent further spread of the virus [97].
562 **Arbidol**, also known as **Umifenovir** is a drug on influenza virus, which is also reported to inhibit
563 multiple viruses. Arbidol interferes in binding of SAS-CoV2 S protein to host receptor and also
564 targets intracellular vesicle trafficking. Arbidol may bind to lipid membranes thus changing the
565 membrane configuration of the cytoplasm and endosome which may also impact the virus
566 attachment and fusion [98]. **Baricitinib** inhibits Janus kinase, on which cytokine receptors (type
567 I and II) family depends for phosphorylation and may control the cytokine storm in the lung
568 tissue. The summary of promising drug candidates against COVID-19 is given in Table 1.

569 **7.2 Immunotherapy**

570 Historically immunotherapy has provided potential solutions during outbreak of SARS-CoV and
571 MERS-CoV. This approach uses plasma from recovered patient, vaccines and cell based
572 therapies which rely on mesenchymal stomatal cells with their derivatives. Development of
573 vaccine is the major key driver in combating SARS-CoV2, as it will provide long term protection
574 to the human community. Considering effort and necessity to develop vaccine, we have
575 discussed vaccine development in a separate section.
576

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7.2.1 Convalescent plasma (CP) therapy

CP therapy has proven as a lifesaving tool for many acute infections. It is an acquired passive immunity [99]. It has been used in SARS-CoV outbreak and recently recommended by WHO to treat Ebola virus infection. It is an empirical tool especially when precise treatment or vaccine is not available. In previous SARS and MERS epidemic CP therapy results were promising. As there are lots of commonalities between these two and SARS-CoV2, including common genera of the pathogenic hosts, CP therapy may provide potential solution to the SARS-CoV2 outbreak. [100]. Patients recovered from COVID-19 with the specific antibodies may be valuable donor source for CP. Although promising, CP therapy has not yet been shown to be effective in COVID-19 in large scale [100].

7.2.2 Cell-based therapies

Cell based therapy is a distinguishable advanced approach and of priority for the disease like cancer. Cell based therapy uses viable cells to inject or graft/ implant into the patient [101]. Best example of cell based therapy is of transplanting T-Cells which can fight cancer cell through cell mediated immunity [102].

Mesenchymal stomatal cells (MSC) has shown promising results against influenza A. In the influenza A patients transplantation of MSCs has reduced the mortality significantly, at least in experimental population. There are common clinical conditions in influenza A and COVID-19 including acute respiratory distress syndrome (ARDS) and lung failure. MSC based therapies specifically in lung tissue has inhibited alveolar collapse, collagen accumulation and cell apoptosis. Self-renewal inside the host body and their multipotency are the added advantages of cell based therapy. Thus cell based therapy may provide improvements in the COVID-19 patients too [103]. Although mode of action of MSC is not clearly understood, it is predicted that

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in lungs tissue MSC may release soluble secretions including anti-inflammatory cytokines, antimicrobial peptides and angiogenic growth factors. Considering these benefits, cell based therapy approach has been initiated to treat SARS-CoV2 at various institutes [104]. MSCs, MSC-derived conditioned media (CM) or extracellular vesicles (EVs) can be used in the therapy. Ongoing trials include various cell types like MSC-derived CM or EVs, adipose-derived MSCs, umbilical cord blood derived mononuclear cells, cytotoxic, dendritic cells, natural killer cells, cord blood stem cells and cytokine-induced killer cells. [101,105].

7.2.3 Recombinant proteins and antibody therapy

Human recombinant soluble ACE2 receptors (rhACE2) is a genetically modified variant of ACE2, which shows reduced infection and viral growth in cell cultures and organoids by acting as a decoy for SARS-CoV2 [106]. ACE2 has been identified as the receptor for SARS-CoV2 binding and it has been proposed that inhibiting this interaction might be effective in treating COVID-19. The rhACE2 binds with the spike proteins on SARS-CoV2 surface and avoids its interaction with the membrane ACE2 protein and thus preventing the virus to enter the cell. It has been shown that clinical grade rhACE2 reduced SARS-CoV2 recovery from vero cells by a factor of 1000-5000. This molecule has undergone phase 1 testing in healthy volunteers and phase 2 testing in some patients with ARDS [106]. Recombinant human plasma gelsolin (rhu-pGSN) can prevent the lung injury observed in SARS-C0V2 infection. Trials for anti-inflammatory action of Ruconest, a recombinant human C1 esterase inhibitor in SARS-CoV2 infected patient is also under progress,

SARS-CoV2 infection leads to cytokine storm resulting into ARD syndrome. Cytokines and chemokines including interleukin (IL)-1β, tumor necrosis factor (TNF)-α, granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF),

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623 interferon (IFN), chemokine 10 (CXCL10), chemokine ligand 2 (CCL2), CCL3 and CCL4
624 shows elevated level in COVID-19 patients [107,108]. Increased IL-6 results into lung tissue
625 damage [109]. Trials are in progress to stop the IL-6 storms using monoclonal antibodies
626 tocilizumab, human monoclonal antibody sarilumab and siltuximab.
627 GM-CSF glycoprotein is secreted by macrophages, T cells, mast cells, natural killer cells,
628 endothelial cells and fibroblasts which functions as a cytokine and modulated by SARS-CoV2. It
629 enhances granulocytes and monocytes flux resulting into inflammatory responses. Anti-GM-CSF
630 IgG1 monoclonal antibody-TJ003234 are being tested to control SARS-CoV2 mediated
631 inflammation. Similarly, infliximab binds to another SARS-CoV2 target, TNF-α and
632 emapalumab binds to IFN-γ. Emapalumab is fully human IgG1 monoclonal antibody and hence
633 getting special attention. Another intracellular non-receptor tyrosine kinases such as Janus
634 kinase (JAK) is involved in transduction of cytokine-mediated signals through JAK-STAT
635 pathway. Anakinra is a recombinant human IL-1RA, Janus kinase 2 inhibiting antibody, is also
636 under trial for COVID-19 treatment. Fedratinib (Inrebic) antibody can also work in the similar
637 way to suppress inflammation [109,110]. Cytokine IL-17 is known to be active in development
638 of lung cancer. Interestingly, it was also observed elevated in SARS-CoV2 patients too. Thus,
639 secukinumab monoclonal antibody used in treating lung cancer patient can also be useful for the
640 treatment of SARS-CoV2 patients [111].
641
642 **7.3 Vaccines**
643 Since its discovery, vaccines have provided the most efficient and economical means to combat
644 deadly infectious diseases. At present multiple treatments are on trial to cure the patients infected
645 with SARS-CoV2. But yet there is no precise medication available and virus is spreading at

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alarming rate. Vaccination provides long-term protection as it triggers adaptive immunity. Thus the development of an effective and safe vaccine against SARS-CoV2 infection is of prime importance to date. So worldwide, more than 40 pharmaceutical companies and academic institutions are engaged to try multiple approaches for the development of vaccine against SARS-CoV2. Antigen selection is the first crucial step in vaccine development and there are several candidates in SARS-CoV2, which can serve as the antigen. S protein is the prominent target for vaccine development as it consists of signal peptide, extracellular, trans-membrane as well as intracellular regions. Along with full length S protein, RBD and NTD of S protein are also under study. Other proteins including N, M, E protein and accessory proteins are also capable antigens for vaccine development [112]. Today vaccine research has reached to the next level and novel types of vaccines are being introduced against different infections. Thus there are wide vaccine options being investigated against SARS-CoV2 which includes attenuated vaccines, live vaccines, DNA vaccines, RNA vaccines and recombinant protein vaccines [113]. Each type comes with their own pros and cons, and best option would be available after efficacy and toxicity studies are done. Almost 133 vaccines against SARS-CoV2 are in pipeline (https://milkeninstitute.org/covid-19-tracker). Type-wise vaccines in progress are discussed below.

7.3.1 Whole cell vaccines

This is a classical type of vaccine. It includes live-attenuated as well as inactive whole virus vaccines. They cause a strong and long-lasting immune response against targeted pathogen. In case of SARS-CoV2 these vaccines were primarily advanced for clinical trials, as most of the vaccine producing firms possess the infrastructure required for the development of these vaccines. In live attenuated vaccine, virus is made non or less infectious by the virtue of

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669 mutations in its genome. For this, virus is passaged multiple times or through different hosts
670 resulting into mutations. Although the virus exhibits reduced infectivity, it can induce the host
671 immune response efficiently. Previously, whole cell vaccine has given tremendous success
672 against polio. Codon deoptimization is technology aims to substitute viral codons by human 'less
673 preferred codons'. By using this technology for virus attenuation, Codagenix in collaboration
674 with Serum Institute India Ltd is in process to develop vaccine against SARS-CoV2 [114].
675 Advantage of whole cell vaccine is that it presents all antigens which can efficiently induce toll
676 like receptors in host cell and adaptive immunity.

677 **7.3.2 Subunit vaccines**

678 Subunit vaccine is a comparatively safe approach. It utilizes pathogen derived small subunits,
679 mostly a surface protein from virus to elicit the immune response and induce the acquired
680 immunity against the virus. Sometime these small subunits may not contain the native danger
681 signal, for which equivalent signal must be incorporated during the vaccine designing.
682 Immunologic adjuvants are generally added to elicit a stronger immune response [115]. As of
683 now, more than 28 institutions have initiated programs on subunit vaccines. Most of the institutes
684 are targeting entry of SARS-CoV2 by preferring S protein as an antigen. In case of SARS-CoV,
685 subunit based vaccine had shown promising results in monkey. As mentioned earlier, fusion of
686 viral and host cell membranes allows the entry of virus in the host cell. Viral fusion protein (S
687 protein in case of SARS-CoV2) undergoes structural rearrangements from a 'pre-fusion'
688 conformation to a highly stable 'post-fusion' conformation. Pre-fusion form acts as important
689 epitopes essential for vaccination. Molecular clamp technology uses polypeptide which clamps
690 to these unstable epitopes and efficiently stabilizes viral fusion proteins, resulting into strong
691 immune response [116,117]. By using this 'Molecular Clamp' technology, the University of

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692 Queensland is developing a subunit vaccine [114]. In a novel approach Novavax developed
693 virus-like nanoparticles based vaccine using recombinant expression of the S-protein from
694 SARS-CoV. The application of these particles with adjuvant matrix M1 has boosted the
695 immunity in mice [118]. Novamax has initiated same technique to develop vaccine against
696 SARS-CoV2. Use of RBD of S protein alone is one of the noticeable attempts made by the
697 University of Texas Medical Branch, Texas Children's Hospital Center for Vaccine
698 Development and New York Blood Center. Clover Biopharmaceuticals has produced its trimeric
699 S protein subunit vaccine candidate using a mammalian cell expression system. Advantage of
700 this vaccine is the utilization of TRIMER-TAG technology which increases the affinity of
701 secreted protein to their target.

702 **7.3.3 Nucleic acid vaccine**

703 Nucleic acid vaccine is novel approach of vaccine development. Both DNA and RNA can be
704 utilized to develop nucleic acid vaccine. These vaccines do not require any infectious virus to
705 handle. Their large scale production is easy as compare to traditional vaccines. DNA vaccines are
706 composed of plasmid DNA molecules derived from the non-infectious bacteria harboring one or
707 more genes encoding antigens from virus. Proteins encoded by this plasmid are considered as
708 foreign molecules and presented as antigens to the host cell inducing immunity against the virus
709 [119,120]. There are almost 11 DNA based vaccines in progress. Most of these vaccines target S
710 protein of SARS-CoV2. The Inovio pharmaceuticals has developed DNA vaccine candidate
711 (INO-4800), which is in preclinical studies and soon will enter phase I clinical trials. Inovio has
712 used same platform to develop vaccine against Nipah, HIV, Filovirus, HPV and Zika [114].
713 Applied DNA Sciences subsidiary, LineaRx and Takis biotech also has collaborated to develop a
714 linear DNA vaccine candidate against SARS-CoV2, which has reached to preclinical studies

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[121]. Zydus Cadila, BioNet Asia, Karolinska Institute in collaboration with Cobra Biologics and Osaka University collaborated with Takara Bio, are the few organizations developing DNA vaccine against SAR-CoV2.

For mRNA vaccine, RNA sequence coding for the antigen or protein identical to virus epitopes is used to develop immunity. Once administrated in the body, the injected RNA sequence is translated to the antigen by host machinery. The situation resembles to the virus entry displaying the antigenic proteins to the host immune system and hence stimulates adaptive immunity against the related virus [122]. The mRNA vaccines are safe, can be produced at higher rate and cheaper as compare to other types of vaccines. [123]. The mRNA vaccine can be delivered as naked mRNA or in the form of encapsulated RNA. For encapsulation, lipid nanoparticles can be used. This idea looks impressive as lipid capsule provides protection to RNA and more importantly, lipid layer can be customized to target specific cell types [124,125]. Ex-vivo administration through dendritic cells is also another option which is costly and time consuming, hence not much preferred [126]. Until now, no mRNA vaccine has been approved for human administration, but some are in clinical trial phase showing promising outcomes. Firstly, Moderna has developed mRNA vaccine named as mRNA 1273 which encodes S protein hence supposed to inhibit virus entry. mRNA 1273 is expected to enter phase III of clinical trials by Jul- 2020. BNT162 is another mRNA vaccine developed through collaboration between BioNTech and Pfizer corporation [121]. There are almost 18 vaccine candidates in development out of which vaccines candidates developed by Stemirna Therapeutics, Imperial College London, Arcturus Therapeutics and Curevac are at phase of clinical trial.

7.3.4 Virus vector vaccines

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737 Since decades, virus has been used in development of vaccines. In 1984 first report of
738 recombinant vaccinia virus was used to express hepatitis B surface proteins and it developed
739 induced immunity in chimpanzees [127]. Virus vectors show highly efficient gene transduction
740 and high target specificity, due to which virus vector vaccine develops potent immune response
741 through B and T cells [128,129]. There are two types of virus vector vaccines, replicating and
742 non-replicating virus vector. Vaccine using replicating vectors gives long term shield and shows
743 strong immunity, while booster doses of non-replicating vector vaccines are required to retain the
744 immunity [130].
745 CanSino Biological Inc in collaboration with Beijing Institute of Biotechnology has developed
746 non-replicating viral vector vaccine using Adenovirus Type 5 Vector. This vaccine is in stage of
747 clinical evaluation phase. University of Oxford has also initiated non-replicating viral vector
748 vaccine using ChAdOx1, a weakened common cold virus and named vaccine as ChAdOx1 n-
749 CoV-19. University of Oxford previously attempted vaccine development for MERS, influenza,
750 TB, Chikungunya and Zika based on the same technique. ReiThera is developing non-replicating
751 vector vaccine using replication defective Simian Adenovirus (GRAd) which encodes SARS-
752 CoV2 S protein. Vaxart has developed oral vaccine while University of Manitoba is developing
753 dendritic cell based vaccine. There are approximately 18 consortiums engaged in non-replicating
754 virus vector vaccines development including GeoVax-BravoVax, Janssen Pharmaceutical and
755 Greffex.
756 Consortium of Institute Pasteur, University of Pittsburgh and Themis Biosciences are developing
757 replicating-virus vector vaccine with measles vaccine virus vector. In past, measles virus vector
758 vaccines have proven their potential as they infect the antigen-presenting dendritic cells and
759 macrophages resulting into long term immunity [113]. Tonix Pharma and Southern Research are

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using replicating Horsepox vector which expresses SARS-CoV2 S protein. University of Wisconsin-Madison, FluGen and Bharat Biotech in collaboration has developed nasal vaccine named as CoroFlu. It is a live viral vectored vaccine based on attenuated influenza virus backbone. University of Western Ontario, IAVI-Batavia and University of Hong Kong are also using replicating virus vector to target S protein or its RBD domain for development of vaccine against SARS-CoV2.

7.3.5 Epitope and VLP vaccines

Epitopes are present on the antigen, specifically to which antibodies bind. Immunity of host cells can be primed by exposing them to small epitopes or virus-like particles (VLPs). They are usually prepared by chemical synthesis technique hence these vaccines are also considered as synthetic vaccine and peptide vaccine. Preparation and quality assessments are easy as compare to other forms of vaccine [131]. But low molecular weight of epitopes may result into low immunogenicity. Thus structural modifications and delivery systems with application of adjuvants are additionally required in formulation [121]. Recently, researchers from the Hong Kong University of Science and Technology have screened a set of B and T epitopes for S and N proteins of SARS-CoV2. The potential epitopes can be used to develop vaccine [121]. VLPs derived from cucumber mosaic virus have shown RBD like residues. Vaccine (RBD-CuMVTT) developed from these VLPs has induced antibodies in mice blocking the binding of S protein to ACE2 receptor [132].

As seen in case of H1N1, VLP for SARS-CoV2 can be synthesized using plant system. Medicago Inc. has initiated such program to synthesise VLP against SARS-CoV. They have synthesized VLPs for flu, Rotavirus, Norovirus and West Nile virus in plant system previously

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[133]. Doherty Institute and Imophoron Ltd with Bristol University's Max Planck Centre are also working on development of VLP base vaccine for SARS-CoV2.

7.3.6 *In silico* approaches

To speed up the vaccine development, number of research teams are using *In silico* approach to facilitate the process. Saha et al has identified 5 MHC I and 5 MHC II B-cell derived T-cell epitopes. They generated tertiary structure of the vaccine protein using 3Dpro of SCRATCH suite with combining adjuvants and linkers, which results into multi-epitope vaccine which may potentially work against SARS-CoV2 [134]. Similarly by using reverse vaccinology approach, a novel multi-epitope vaccine against SARS-CoV2 triggering $CD4^+$ and $CD8^+$ T-cell immune responses was synthesized *In silico*. From viral N, ORF3a and M protein, a multi-epitope vaccine candidate having five rich-epitopes domain were identified. This *In silico* study proposed that multi-epitope, chimeric protein vaccine can generate humoral and cell-mediated immune responses [135]. Similar study identified vaccine with 242 residues which are immunogenic epitopes for B cells [136]. By comparing experimentally-determined SARS-CoV-derived B cell and T cell epitopes from structural proteins of SARS-CoV and SARS-CoV2, identical epitopes were mapped [137]. Similarly study using two different bioinformatics approaches identified common antigenic region between SARS-CoV and SARS-CoV2, which can serve as a candidate for vaccine development with high degree of confidence [140]. The list of various vaccines under development against SARS-CoV2 is summarized in Table 2. Research and its outcomes about SARS-CoV2 are continuously evolving and parallelly the vaccine development. Recent updates on treatments and vaccine development can be obtained from https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports, https://milkeninstitute.org/covid-19-tracker.

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CONCLUSION

During this initial period of the outbreak of COVID-19, several studies have been published highlighting the characterization, genetic evolution, receptor binding, pathogenesis, clinical manifestation of SARS-CoV2. Many scientists are working vigorously on the prevention and control of this novel coronavirus, as studies in this area are of high priority to reduce the impact of this outbreak. This review is a summative overview of the current knowledge on SARS-CoV2. The sequence based analysis suggested horseshoe bat to be the natural reservoir and primary evidences suggests Malayan pangolin as an intermediate host. The spike protein plays a vital role in determining the host range and the analysis of RBD of spike protein concluded that SARS-CoV2 and Malayan pangolin CoV share identical binding residues to ACE2. However the knowledge regarding some aspects of this virus remains limited, such as many of the accessory proteins are still uncharacterized. Till now, no promising drug or treatment has been developed against the virus but of all the drug molecules screened for blocking the coronavirus mechanism of invasion and action, Remdesivir has come out as one potential treatment for COVID-19 after completing phase 3 trials to study its effectiveness in the COVID-19 patients. Researchers from different industries and universities across the world are employing different technologies for the development of an efficient and safe vaccine for SARS-CoV2, in which S Protein is serving as the promising antigen for vaccine development. By the mid of May 2020, five candidate vaccines are under clinical evaluation and 133 vaccines are in different stages of development. Hence, for the time until an empirical vaccine is not out in the market for human use, one can lower the possibility of human to human virus transmission by following physical distancing, contact tracing and large scale testing of high risk group individuals and quarantine to cope up with this prevalent threat.

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CONFLICT OF INTEREST STATEMENT

All authors declare we do not have any financial/commercial conflicts of interest.

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AUTHORS' CONTRIBUTIONS

SBK, GS and PB drafted the primary version of manuscript. AAP and VTB designed, supervised this study and carried out the bioinformatics analysis and drafted the manuscript. All the authors read and approved the final version of manuscript.

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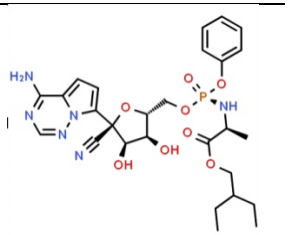
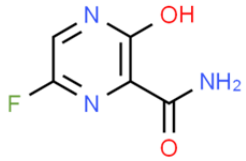
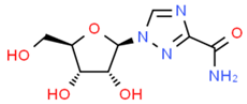
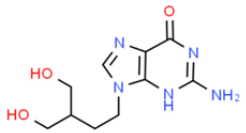
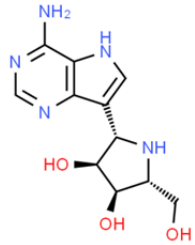
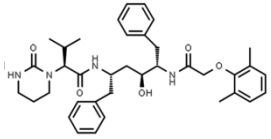
TABLE LEGENDS

Table 1: Summary of promising drugs with their molecular targets against SARS-CoV2.

Table 2: Ongoing efforts of vaccine development against SARS-CoV2.

Sr. No.	Antiviral agents	Drug targets	Reported mechanism of action	Status	Molecular formula and mass (in Dalton)	Structure
1	Remdesivir	RdRp	Inhibits RNA replication by delayed chain termination.	Phase 3 for SARS-CoV2	C27H35N6O8P 602.2254	
2	Favipiravir	RdRp	Inhibits RNA dependent RNA polymerase and causes incorrect RNA synthesis	Undergoing clinical trials in Japan	C5H4FN3O2 157.0287	
3	Ribavirin	RdRp	Inhibits viral RNA synthesis and mRNA capping.	Affirmative Pre-clinical trials.	C8H12N4O5 244.0807	
4	Penciclovir	RdRp	Inhibits RNA dependent RNA polymerase.	Randomized trials for SARS-CoV2 Approved for HSV.	C10H15N5O3 253.1174	
5	Galidesivir	RdRp	It blocks viral RNA polymerase which leads to premature chain termination.	Randomized double-blind, placebo-controlled trials for SARS-CoV-2.	C11H15N5O3 265.1174	
6	Lopinavir	$3CL^{pro}$	Inhibits 3C-like protease of the virus and Papain-like protease.	Phase 3 clinical trials for SARS-CoV2	C37H48N4O5 628.3624	

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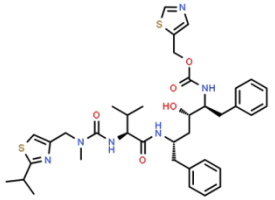
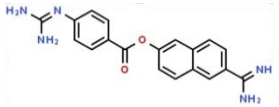
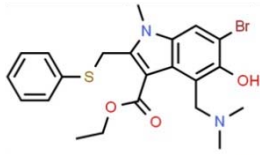
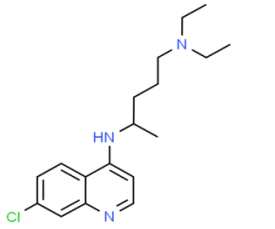
7	Ritonavir	$3CL^{pro}$	Inhibits 3C-like protease of the virus and Papain-like protease.	Phase 3 clinical trials for SARS-CoV2	C37H48N6O5S2 720.3127	
8	Nafamostat	TMPRSS2	Inhibits spike-mediated membrane fusion.	Undergoing interventional clinical trials.	C19H17N5O2 347.1382	
9	Umifenovir	Inhibits membrane fusion	Inhibits viral entry into target cells	Phase 4 for SARS-CoV2	C22H25BrN2O3S 476.0769	
10	Chloroquine	Sialic acid and gangliosides	Lower the virus attachment to host cell surface. Inhibits terminal glycosylation of the ACE2 receptor which interrupts binding of S protein to ACE2	Open-label trail for SARS-CoV2	C18H26ClN3 319.1815	

doi:10.20944/preprints202005.0519.v1

Platform	Target	Consortium	Status	Advantages	Disadvantages
RNA Vaccines	S protein	Moderna CureVac Stemirna Therapeutics, Imperial College London, Arcturus Therapeutics	Phase 1 Clinical trial	No infectious virus needs to be handled; vaccines are typically immunogenic, rapid production possible.	Safety issues with reactogenicity have been reported.
DNA Vaccines	S protein	Inovio Adnas/LineaRX/Takis Biotech. Advaccine Biotech Co./CEPI.	Preclinical studies Preclinical studies	No infectious virus needs to be handled, easy scale up, low production cost, high heat stability.	Vaccine needs specific delivery devices to reach good immunogenicity.
Recombinant protein Vaccines	S protein	University of Queensland/CEPI. Novavax Clover Biopharmaceuticals.	Pre-clinical testing	Adjuvants can be used to increase immunogenicity.	Global production capacity might be limited. Antigen and/or epitope integrity needs to be confirmed.
Viral vector-based Vaccines	S protein	CanSino Biological Inc/ Beijing Institute of Biotechnology/ Janssen Pharmaceutical/Greffex.	Clinical evaluation	No infectious virus needs to be handled, excellent preclinical and clinical data for many emerging viruses, including MERS-CoV.	Vector immunity might negatively affect vaccine effectiveness (depending on the vector chosen)
Whole cell Vaccines	Whole virion	Codagenix/Serum Institute of India	Under development	Straightforward process used for several licensed human vaccines, existing infrastructure can be used.	Creating infectious clones for attenuated coronavirus vaccine seeds take time because of large genome size. Safety testing will need to be extensive.

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Inactivated Vaccines	Whole virion	Wuhan Institute of Virology	Under development	Existing infrastructure can be used, has been tested in humans for SARS-CoV, adjuvants can be used to increase immunogenicity.	Large amount of infectious virus need to be handled. Antigen and/or epitope integrity needs to be confirmed.
Epitope Vaccines	Epitope	Hong Kong University of Science and Technology Medicago Inc.	Under development	Easy preparation and quality assessments	Low immunogenicity because of low molecular weight of epitopes

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