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Review Article

Hurdles and Directions in the Fight against COVID-19

Sulagna Mandal[1], Debprasad Chattopadhyay[2,3], Ishwar Singh[2] and Aparna Mukhopadhyay[1*]

[1]Department of Life Sciences, Presidency University, Kolkata, India

[2]ICMR-National Institute of Traditional Medicine, Nehru Nagar, Belagavi, India

[3]ICMR-National Institute of Cholera and Enteric Diseases, Kolkata, India

***Corresponding author:** Department of Life Sciences, Presidency University, 86/1 College Street, Kolkata-700073, India, Email: aparna.dbs@presiuniv.ac.in

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Abstract

COVID-19 has ushered in a new decade with potential to leave a permanent mark in the history of mankind. While some vaccine candidates are in circulation, we are still largely ignorant on SARS-CoV-2. This review summarizes information on the SARS-CoV-2 as revealed by the innumerable publications that have emerged. Parallels have been drawn with the more studied predecessors SARS-CoV and MERS, while the areas that still pose challenges in vaccine, prophylactic or therapeutic development have been highlighted. Thus, this review attempts to provide a holistic understand of the virus along with possible areas where successful therapeutic development can proceed.

Keywords

SARS-CoV-2, Spike, Mutation, Cytokine, Vaccine

Introduction

With an alarming and rising number of morbidity and mortality, the COVID-19 pandemic has made a permanent mark on the history of mankind/human evolution. The disease originally emerged from Wuhan, China at the end of December, 2019, has wiped out over 2.7 million of world population and infected over 124 million globally, as of March 23rd, 2021, as per WHO reports. Owing to frequent international travel this 160 nm pathogen could not be contained; rather transmitted from country to country, rapidly infecting more than 200 countries across the seven continents, as per WHO. Living in an era of advanced science and technology, it was unimaginable that a tiny ultramicroscopic (160 nm) nucleoprotein particle could wreck an unprecedented havoc to bring the entire world to a standstill, crashing the global economy, affecting the livelihood and careers of millions.

Although, coronaviruses has been studied since mid-1960 [1], it gained prominence during the Severe Acute Respiratory Syndrome (SARS) outbreak in China (November 2002-July 2003) [2] followed by Middle-East Respiratory Syndrome(MERS) outbreak (2012-2015) in the Middle-East countries [3]. While researchers were still pursuing studies on SARS, underlying mechanisms of pathogenicity of MERS and investigating a tool that would either act as a pan-coronavirus inhibitor or a novel therapy that would efficiently target SARS- and MERS-CoV, the highly pathogenic variant (initially named as 2019-nCoV by WHO) emerged. Among the seven human coronaviruses, namely, 229E, NL63, OC43, HKU1, SARS, MERS and SARS-CoV-2, the newest and the seventh member, SARS-CoV-2 has the most sequential resemblance to bat-derived SARS-like coronaviruses with 88-89% similarity followed by SARS (79.5%) [4]. Like its predecessors SARS-CoV and MERS-CoV, SARS-CoV-2 have shown different degrees of pathogenicity and virulence, such as, bronchitis, pneumonia with renal involvement [5], however proving to be most virulent among the three. COVID-19 is associated with a broad range of clinical manifestations ranging from asymptomatic pneumonia to severe acute respiratory diseases, enteric, hepatic and neurologic diseases [6]. Patients with co-morbidities and immuno-incompetence have the worst prognosis [7]. While SARS infects ciliated bronchial epithelial cells and type-II pneumocytes through angiotensin-converting enzyme 2 (ACE2), MERS infects un-ciliated bronchial epithelium and type-II pneumocytes through dipeptidyl peptidase 4 (DPP4) receptor, also known as CD26 [8,9]. SARS-CoV-2, just like its predecessor SARS-CoV also uses ACE2 as cellular receptor [10].

The urgency to develop specific drugs and vaccines which started since SARS outbreak, gained prominence and boost since the COVID-19 pandemic started. Accelerated efforts were aimed towards therapies which would either show potent antiviral activity against pan-coronaviruses or be targeted against SARS-CoV-2, thereby lowering the severity of the pandemic and also trying to eliminate the probability of another outbreak. Although, wait for vaccine seems to be over, it is too soon to arrive at the conclusion about potency and safety of these vaccine candidates. In this review, we focus on the molecular virology of SARS-CoV-2 along with possible hindrances faced by scientists in the venture of drug development to fight the pandemic with respect to its transmission and mutation of the virus. Further attention is drawn towards viral immunology and mechanism of pathogenesis that evolved to evade the most crucial antiviral immune response. Therefore, this review might provide new direction in therapeutic development against COVID-19 by furthering our knowledge on the above mentioned aspects of SARS-CoV-2.

Molecular Virology

Structure and Genome Organization of SARS-CoV-2

Belonging to the order *Nidovirale*, family *Coronaviridae,* SARS-CoV-2 is an enveloped virus containing non-segmented positive sense, single strand RNA genome of ~30 kilobases (Figure 1) [11-13]. Coronaviruses are divided into 4 genera, α-, β-, γ-, and δ-coronaviruses. The β-Coronaviruses are further divided into A, B, C and D lineages. Among the seven identified human coronaviruses so far, SARS-CoV and SARS-CoV-2 belong to the β-coronaviruses lineage B [14].

The term "corona" was derived from the appearance of a crown-like spike projecting from the surface

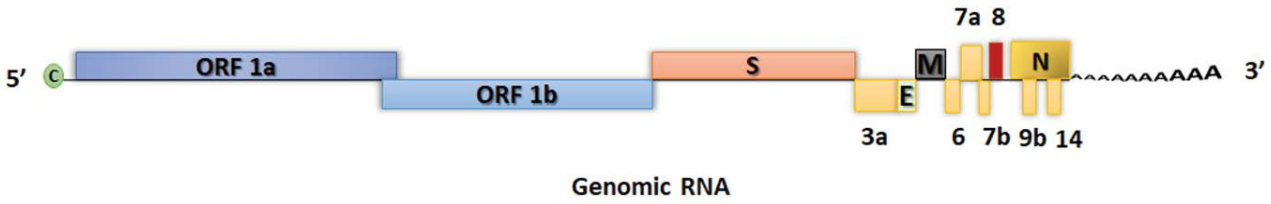

Figure 1: Genomic Organisation of SARS-CoV-2.

The ~30 kb RNA genome encodes for two overlapping ORFs (1a and 1b). These are translated to produce two large polyproteins pp1a and pp1b. The rest of the genome is transcribed into a nested set of subgenomic mRNAs. These viral polyproteins are further processed by virally encoded cysteine proteases, papain-like protease (PLpro) and 3-chymotrypsin-like protease (3CLpro). Sixteen (16) non-structural proteins (nsp1-nsp16) are encoded by ORF 1a/b at the 5' end, followed by structural proteins spike (S), membrane (M), envelope (E) and nucleocapsid (N), all of which are encoded by ORFs at the 3' end. Each structural protein is depicted by its single letter code. Numbers indicate the non-structural elements.

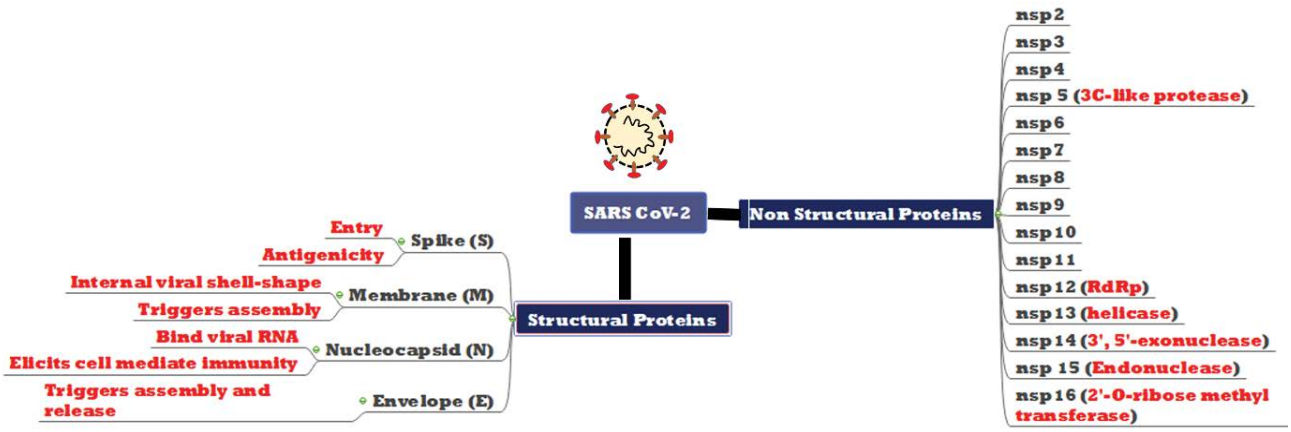

Figure 2: Structural and non-structural proteins encoded by SARS-CoV-2.

The primary function of each protein is depicted in red. Function of all 16 non-structural proteins is not clear. ORF1b encodes the RNA dependent RNA polymerase (RdRp), helicase and an mRNA cap-1 methyl-transferase as predicted by computational analysis. An exo-ribonuclease is also encoded by nsp14 of ORF1b, which is a unique feature of coronaviruses, contributing to high replication fidelity. Among four structural proteins, the S protein is required for species specificity, receptor binding and fusion. The M protein is a central organizer of viral assembly and defines the shape of viral envelope together with E protein. However, the majority of the E protein is localized at the site of intracellular trafficking, where it is found to participate in viral assembly and budding. Apart from these proteins, the N protein is actively involved in complete virus formation.

of the envelope. All coronaviruses share the common features in the organization and expression of their genome, a very large open reading frame 1 (ORF 1), comprising about the two-third of the genome that encodes two large overlapping ORF1a and ORF1b as depicted in (Figure 1). Other ORFs encode the structural proteins. Additionally, an ORF8 is found to exist in SARS-CoV and SARS-CoV-2, whose exact function is unknown, but is predicted to facilitate host shifts [15].

Both structural and non-structural proteins play a very crucial role in the viral life cycle as well as pathogenesis, depicted pictorially in (Figure 2) [16-18]. Therefore, these proteins always serve as potent targets for antiviral therapy. Primary candidates include the Spike (S) protein which forms the surface 'corona' of the coronavirus, as it's primarily responsible for entry and eliciting an adaptive antiviral response. Other targets include RdRp and nsp3 which are essential proteins involved in the replicase complex. Presence of viroporins encoded by ORF3a is unique to SARS-CoV and SARS-CoV-2 suggested by structural analysis [19,20]. These accessory proteins form membrane pores which regulate cellular ion-channels, which make them invaluable tools for anti-viral therapy and need to be explored further.

Here we highlight features of SARS-CoV-2 in comparison to the relatively better studied SARS-CoV (Table 1). Innumerable publications so far have focused on the Spike protein (because of its obvious importance in host entry and innate immune response) and many of the structural peculiarities have been well documented. Structural analysis reveals that the spike (S) protein of SARS-CoV-2 is closely related to SARS-CoV, sharing about 89.8% sequence similarity in their S2 subunit [14], an important target of fusion inhibitors at present. This type-I glycoprotein binds to the cellular receptor Angiotensin-Converting Enzyme-2 (ACE2), present in almost all major organs like lungs, heart, kidney and intestine [21]. The main physiological role of ACE2 is in the maturation of angiotensin, a peptide hormone involved in regulation of vasoconstriction and blood pressure [22]. The S protein of both SARS-CoV and CoV-2, has two extracellular structural domains S1 and S2. Receptor binding is mediated by receptor binding domain (RBD) of S1 subunit, followed by fusion via S2 subunit. After receptor binding, the S protein undergoes conformational changes followed by cleavage at its S1/S2 junction by the host cell protease-transmembrane protease serine 2 (TMPRSS2) [23]. This priming leads to destabilization

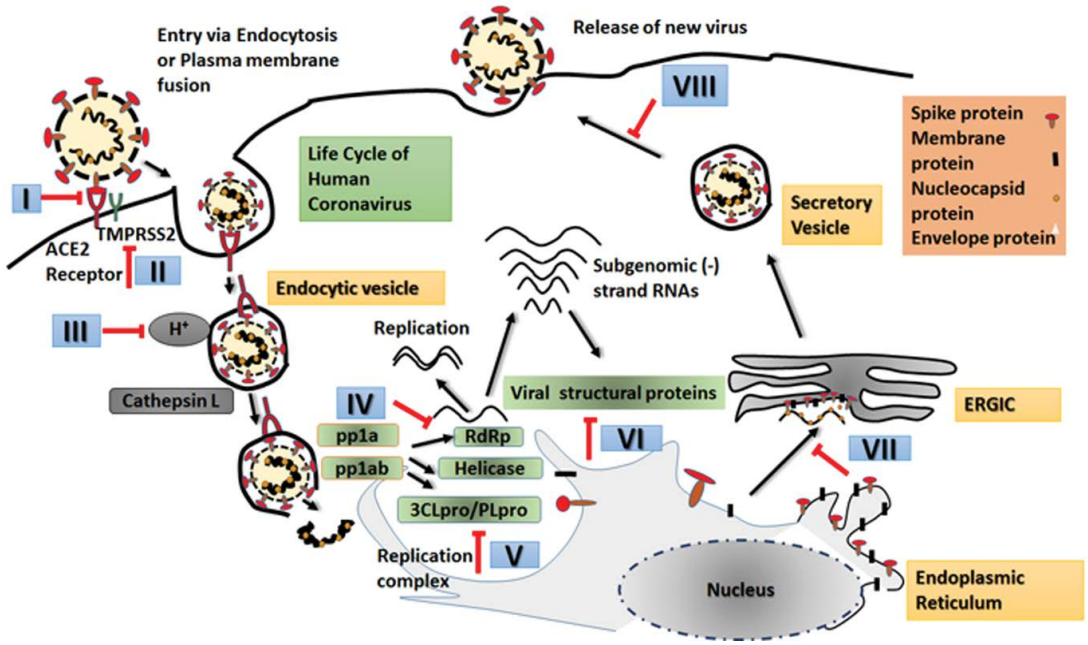

Figure 3: Life Cycle of SARS-CoV-2.

Life cycle begins immediately after binding of viral spike protein with host ACE2 receptor. Internalization by endosome. Release of viral genome into host cell cytoplasm followed by translation of ORF1a and ORF1b into two polyproteins (pp1a and pp1ab) by the host translational machinery. The pp1ab synthesis requires a -1 ribosomal frameshift but is formed from both ORFs. Further modifications occur when virally encoded proteases ($3CL_{pro}$, PL_{pro}) cleave these polyprotein to produce downstream proteins involved in viral replication in which a full length negative strand RNA is synthesized. Other downstream structural and non-structural proteins are synthesized via a nest of sub-genomic negative sense RNAs. Incorporation of structural proteins in endoplasmic reticulum (ER) followed by assembly in endoplasmic reticulum-Golgi intermediate compartment (ERGIC). Budding and release of new virus particles. Potential therapeutic targets are indicated by red arrows. Numbered steps are areas where intervention of the life cycle can be done for development of therapeutics. Some of them have been tapped and several are already in clinical trial as detailed in text.

of the prefusion structure, cleaving S1 subunit and transition of S2 subunit to a stable post-fusion conformation [24,25]. After binding through RBD, the heptad repeat 1 (HR1) and 2 (HR2) domains in S2 subunit interact and a six-helix bundle (6-HB) fusion core is formed, bringing viral and cellular membranes in close proximity to accelerate fusion and infection, documented by X-ray crystallography. Studies also suggest cathepsin-L driven processing of spike protein in endosomes, indicating receptor-mediated endocytic entry of both SARS-CoV and CoV-2 (Figure 3). Importance of other cellular proteins like PIK_{FYVE} (Phophatidylinositol 3-phosphate 5-kinase), TPC2 (Two pore channel subtype 2) in target cells that largely influence the viral entry have been highlighted in a recent study [26,27]. However more in-depth knowledge is needed on exact viral entry mechanisms to develop preventives and could be the Achilles' heel faced by modern science in developing antivirals with concerted efficacy (Box 1).

Life cycle of human coronavirus: A challenge

An in-depth knowledge of virus biology is essential to therapeutic development (Figure 3) in this fight against COVID-19. The life cycle of the virus begins with viral entry (attachment fusion and penetration) into the target cell (goblet and ciliated cells in lungs and bronchioles) following membrane fusion, via Ca^{2+} dependent mechanism [32] or endocytosis, as described in (Figure 3). The complete virus is released from the host cell by fusion of virion-containing vesicles with the plasma membrane [33]. Virus replication possibly induces ER stress thus activating the unfolded protein response (UPR) signalling pathway [34]. Further investigations might make use of the UPR pathway as potent therapeutic target against emerging pathogenic human coronaviruses like SARS-CoV-2.

Transmission and Spread

Having originated from bats, SARS-CoV-2 has successfully crossed the species barrier and adapted human to human transmission, just like its predecessors [35]. The major route of transmission for SARS-CoV-2 include droplet transmission, aerosol of close room and perhaps feco-oral [36,37]. A possible nosocomial transmission is also involved, as evidence exists for past nosocomial in-

Abbreviation	Full Form
IFN	Interferon
TLR	Toll-like receptor
RIG-1	Retinoic acid-inducible gene-1
MDA-5	Melanoma differentiation associated protein
PKR	Protein Kinase R
TRIF	TIR-domain containing adapter inducing IFN-β
TRAF	Tumor necrosis factor-receptor associated factor
IRF	Interferon regulatory factor
TBK	TANK binding kinase
MyD88	Myeloid differentiation factor 88
PRR	Pathogen Recognition Receptors
PAMP	Pathogen Associated Molecular Patterns
OAS	2', 5'-oligoadenylate synthetase
ORF	Open reading frame
M	Coronavirus Membrane (M) protein
N	Coronavirus Nucleocapsid (N) protein
nsp	Non-structural protein
IL	Interleukin
IFNAR	Interferon α receptor
TNF	Tumour Necrosis Factor
RLR	Rig-1 like receptors
NLR	Nucleotide-binding oligomerization domain (NOD)-like receptor
IKK	Inducible IκB Kinase
MAVS	Mitochondrial-Antiviral Signalling
ISGF3	Interferon Stimulated Gene Factor 3
NFκB	Nuclear Factor kappa-light-chain-enhancer of activated B-cells

Box 1: List of Abbreviations and Full Forms

Table 1: Comparative features between SARS-CoV and CoV-2.

Features	SARS-CoV-2	SARS-CoV	References
Cellular receptor	ACE2	ACE2	[23], [27], [28], [29], [30], [31]
Spike-ACE2 binding affinity	10-20 fold higher than SARS-CoV	Less than SARS-CoV-2	
Furin cleavage site	Prominent multi-basic furin cleavage site	Monobasic cleavage site only	
Role of furin cleavage site	Expansion of viral tropism, cell-cell fusion, enhances infectivity. Also cell specific.	No prominent role found.	
Reservoir host	Bat	Bat	
Intermediate host	Pangolin (in all probability)	Civet cat	
Surface stability	High. Upto a day or more in stainless steel and plastic.	Less than that of SARS-CoV-2	
Thermostability of spike (S) glycoprotein	Low thermostability is speculated to lower energy barrier to exert conformational change of S protein	Thermostability is comparatively high.	

fections of SARS [9]. The zoonotic source of this virus is still debatable, though phylogenetic analysis repeatedly relates it to bat-derived SARS-like coronaviruses (bat-SL-CoVZC45 and bat-SL-CoVZXC21) with 88-89% homology. The disease manifestation is reported in cats and tigers, but not in dogs and pigs, owing to low expression of ACE2 in the airways of dogs and pigs compared to the other susceptible species as well as homology of ACE2 between humans and cats [38]. Investigation of changes in ACE2 across species have identified many key changes between those that are infected vs. those that aren't. Consistent with studies related to SARS-CoV, the super spread of COVID-19 is also linked to surface stability and viability of SARS-CoV-2, mentioned in (Table 1). Higher binding affinity of SARS-CoV-2 S protein with host ACE2 indicates that the organs with high expression of ACE2 are the potential targets of SARS-CoV-2. Since most of the vital organs of the human body express ACE2, the morbidity and mortality rate is further enhanced as the virus can potentially infect multiple organs. Consistent with the wide spread of infection, we can assume the presence of more than one type of cellular receptor for viral entry besides ACE2. Hence, further studies should investigate the role of any other cellular receptor, if any, on viral pathogenesis. It is probable that this missing piece of knowledge might be a key to targeted therapeutics.

A major drawback in COVID research is the absence of an established animal model. A few recent studies claim that ferrets might be used as potential animal model to study viral pathogenesis, evidenced by very low titre of viral RNA in nasal turbinate, rectal swab and shedding of infectious virus in faeces. Another study also claims humanized mice to be a model to study viral pathogenesis. Infection in ferrets exhibit limitations due to the low viral titre in lungs than that exhibited by SARS-CoV and MERS-CoV infected hACE2 and hDPP4 transgenic mice, indicating that rigorous research is needed to establish an animal model [39,40]. Apart from these, Syrian hamsters may also serve as good model for SARS-CoV infection.

Mutation of SARS-CoV-2: Yet another obstacle.

Mutations are constant, inevitable and an integral part of evolution for all life forms, especially in RNA viruses. Coronaviruses show varying mutation rates compared to other single-stranded RNA viruses. On an average coronaviruses have ~10^{-4} substitutions per year per site [5]. The hotspots of mutation involve regions such as ORF8, ORF3, spike protein and nsp3 [41]. Its large genome size contributes to extra plasticity in genome modifications by mutation and recombination which increases the probability of intra-species variability, interspecies host jump and emergence of novel coronaviruses under right conditions [42]. Although, occurrence of erroneous mutations during replication is comparatively lower in coronaviruses compared to other RNA viruses due to high proofreading activity carried out by 3'-5' exonuclease encoded by nsp14 [17], evolution of coronaviruses is in progress as evident by the newly emerging strains in UK, Brazil and other parts of the world. Increased recombination during RNA replication might also lead to mutations. Generation of nested set of sub-genomic mRNAs is also responsible for increased homologous recombination among closely related genes from different coronavirus lineages. Besides circulation in multiple hosts which serve as mixing vessels, is likely to increase the recombination events. Evidence suggests that SARS-CoV shares its recombination events with α and γ lineages. A number of specific breakpoints and small recombinant regions have been found in the RdRp (nsp12), parts of nsp9, nsp10 and nsp14, based on sequence analysis of different strains of SARS-CoV [43]. Studies have shown recombination between SARS-related bat-associated CoV generated an entirely new strain in civets with a breakpoint at the nsp16/spike and S2 region. Furthermore, SARS-CoV and SARS-CoV-2 [44] is shown to have acquired an ORF8 by gene gain mech-

anism that may facilitate host shift. Additionally, gene gain and gene loss leads to rapid evolution in these viruses [45]. Plasticity of SARS-CoV and other bat-derived SARS-like coronaviruses spike protein is yet another attributable factor to viral mutation. Multiple substitution events allow escape of the virus from antibody neutralization, while retaining receptor specificity. This flexibility allows usage of a wide range of zoonotic receptors to human ACE2 receptors [46].

Recent phylogenetic network of analysis suggests that ancestral SARS-CoV-2 has undergone mutations leading to different variants. Some studies classify them into various lineages namely A, B and C distinguished by amino acid changes [47]. Both synonymous and non-synonymous mutation has led to different lineages, as lineage B supposedly diverged from parent lineage A and C from B. Lineage A and C is speculated to be prevalent in the USA and European countries while B lineage is more prevalent in East-Asian countries. Another study classifies them into two major genotypes- type I and type II, which are further subdivided [48]. Type II emerged from type I and is prevalent. Genomic analysis reveals synonymous mutations in two sites, which is correlated to high translational efficiency in type II strains than type I, thus implying their rapid transmission and infection amplitude. Further genotyping showcases high frequency SNP mutation sites in the critical proteins like S protein, RNA polymerase, primase and nucleoprotein of SARS-CoV-2 [49]. Similar to its predecessors, several amino acid substitutions were identified in RBD of S protein of SARS-CoV-2, suggesting its changing tropism and increased pathogenesis [50]. Further evidence suggests involvement of many mutation events including deletion and mis-sense mutations in parts of the genome encoding the major structural and non-structural proteins, except envelope protein [51,52]. Two mutations reported in nsp6 might favour evasion of CoV-2 from cellular immunity, specifically autophagy, a critical stress-response pathway of the cell [53]. Autophagosomes are formed but lose their ability to deliver the viral components to lysosomes for degradation. Nevertheless new variants were also identified in India by *in silico* analysis [54]. Non-synonymous mutation was observed in nsp3 gene that might be related to its transmission rate. However, experimental validation is unavailable to support this *in silico* study. Evidences only suggest gradual evolution of SARS-CoV-2 leading to different variants of the original Wuhan strain. Among the new variants in circulation, D614G variant gained most prominence. Through its evolution in humans,

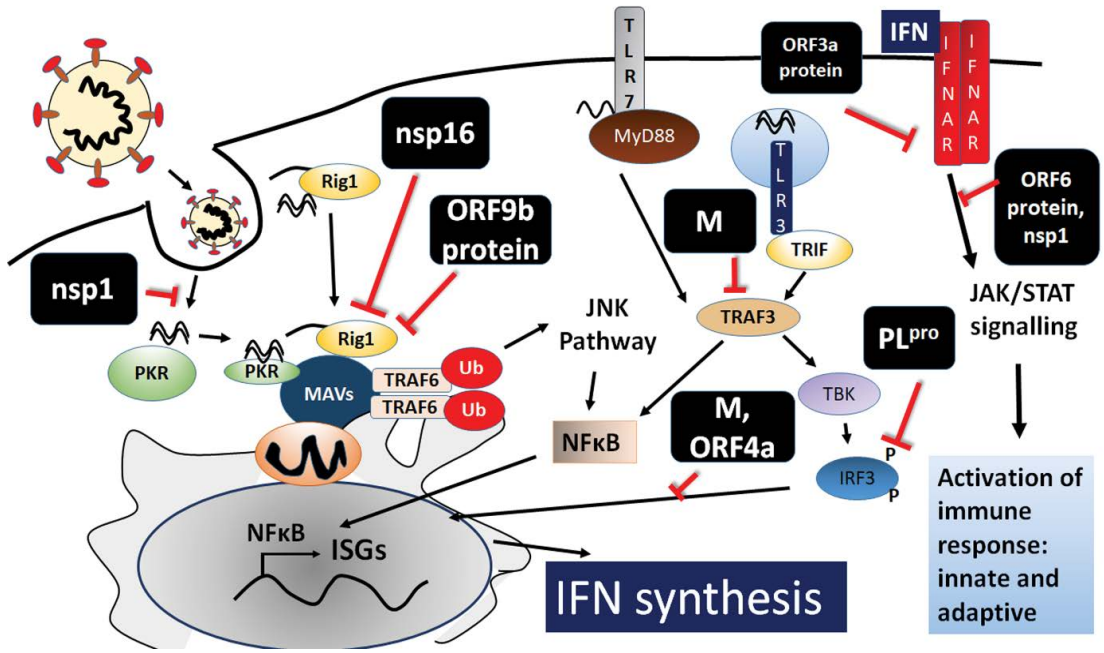

Figure 4: Innate Immune response in COVID-19.

Activation of interferon signalling system is depicted. The dsRNA (PAMP) intermediate during viral replication can trigger TLR3 (on endosomal membranes), RIG-1, MDA-5 and PKR all in the cytoplasm. GU-rich ssRNA can trigger TLR-7 which is present on endosomes and plasma membranes. TLR3 engages TRIF and TRAF3 which activate TBK, which phosphorylates IRF3 leading to its activation and translocation to the nucleus to activate transcription of interferons. Activation of TLR7 by MyD88 and TRAF3 activates IKKα resulting in the phosphorylation and activation of IRF-7, which too acts as a transcription factor similar to IRF3. Interferon genes are also activated by the NFκB pathway. MyD88 along with RIG-1 and MDA-5 converge on the MAVS (an outer mitochondrial membrane protein) coordinates activation of numerous TRAFs ultimately resulting in activation and translocation of NFκB to the nucleus. PKR also activates the NFκB pathway, apart from shutting down host mRNA translation. Upon release IFN signalling acts via JAK-STAT pathway. Various areas of interference by viral proteins (as predicted by studies in SARS-CoV and MERS) are also represented.

the aspartate residue at 614 position got replaced by glycine at the carboxy terminal domain of S1 subunit of spike protein of SARS-CoV-2 and epidemiological study suggested higher transmission of the G614 variant [55]. Further investigation led researchers to claim higher replication of the new variant *ex-vivo* using primary human nasal epithelial (HNE) cells, large airway epithelial (LAE) cells and distal lung small airway epithelial (SAE) cells from multiple donors [56]. Besides, increased viral load and fitness of G614 has been suggested by few *in vivo* studies [57,58].

Attention should be drawn to the latest variant in circulation and mostly spoken of is the UK variant or B.1.1.7. Although, this variant is mostly predominant in the UK, it has been detected in several other countries including India, the USA and Japan [59]. Unlike the other variants, it has acquired multiple mutations in the spike protein including deletion 69-70, deletion 144, N501Y, A570D, D614G, P681H, T716I, S982A and D1118H [60]. Almost 70% higher transmission rate of the UK variant has been speculated, despite lack of experimental evidence. In light of exceptionally large number of mutations in the spike region, pathogenicity of this variant needs to be determined immediately.

High frequency of recombination as well as continuous interaction between multiple species of coronaviruses amongst animal host pool leads to emergence of pathogenic zoonotic coronaviruses and potential outbreaks already seen and may again happen in near future. The question that concerns scientists is whether these variants are more transmissible or more pathogenic. Emergence of these variants and their increasing prevalence have kept virologists busy in studying how these variants can lead to altered pathology, transmission, spread and prognosis of the disease. This also questions the efficiency of current vaccine candidates making the fight against this virus challenging.

Immune Response and Its Evasion

Commonly, patients exhibiting poor prognosis have been reported to experience a cytokine storm which brings about an overwhelming immune response that often results in a wide spread and uncontrolled inflammatory response in the body. In patients presenting acute respiratory syndrome (ARS) due to COVID-19, circulating levels of pro-inflammatory cytokines (IFNα, IFNγ, IL-1β, IL-6, IL-12, IL-18, Il-33, TNFα, TNFβ and chemokines CXCL10, CXCL8, CXCL9, CCL2, CCL3, CCL5 along with MCP-1 (monocyte chemoattractant protein 1) have been reported, similar to what has been reported in SARS and MERS infections [61-63]. Although many reports have extensively reviewed the pathology of a typical cytokine storm, especially in the context of SARS and MERS infections, details on COVID-19 are restricted to correlation between cytokine levels found in patients with mild symptoms to those requiring ventilation and scanty reports from autopsies [64,65]. This review briefly explains the basics of a cytokine storm in the context of COVID-19 as many extensive reviews are available. Cytokines are released from activated immune responsive cells which include interferons, interleukins, chemokines and TNF-α [66,67]. Of these interferons offer the first line of defence against viral infection and have been discussed below.

Interferon signaling system

As depicted in (Figure 4), abbreviations stated in Box 1, the innate immune response is triggered by the presence of viral RNA that acts as PAMPs to activate specific PRRs such as TLR-3 and TLR-7. Upon activation of the PRRs, numerous signal transduction pathways are activated, which together leads to activation of interferon genes. Type I and II interferons upon release bind to their receptors on specific cells to trigger the formation of ISGF3 that act as transcription factor to initiate synthesis of numerous products that would initiate an antiviral response by promoting immunomodulatory, antiviral and anti-proliferative activity. IFN signalling primarily acts through the JAK-STAT pathway. Amongst others, such products produced as a result of IFN signalling include synthesis of 2', 5'-oligoadenylate synthase (OAS) that cleaves viral ds and ssRNA via activation of the OAS/RNase L pathway. Other effects include activation of NFκB leading to promotion of T-cell differentiation, NKC activation, DC maturation, and apoptosis of the infected cell and even of the bystander uninfected cell.

Evasion strategies by coronaviruses

Coronaviruses have also developed many ingenious means to tamper with the innate immune signalling response [68]. Primary evasion strategy by most RNA viruses replicating in the cytoplasm is to create a viroplasm of cellular membranes and hence sequester their genomic RNA from the cellular surveillance PRRs [69,70]. Furthermore, the N protein in SARS-CoV has been reported to sequester IFN-inducing RNA, while nsp1 mediates host mRNA degradation and blocks host mRNA translation and phosphorylation of STAT1 [71-73]. The M protein inhibits TRAF3/TBK1 complex formation whereas ORF9b product is known to induce proteosomal degradation of MAVs, TRAF3 and TRAF6 [74] (Figure 4). PLpro can inactivate IRF3, RIG-1, TBK1 and IRF3 [75] (Figure 4). Similar reports are also available about MERS in inhibiting various aspects of interferon synthesis and signalling molecules [76]. Furthermore, several protein products such as SARS-CoV ORF3a and ORF6 disrupt interferon-induced signalling by inducing degradation of the interferon α receptor (IFNAR) and inhibiting JAK/

STAT signalling respectively [71]. JAK/STAT signalling is also disrupted by nsp1 of SARS-CoV [72]. Some zoonotic coronaviruses encode phosphodiesterase (PDE), which cleaves 2', 5'-oligoadenylate to block activation of cellular endo-ribonuclease RNase L, thereby inhibiting interferon-induced antiviral response, which is another intriguing feature that enhances virulence.

How does a cytokine storm happen?

The pathology of a cytokine storm [77] is a complicated event but we attempt to simplify it. As a result of interference at the first level of interferon signalling by the viral proteins, early stages of infection results in delayed release of cytokines and chemokines in the respiratory epithelial and dendritic cells [78,79]. During the later stages, low levels of interferons with higher levels of pro-inflammatory cytokines such as IL-β, TNF-α, and chemokines CCL-2, CCL-3 and CCL-5 [80-83] have been reported in SARS and MERS infections. This results in an accumulation of neutrophils and monocytes in the lung tissue and peripheral blood of the patient. An excess infiltration of these cells leads to lung injury, caused due to apoptosis of the infected cell. Furthermore, accumulated macrophages receive activating signals and produce more chemoattractants such as CCL-2, CCL-7, and CCL-12 that leads to a further accumulation of more immune competent cells. This results in an elevation of secreted TNF-α, IL-6, IL-1β [68,84] that induces apoptosis of T cells and damage to the pulmonary microvasculature, vascular leakage and alveolar edema.

A high prevalence of COVID-19 disease has been observed in immunocompromised population and patients with co-morbidities [85]. In case of obesity which is also a comorbid factor, it is proposed that cytokines are released upon inflammation leading to a severe condition in COVID-19 [86]. However, the underlying relation between comorbid factors and imbalanced immune response such as cytokine storm needs to be delved into further.

Adaptive Immune response to COVID-19: The adaptive or humoral response to SARS-CoV-2 forms the basis for the development of plasma therapy where plasma from cured patients are being administered to critical patients [87], as well as rapid ELISA based tests that don't require access to a sophisticated laboratory. The adaptive response develops as a result of signalling from the innate immune response arm but typically becomes prominent only after a few days of the onset of symptoms. In SARS-CoV, MERS as well as SARS-CoV-2, delayed and weak onset of the adaptive immune response has resulted in poor outcome [88,89]. Profiles of appearance of specific immunoglobulins from patients have revealed the appearance of acute phase antibodies, IgA, and IgM early after the onset of symptoms with later appearance of IgG. Consequently several ELISA detection kits are being developed, based on the detection of IgG [90].

There is a great volume of literature investigating whether a previous exposure to any of the human coronaviruses can generate immunity against SARS-CoV-2. Antibody profiling to check for cross reactivity between those generated against viral proteins of other coronaviruses with SARS-CoV-2 has almost consistently revealed mixed cross-reactivity results. While minimal cross reactivity has been observed between the S1 region of SARS and SARS-CoV-2 [91], a more conserved region of S2 has the possibility of more cross reactivity. Few other studies have also reported the effectiveness of neutralizing antibodies against SARS-CoV in inhibiting entry of SARS-CoV-2 [23]. Furthermore, antibodies against N protein has also shown certain amount of reactivity [90]. There have been reports on T-cell and B-cell response inducing epitope profiling and few epitopes are found to be common in the S and N proteins across SARS and SARS-CoV-2 [92]. Recently, a study suggests that an antibody cocktail consisting of four antibodies raised against spike variants have more effective neutralizing activity compared to individual antibody raised against specific spike protein of SARS-CoV-2. It prevents rapid mutational escape of the virus as seen with individual antibody [93].

Vaccine development and its effectiveness depend on the onset of adaptive immune response and hence ideally a perfect vaccine would induce long-lasting immunity against all coronaviruses. Hence, research has focused on a variety of targets, most common being the Spike protein as it is abundant and presented on the viral surface to be recognized early in infection. Furthermore, an effective vaccine against S protein could kick start the immune response instead of wait for the innate signalling arm to activate it. However, this depends on the level of conservation being maintained by Spike protein in all serotypes that are being generated and the epitopes being used in vaccine design.

An overview of antibody dependent enhancement (ADE) and its role in Coronavirus entry

Besides cross-species barriers, there are many other factors that throw challenges in developing effective vaccines and drug strategies against a zoonotic pathogen and coronavirus is no exception. Among other factors, antibody dependent enhancement of coronavirus entry is another issue which is of utmost importance in the fight against SARS-CoV-2.Over the last few years, scientists studied the mechanism of antibody dependent enhancement of pathogenicity and severity of Dengue infection (or any other Flaviviruses). Primary infection of dengue produces neutralizing antibodies against the

Table 2: Prophylactics.

Name of the drug	Mode of action	Current status of Clinical trial	References
Hydroxychloroquine	1. Inhibition of virus fusion caused by elevated endosomal pH. 2. Impairment of terminal glycosylation of ACE2.	1. Controversial results. 2. Removed from clinical trial.	[99] [100]
Azithromycin	1. Increased endosomal pH inhibits viral fusion. 2. Also an immunomodulator.	1. Moderate efficacy. 2. Controversial results.	[101] [102]
Arbidol hydrochloride	Inhibits viral entry by inhibition of endocytosis or membrane fusion. It also inhibits viral RNA synthesis.	Not yet approved by FDA.	[103] [104]

Table 3: Curatives.

Name of the drug	Mode of action	Current status	References
Remdesivir	1. An adenosine analogue prodrug. 2. Interferes with viral replication by potentially inhibiting the RNA-dependent RNA-polymerase (RdRp) (Figure 3, step IV).	1. Although approved by FDA for emergency use in severe patients, NIH stopped its clinical trials.	[105], [106], [107], [108], [109], [110]
Favipiravir	1. It also interferes with viral replication by potentially inhibiting the RNA-dependent RNA-polymerase (RdRp). 2. Initially approved for influenza treatment in 2014, it is a guanosine analogue prodrug.	1. Initial results were promising. 2. Clinical trial is ongoing in many countries including India.	
Lopinavir/Ritonavir	1. Potential anti-retroviral protease inhibitor. 2. Ritonavir is speculated to inhibit other host proteases thereby protecting Lopinavir.	1. Results not consistent and unsatisfactory.	
Oseltamivir	1. A neuraminidase inhibitor. 2. Exact mode of action against SARS-CoV-2 has not been established.	1. Removed from clinical trial.	
Dexamethasone	1. A glucocorticoid drug. 2. Anti-inflammatory. 3. Decreases expression of pro-inflammatory cytokines, chemokines and adhesion molecules.	1. Shown promising result in RECOVERY clinical trial. 2. Conflicts remain regarding benefit-risk profile of the drug across full-spectrum of critical patients.	

same serotype. However, when the same patient if infected a second time by a different serotype, these neutralizing antibodies fail to protect but rather enhance the infection by many fold [94]. Such mechanism has also been observed in viruses like HIV and Ebola subverting antiviral immune response.

Antibody dependent enhancement or ADE is a phenomenon by which virus-specific antibodies accelerate the entry and/or replication of viruses into monocytes and granulocytes via interaction with FcR of IgG or complement receptors. It is facilitated either by uptake of antibody-virus complexes or by increasing viral nucleic acid and protein synthesis. This mechanism might also interfere with the cellular signalling pathways [94] or enhance stronger anti-inflammatory responses disproportionally [95]. Similarly, antibody dependent enhancement of infection has been observed in coronaviruses. Anti-sera against SARS-CoV spike enhances virus entry into FcR expressing cell [96]. This study reported that human pro-monocyte cell line (HL-CZ) which also expressed ACE2 was successfully infected by SARS-CoV in presence of monoclonal antibody targeted against the virus spike protein. Furthermore diluted anti-sera enhanced infection and induced apoptosis. It is important to note that virus-induced cytopathic effects and increased level of pro-inflammatory cytokines was also detected by quantitative RT-PCR. Additional studies indicate that immunization of cats with FCV spike worsens future infection due to ADE [97]. Another study experimentally demonstrated that neutralizing monoclonal antibody targeted against the RBD in spike protein of MERS and SARS coronaviruses catalyzes pseudovirus entry into FcR expressing host cells [98]. During ADE, the antibody likely binds RBD and leads to conformational changes, exposing S2' to protease cleavage. However, in case of SARS and MERS coronaviruses, receptors for ADE remain the same as for the normal viral entry, following ACE2 and DPP4-dependent viral entry. This in-

Table 4: List of 10 frontline vaccine candidates.

Vaccine Candidate	Developer/ Manufacturer	Vaccine type and mode of action	Outcome (if known any)	Clinical trial phase	References
ChAdOx1/ (COVID-19 vaccine AstraZeneca)	University of Oxford/ AstraZeneca	Non-replicating chimpanzee adenovirus vector with SARS-CoV-2 spike proteins on its surface.	• Immunogenicity response. • Authorized for use in the UK, India, Argentina, Mexico, Morocco, Dominican Republic and El Salvador.	**Phase 3**	[111]
Covaxin	Bharat Biotech	Inactivated whole-virion vaccine	• SARS-CoV-2 specific antibody production. • Reduced viral load in nasal cavity, lungs.	**Phase 3**	[112]
Zycov-D	Zydus Cadila	Plasmid DNA vaccine targeted against viral membrane protein	• Elevated neutralizing antibody in animal studies. • Safe and well-tolerated in Phase 1.	**Phase 2**	[113]
BNT162 (Comirnaty)	Pfizer/BioNTech/ Fosun Pharma	4 LNP-encapsulated mRNAs.	• Strong immune response. • Regulatory approval in the UK, Canada, Mexico, US, Bahrain, Chile, Singapore, Oman, Saudi Arabia, Kuwait.	**Phase 3**	[114], [115]
mRNA-1273	Moderna/NIAID	LNP-encapsulated RNA vaccine	• Immunogenic. • Authorized for use in US, Canada and Israel from January 2021.	**Phase 3**	[116]
CoronaVac	Sinovac	Formalin-inactivated and alum-adjuvant vaccine	• Immunogenic. • Emergency use for high-risk patients in China.	**Phase 3**	[112]
NVX-CoV2373	Novavax	Prefusion protein nanoparticle adjuvant with matrix M.	• Immunogenic.	**Phase 3**	[117]
Sputnik V	Gamaleya Research Institute	Non-replicating Adeno-viral vector	• Elevated humoral and cellular immune response. • Lack of clinical data. Received regulatory approval in Russia.	**Phase 3**	[118]
EpiVac Corona	Federal Budgetary Research Institution State Research Center of Virology and Biotechnology	Peptide vaccine	• No clinical data available but received regulatory approval in Russia.	**Phase 1/2**	[112]
BCG	University of Melbourne and Murdoch Children's Research Institute; Radbound University Medical Center; Faust man Lab at Massachusetts General Hospital	Live-attenuated vaccine	• Strong humoral as well as cellular immune response.	**Phase 2/3**	[119] [120]

dicates that neutralizing monoclonal antibody mediates ADE of coronavirus entry by acting as functional viral receptor mimic.

In line with humoral immune response, ADE becomes a major area of concern in vaccine design and antiviral drug therapy. At present there is no literature available on ADE of SARS-CoV-2. It can be perceived that SARS-CoV-2 might exhibit antibody dependent enhancement of viral entry and pathogenesis similar to its predecessors. Therefore, in this review we suggest to have research done in this area. If ADE is found in SARS-CoV-2, then strategy for antibody-dependent drug delivery needs to be modified accordingly. The current scenario where widespread vaccination is underway will also tell us about ADE in SARS-CoV-2 infections.

Current Therapeutic Approaches and Challenges

Repurposing drugs

With each passing day, the pandemic's toll on life and economy worldwide increases. Scientists across the globe are working tirelessly to find a cure, a solution to this pandemic. During this time of emergency, it is difficult to develop novel antiviral compound as drug testing takes time, thus prolonging start of a treatment regime. Therefore, researchers and public health agencies are screening the efficacy of existing drug combinations and therapies, those that have already been proved to be safe for other diseases. In this review a few most commonly used prophylactics and curatives have briefed in (Table 2) and (Table 3) respectively.

Race towards vaccine

The WHO has recognized about 48 candidates in the race towards development of an effective vaccine which are in the clinical evaluation and authorized globally. This article will aim to summarize some of the front runners in the following (Table 4).

Since January 16, two COVID vaccine candidates Covishield and Covaxin were rolled out in India among the other frontline vaccines. Having vaccinated ~30 million people already, the country is now administering to people over 60 years of age [121] and those younger with comorbidities. Although both the vaccines have now accelerated the wheel, controversies over authorization of Covaxin, an inactivated whole virion vaccine continues. Also, the vaccines' effectiveness in high risk population have not been reported separately. Besides India, several other countries across the globe including Australia, South Africa, UK, Bangladesh, and Brazil authorized roll-out of Covishield (also known as ChAdOx1) since January 2021. However, several countries suspended the use of the vaccine after reports of blood clotting and thrombotic events began to circulate in March [122], which once again brings a pitfall in the fight against the virus and the pandemic. Other noteworthy vaccine candidates which are now in circulation are Comirnaty, mRNA-1273, SputnikV, COVID-19 vaccine Janssen and Coronavac. After its circulation, Comirnaty was claimed to be effective against the new variant B.1.1.7 but later it was proved wrong [123]. Even when we are chasing after the most promising treatment to fight against SARS-CoV-2, it is extremely important to keep in mind that unbiased use of so many therapeutics at the same time might complicate the treatment. When we are still at an early stage of vaccination, an upsurge of fresh infections in many countries including India, UK, Brazil, USA makes the fight against the pandemic even more cumbersome [124]. It might be possible that the pathogen has already developed a resistance against these therapeutics, gaining an advantage of high transmissibility and rapid disease progression. Therefore, more and more accurate clinical trials are needed before declaring the drug or vaccine, a promising one. Hence our fight against the disease seems to be a very tough one because as soon as we gain a miniscule edge against it, the virus seems to pose another challenge.

Plasma therapy

Since the MERS outbreak, plasma therapy has been the leading proposed treatment. Convalescent plasma (CP) therapy is a classical adaptive immunotherapy used to treat and prevent extremely infectious diseases like SARS, MERS, H1N1 swine flu [9]. In absence of a promising and approved specific antiviral drug against SARS-CoV-2, focus had shifted to alternate strategy to fight COVID-19, especially in severe patients. A study performed on a small group of patients demonstrated hopeful results. CP from recovered patients who had developed humoral immunity against SARS-CoV-2, with a large quantity of neutralizing antibodies raised against SARS-CoV-2 was administered to patients with severe disease. This therapy exhibited a promising change in the treatment procedure with little or no viral load in serum after CP transfusion, an increase in oxygen saturation and improvement of liver function and CRP level. The two key factors in plasma therapy were highlighted to be neutralizing antibody titre and treatment time point [88]. Another trial was conducted in China on a group of 5 critically ill COVID-19 patients with acute respiratory distress syndrome (ARDS). Although, viral load decreased after the standard antiviral treatment, it became undetectable only after plasma transfusion into recipients. Consistent with previous reports, there was a marked increase in neutralizing antibody titre, which highlights the possible clearance of the virus and clinical improvement [125]. Other countries like India and the UK is also continuing clinical trials with convalescent plasma therapy [126]. Central Drugs Standard Control Organization (CDSCO) of India also permitted

Indian Council of Medical Research (ICMR) to conduct a series of trials of plasma therapy in certain states to fight against SARS-CoV-2 [127]. However, there are limitations to this therapy as well, which includes the small number of patients being treated and possible synergistic effect of other drugs with CP.

Discussion

Even after a year the entire world including more than 200 countries is still witnessing the rage of the pandemic COVID-19, a disease that has brought our life and livelihood to a halt or "new normal life" scientists are rigorously working to develop an effective antiviral therapeutic to defeat it. The fight has been multifaceted since the very beginning, whether fighting against economic recession or fight to adjust to the "new normal" life or fight to defeat the pandemic by designing effective therapeutics and vaccines. Some repurposed drugs have been approved but each drug has its own limitation. Therefore, science cannot rush; it can only make rational use of the potential drugs or vaccines. The world is in dire need of an effective vaccine. Although, the wait seems to be over since already a few vaccines have been authorized for use in several countries. However, it should be kept in mind that science meets with innumerable barriers, primarily due to lack of our understanding about the pathogen in depth, the complexities in human system, probable ADE of SARS-CoV-2 entry, cluster of mutated strains of the virus across the globe and lack of knowledge on virus life cycle, pathogenesis, high transmission rate and above all, time. Furthermore, unavailability of peer-reviewed data and clinical evidences on the already authorized vaccine candidates makes us repeatedly question on their efficiency and safety profile. Our only ray of hope lies in the fact this is not the first instance of a pathogen affecting humans and we have survived as a race either by developing therapeutics or by evolving our immunity to co-exist. It is not seldom that respiratory viruses have encountered humanity, and we shall overcome COVID-19 too taking all the lessons this pandemic has taught us and will be prepared for such pandemic outbreaks in coming days.

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