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SPECIAL ISSUE: COVID-19: A HOLISTIC INSIGHT

Coronavirus Disease (Covid-19) Proteins and Potential Drugs: What We Know So Far

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ABSTRACT

Wuhan, Hubei province, China became an epicentre of an outbreak pandemic SARS-CoV-2 in December 2019. As per World Health Organization (WHO) China expressed its first case of SARS-CoV2 sequence on 12 January 2020 and the first case of SARS-CoV-2 have been reported outside China was on the 13 January 2020 at Thailand. Form the day one to till today there are no outcome of effective drugs against SARS-CoV-2 pandemic. As of 8:40 PM IST, 11 May 2020, the global count of confirmed COVID-19 cases has reached 41,32,365, with as many as 2,83,387 victims succumbing to the same. The SARS-CoV-2 virus shows a high sequence similarity, of about 70-80%, to the SARS-CoV viruses. Additionally, either of them shows an identical clinical symptom such as respiratory illness, fever, dry cough, and with complications like chest pain or pressure, loss of speech and movement. The current review focuses on potential drugs against the SARS-CoV-2 virus, with an emphasis on the major drug interactions, functions and features of the Human Corona 229E strain.

Keywords: Chloroquine, Human Coronavirus, Human Corona229E, Potential drugs, Remdesivir, SARS-CoV-2

INTRODUCTION

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The year 2020 started with a novel pandemic caused by a highly infectious virus the SARS-CoV-2. This disease eventually gained the name Coronavirus disease of 2019 or Covid-19. Persistent findings revealed that the so observed disease was modified version of the 2003 Severe Acute

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Respiratory Syndrome (SARS) and 2019 Middle East Respiratory Syndrome (MERS) pandemics, all caused by viruses of the genus betacoronavirus [1]. In comparison, the Covid-19 was found to be more infectious while the preceding pandemics were more deadly**.** The invisible virus like SARS-CoV-2 has been affected on the human population as well as countries and world economy, climate and global environment. On 30 January 2020, the Covid-19 outbreak was declared a public health emergency of international concern (PHEIC) by Dr. Tedros Ghebreyesus, the Director-General of WHO.

SARS-CoV-2

December 2019, China health authorities went through several pneumonia patients linked to sea food whole sale market in Wuhan, China. Patients were suffering from primary symptoms of pneumonia like disease of unknown cause [2]. The fact is SARS-CoV-2 is highly pandemic virus which spread by droplets, direct contacts from the infected objects whose incubation period lies around 14 days and in some cases it has affected the people asymptomatic ways. An only 5%-10% of infected people's shows complete severe respiratory syndrome known as coronavirus-19. SARS-CoV-2 belongs to betacoronaviruses which shares around 70-80% of its genome with SARS-CoV but it also shares its high sequence similarities with a horse-shoe bat coronavirus along with genome it also shows clinical similarities such as ACE-2 target receptor and virus transmission pathways [3]. Scientists have subcategorised the viruses alpha, beta, gamma, and delta. Total seven of these viruses can cause infection to the people and major four viz., 229E(alpha) NL63(alpha), OC43(beta), HKU1(beta) and three less common are:

- MERS-CoV, a beta virus that causes Middle East respiratory syndrome (MERS)
- SARS-CoV, a beta virus that causes severe acute respiratory syndrome (SARS)
- SARS-CoV-2 also known as COVID-19 [4]

In this review, it's been endeavouring to explain about structural, functional properties and potential drug-target interaction of Human coronavirus 229E strain and its proteins.

Human coronavirus 229E

National Center for Biotechnology Information database reports that there are five major proteins that are taking place under Human coronavirus 229E strain are:

1. HCoV229Egp1
2. N proteins (nucleocapsid protein)
3. S-protein
4. ORF4a and ORF 4b
5. M (Membrane protein)

Data shows that Human CoV shows two serotypes which are represented by HCoV-229E and HCoV-OC43 [6].

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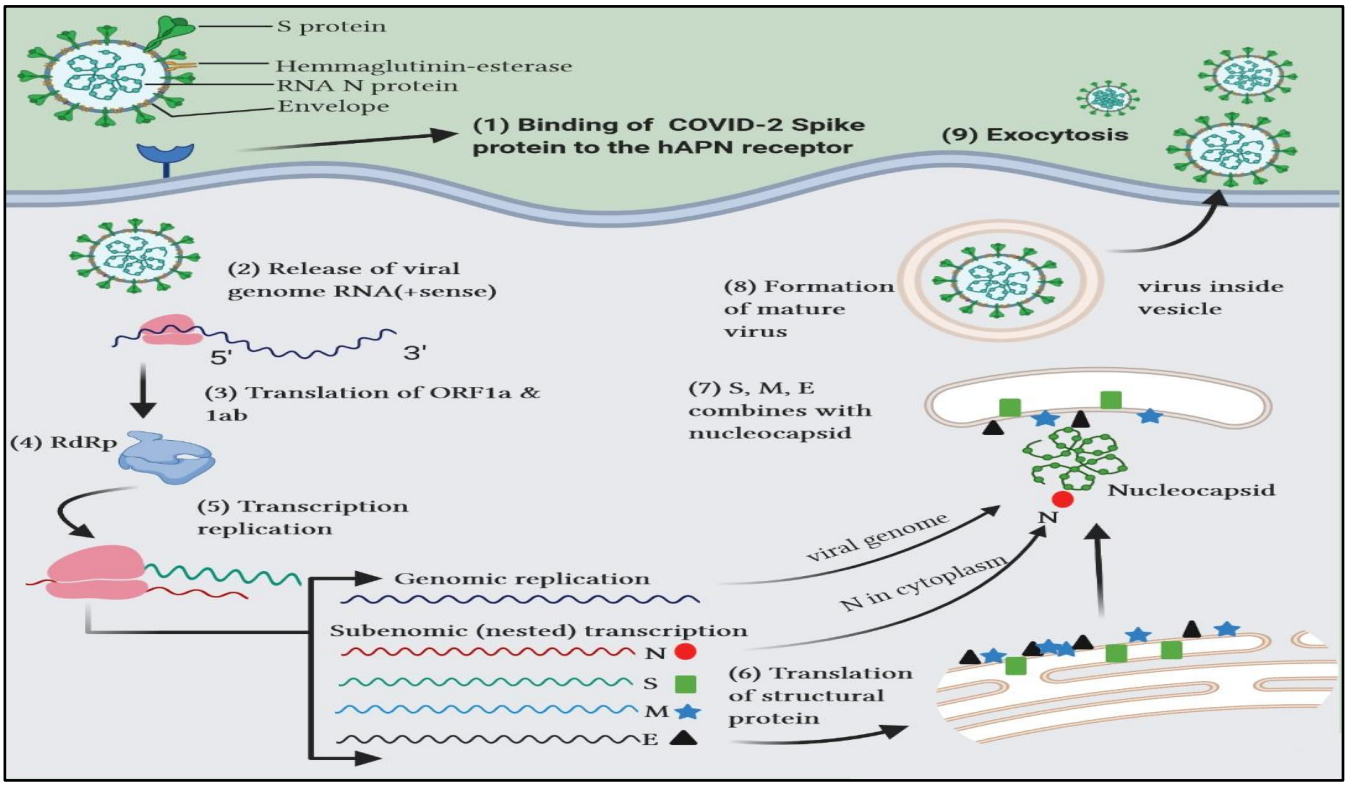

Figure 1*:* Transcription and replication process

When the viral genome enters to the surface of the human cell membrane the viral spike protein makes its interaction with hAPN receptor of the host and bounds to it once after it bounds the receptor a positive sense(5'-3') of viral genome will get released, translation of ORF1a&1b will occurs and virus expresses the RdRp (RNA dependent RNA polymerase) which helps as transcription replication of viral genome this process leads to a formation of genome replication as well as formation of N, S, M, E proteins. The virion progeny will form via the secretory pathway include rough ER, Golgi apparatus, exocytosis [5] (Figure 1).

HCoV229egp1 (replicase polyprotein 1a; replicase polyprotein 1ab)

It's a Replicase polyprotein 1a: replicase polyprotein 1b also known as ORF1ab polyprotein and pp1ab it's about the length of 7073 residues. It contributes itself in the process of 7-methylguanosine mRNA capping, persuade by virus of catabolism of host mRNA, modulation by virus of host protein ubiquitination, mRNA methylation, positive stranded viral RNA replication, protein K48-linked deubiquitination, the viral RNA genome replication,viral transcription and it's composed of host cell endoplasmic reticulum-Golgi intermediate compartment, host cell membrane, host cell perinuclear region of cytoplasm, an integral component of membrane. This enzyme also implies transcription and replication of viral RNAs. Enzyme HCoV229Egp1 (replicase polyprotein 1a; replicase polyprotein 1ab) contains proteinase responsible for cleavage of the polyprotein and several NSPs [7]. This protein has a

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huge multienzyme complex of several cellular proteins and 16nsp (Nonstructural protein) each nsp has its own function and contribution for virus sustainability such as shown in Table 1.

Table 1: Nonstructural protein and functions [8]

Sl. No.	Non Structural Protein	Function Description
i.	NSP1	Inhibits host translation, perform endonucleolytic cleavage near 5′ UTR also by supressing the host gene expression nsp1 facilitates efficient viral gene expression in infected cells and evasion from host immune response
ii.	NSP2	May play a role in the modulation of host cell survival signalling pathway by interacting with host PHB and PHB2. Indeed, these two proteins play a role in maintaining the functional integrity of the mitochondria and protecting cells from various stresses.
iii.	Non-structural protein 4	Participates in the assembly of virally induced cytoplasmic double-membrane vesicles necessary for viral replication.
iv.	Non-structural protein 6	Plays a role in the initial induction of autophagosomes from host reticulum endoplasmic. Later, limits the expansion of these phagosomes that are no longer able to deliver viral components to lysosomes.
v.	Non-structural protein 7	Forms a hexadecamer with nsp8 (8 subunits of each) that may participate in viral replication by acting as a primase. Alternatively, may synthesize substantially longer products than oligonucleotide primers. Alternatively, may synthesize substantially longer products than oligonucleotide primers
vi.	Non-structural protein 9	May participate in viral replication by acting as an ssRNA-binding protein.
vii.	Non-structural protein 10	Plays a pivotal role in viral transcription by stimulating both nsp14 3'-5' exoribonuclease and nsp16 2'-O-methyltransferase activities. Therefore, plays an essential role in viral mRNAs cap methylation.

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Papain-like proteinase

Papain-like proteinase enzyme cleaves the replicase polyprotein located at the N-terminus. Besides that, PL-PRO takeover the deubiquitinating/deISGylating activity and processes both 'Lys-48'- and 'Lys-63'-linked polyubiquitin chains from cellular substrates. Enzyme engage with both nsp4 in the assembly of virally induced cytoplasmic double-membrane vesicles necessary for viral replication and it provoke innate immune induction of type 1 interferon by blocking the phosphorylation [8].

Proteinase 3CL-PRO

Enzyme resulting in cleavageof the C-terminus of replicase polyprotein at 11 sites. Recognizes substrates containing the core sequence [ILMVF]-Q-|-[SGACN]. Also, able to bind an ADP-ribose-1''-phosphate (ADRP) [7]. RNA-directed RNA polymerase: RdRp will help in the process of replication and transcription of the viral RNA genome (8) Compound Helicase: It has a multipurpose activity of protein with a zinc-binding domain presence at N-terminus displaying RNA and DNA duplex-unwinding activities with 5' to 3' polarity. Helicase activity seems to be dependent on magnesium [7]. The Guanine-N7 methyltransferase enzyme will possess two different activities, 1) Exoribonuclease activity acting on both ssRNA and dsRNA in a 3' to 5' direction. 2) N7-guanine methyltransferase activity [7]. Uridylate-specific endoribonuclease: Mn (2+) dependent, uridylate-specific enzyme will leaves 2'-3'-cyclic phosphates 5' to the cleaved bond [8]. 2'-O-methyltransferase: Methyltransferase that mediates mRNA cap 2'-O-ribose methylation to the 5'-cap structure of viral mRNAs. N7-methyl guanosine cap is a prerequisite for binding of nsp16. Therefore, plays an essential role in viral mRNAs cap methylation which is essential to evade the immune system [8].

N Protein (Nucleocapsid Protein)

N protein is also known as the nucleocapsid protein. It's a major structural protein component of CoV-19 it helps to form the virion core by binding the viral RNA genome and leads to the formation of a ribonucleoprotein (RNP) complex/ long helical nucleocapsid structure. A process of forming an RNP is important for supervising the RNA in a conformational state for transcriptionand replication of the viral genome. N protein will bind to the full length of genomic RNA (gRNA) also with six sgRNAs but it has been shown to haveahigh affinity with gRNA. Here the gRNA acts as a template for the viral RdRp (RNA dependent RNA polymerase) also as an alarm for translation. At the period of infection gRNA primarily transcribed by an early polymerase activity to turn into a genome sized negative strand RNA after the early polymerase activity a late polymerase activity will transcribe the negative stranded RNA into a full-length gRNA which bounds to polysomes and shows its presence in a nucleocapsid structure [9] among all of that N protein can be a an important diagnostic marker and immunodominant antigen in host immune responses.

The nucleocapsid protects the genome and ensures its timely replication and reliable transmission. Nucleocapsid has both N-RNA interaction as well as an intermolecular association between disulfide-linked N protein multimers. N protein express 26–30% of homology sequence with coronavirus N proteins of other strains such as SARS and HCoV-OC43. Full stretch of coronavirus N proteins has an

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ability to form high-order oligomers, and the C-terminal domains of the coronavirus N protein are responsible for oligomerization activity [10].

S Protein (Spike Protein)

Spike protein takes responsible for receptor binding and fusion of the viral to the host cell membrane. Virus SARS-CoV is driven into the host cell by the interaction of S(Spike) protein with the host cellular metallopeptidase angiotensin-converting enzyme 2 (ACE2) [11] but the Human corona 229E strain shown to use the human amino peptidase N (hAPN) as receptor enzyme. Its receptor-binding domain (RBD) proved to exhibits an extensive sequence variation. The ectodomain of this protein consist of N - terminal S1 region which harbors the RBD (Receptor binding domain) and C-terminal S2 region of the protein mediates membrane fusion. S protein acts as a trimmer in both pre-fusion and post-fusion confirmation. RBD up conformation may promote conversion to the post-fusion form the post-fusion form of the SARS-CoV S-protein is characterized by a 6-helix bundle formed by the inner HR1 triple-helical coiled-coil around which the HR2 helices are packed in the case of HR1 triple-helical coiled, the coil is formed entirely on the conversion of the post-fusion form and HR2 helices may form a triple-helical coiled-coil in the pre-fusion confirmation, it must dissociate before taking part in 6-helix bundle formation [12].

4ab protein

All group 1b coronavirus exhibits to code a single accessory protein between the S protein and E protein, except human coronavirus 229E strain a template or first model virus has a split gene which codes for ORF4a and ORF4b proteins (Figure 2). A numbering to the Open Reading Frames in Human coronavirus 299E is based on the northern blot analysis of sgRNA. With the company of an additional sg mRNA in Human corona 229E-infected cell will lead to shifting the numbering from ORF3 to ORF4a/b. The location of ORF4a/b genes in the genome and sequence shows a high similarity with the 1b ORF3 genes since they exhibit homologous. The two ORFs of HCoV299E 4a and 4b presence in between the S and E protein of the virus. The both ORF4a and ORF4bshares a common sg mRNA for the expression of ORF4b also it would require an alternative mechanism of translation and those can be internal entry, leaky scanning or translation re-initiation off ribosomes. The aligned 5 clinical isolates sequence reveals that it encodes a single, uninterrupted ORF between S and E gene and that single accessory gene is about 660 nucleotide length which encodes an ORF4 protein that length of 219 amino acids with high similarity of the N-terminal and C-terminal domains with ORF4a (93%) and ORF4b (96%) respectively [13].

M (Membrane Protein)

M proteins are key constituents of the viral envelope involved in virus morphogenesis and assemble through interaction with other viral proteins. It has a length of 225 polypeptides of amino acid sequence which a primary translation product, extrapolated from the DNA sequence that codes for formation of membrane protein in Human corona 229E strain. Also, it has been experimentally proven that the polypeptide has 3 potential N-glycosylation sites [14]. M proteins are transported as an

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integral membrane protein to the budding compartment and then later in infection, it backs in the endoplasmic reticulum [15].

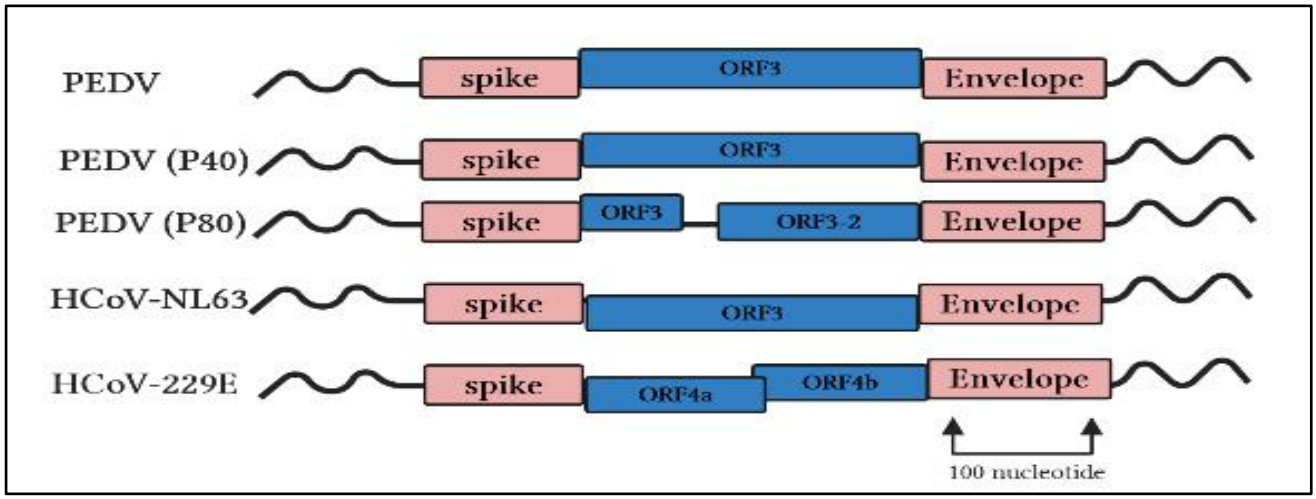

Figure 2: Representation of ORF4a/b split gene in Human corona229E.All the 1b coronaviruses codes a single accessory gene in-between of spike – envelope protein but in HCoV229E case gene have been in split form as ORF4a and ORF4b respectively. Group of 1b coronavirus includes a PEDV (porcine epidemic diarrhea virus), HCoV-NL63, HCoV-229E. PEDV and HCoV-NL63 encode ORF3 protein and HCoV-229E encodes ORF4a & ORF4b proteins [13].

Diagnostic Approaches for SARS-CoV-2

PCR testing

Currently as per the WHO, patients who have been tested positive for the novel coronavirus, PCR test is carried out by taking two different samples from them (like nasopharyngeal and stool). The PCR method must include suitable negative and positive controls in each run, and the results must be as, one negative control for the extraction process and one water control for the PCR run, one positive control for extraction and PCR run, the patient sample will be spiked with a weak positive control to detect PCR inhibitors [16]. If the patient's sample achieves the above parameter disease will confirmed by repeating the PCR using the original sample or same sample must tested for the second time from different laboratory but thecondition is reference laboratory must be identified at national level. For the high-test specificity amplification of second genome region could be done.[16]

SARS Antibody Test for Serosurveillance

Enzyme-linked immunosorbent assay is peptide-based assay which identifies the undetected chain of disease transmission. Also, this method was able to identify the asymptomatic cases of SARS-CoV and similar viruses which has been isolated from the wild mammals. SARS coronavirus immunoassay comprises of the Western blot with an antigen whole virus or recombinant proteins, ELISA, and Immunofluorescence assay (IFA) to determine the neutralizing antibodies [17].

Potential Drugs against Sars-Cov-2

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WHO reported that the death rate of the novel coronavirus was about 3.4% as of March 3, 2020.[18] In India, it has reached to 67,152 confirmed cases and 2,206 deaths. ICMR of India and other research sectors also other country's health organizations, research sectors are trying to come up with the best potential drugs against the COVID-19. But meantime since the number of pandemic cases keeps increasing in each country the pressure of conducting clinical trials of drugs are also increasing to reach a final drug or solution. Currently, Drugbank database shows 16 potential drugs and 30 targets, Table 2.

Table 2: Potential drugs and targets [8]

Sl. No.	Drugs	Targets
i.	Azithromycin	23S ribosomal RNA. Protein-arginine deiminase type-4.
ii.	Bevacizumab	Vascular endothelial growth factor A
iii.	Chloroquine	Tumor necrosis factor. Glutathione S-transferase A2. Toll-like receptor 9. Glutathione S-transferase. High mobility group protein B1. Glutathione S-transferase Mu 1. Angiotensin-converting enzyme 2.
iv.	Darunavir	Human immunodeficiency virus type 1 protease
v.	Elbasvir	Nonstructural protein 5A.
vi.	Favipiravir	RNA-directed RNA polymerase catalytic subunit.
vii.	Fingolimod	Sphingosine 1-phosphate receptor 5. Histone deacetylase 1. Sphingosine 1-phosphate receptor 1. Sphingosine 1-phosphate receptor 4. Sphingosine 1-phosphate receptor 3.
viii.	Galidesivir	RNA-directed RNA polymerase L.
ix.	Human interferon beta	Interferon alpha/beta receptor 1.
x.	Leronlimab	C-C chemokine receptor type 5.
xi.	Lopinavir	Human immunodeficiency virus type 1

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		protease.
xii.	Methyl prednisolone	Glucocorticoid receptor. Annexin A1.
xiii.	Remdesivir	Replicase polyprotein 1ab. RNA-directed RNA polymerase L. Human immunodeficiency virus type 1 protease.
xiv.	Ritonavir	Nuclear receptor subfamily 1 group I member 2.
xv.	TMC-310911	HIV-1 protease.
xvi.	Tocilizumab	Interleukin-6 receptor subunit alpha.

FDA's emergency authorization of Remdesivir after NIH (National Institute of Health) claims to show a promising resultalso it authoriseschloroquine and hydroxychloroquineas anemergency drug, while the authorization permits for the unapproved usage of drug in concern of public health emergency. Chloroquine was developed to treat against Malaria until a new effective antimalaria drug arrives. Chloroquine and hydroxychloroquine have been repositioned for several diseases like rheumatoid arthritis, HIV, systemic lupus erythematosus, and prophylaxis of Zika virus [19,20].

As of 13 May 2020, under the NIH (National Institute of Health) guideline, they are evaluating major drugs for treatment. Remdesivir and Chloroquine/Hydroxychloroquine [21].

Remdesivir (GS-5734) Target drug to the Replicase polyprotein 1ab

Currently, there no such high potential and proper approved drug to treat the SARS-CoV-2 but the clinical development of Remdesivir (also called as GS-5734) have been used in animal studies against MERS and SARS diseases also it has shown limited efficacy. As the antiviral drug, Remdesivir has been proved to reduce the severity of the disease when tested on rhesus macaque model of MERS coronavirus infection, virus replication and damage to the lung data shows that it could be implemented to clinical trials and it may also have potential effects on novel Covid-19 [22]. This drug is presently under clinical development for Ebola virus disease and it is able to inhibit both MERS and SARS coronaviruses replication in *in vitro* also reported its effectiveness against bat coronavirus, pre-pandemic bat coronavirus and circulating existing human coronavirus in primary human lung cells. In a mouse model of SARS-CoVs, GS-5734 exhibits its activity against the virus in reducing lung viral load and enhanced clinical signs of diseases also respiratory function [23].

The nsp14 exoribonuclease (ExoN) of an outbreaking CoVs has involved of an antiviral nucleoside due to its proofreading activities but in the recent days, some of the scientists have stated that nucleoside analogous GS-5734 (Remdesivir) strongly inhibits human and zoonotic coronaviruses in lab

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experiments and in SARS-CoVs mouse model. It describes that nucleoside analogous usually targets the replication of virus, chiefly the viral RNA/DNA polymerase and it's been proved successfully while treating multiple viral infections.

Mode of action

Nucleoside analogous has multiple mechanisms and among those purified RSV (respiratory syncytial virus) polymerase causes the cessation of early nascent RNA transcripts. Data shows that GS-5734 actson primary infection and it reduces RNA levels in a dose-dependent [24]. When the invasion of a virus occurs on the epithelial cell of the host it releases a viral genome and uses the host ribosome to replicates polyproteins. Main 2 polyproteins like papain-like protease (PLpro) and the coronavirus main proteinase 3CLpro cleaves the polyproteins into smaller to use as replicating new viruses. As a means of synthesizing the daughter RNA genome, the virus expresses an RNA dependent RNA polymerase (RdRp) which is responsible for the synthesis of a complementary RNA strand by using virus RNA as a template. RdRp is able to allow the binding of Remdesivir drug molecule to it which exact mirror action of RNA building blocks ATP, into new RNA strands. Once Remdesivir binds to RdRp it stops the following replication process and integrating to RNA subunits [25,26,27] (Figure 3).

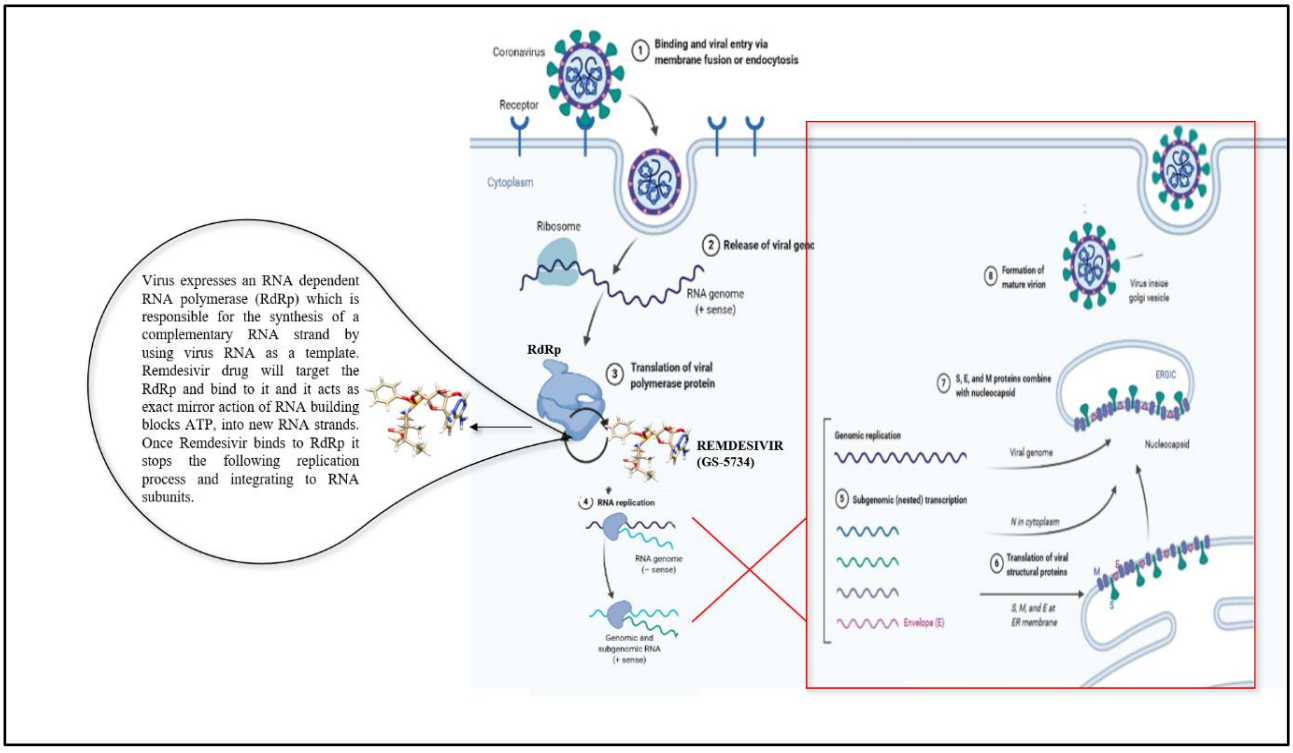

Figure 3: Remdesivir binding to RdRp and terminating the process of transcription replication of SARS-CoV-2

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Chloroquine

There are no such huge data and reports about chloroquine and SARS-CoV-2 virus interactions, but Chloroquine has been used to treat Malaria disease [28]. It eliminates or blocks the viral by increasing the endosomal pH which is necessary for cell fusion also mediates with glycosylation of SARS-CoV-2 cell receptors [29]. So, Savarino et al. (2006) hypothesized that chloroquine may inhibit replication of this virus and also proved in two in-vitro studies [30]. Also, at the effective concentration of Remdesivir (EC50=0.77 μM) and chloroquine (EC50=1.13 μM) were able to block the infection. It performs as immune-modulating activity collaboratively enhances the antiviral effect too in vitro [29]. Apart from Remdesivir chloroquine were proven its efficacy at the effective concentration (EC50) of approximately 8 μM, in vitro [31].

Mode of action

Chloroquine can affect the virus in several mechanisms such as pre and post-infection antiviral agent. Chloroquine is a 9-aminoquinoline compound having a state of weak base; it increases the pH of vesicles. If the drug is treated to an extracellularly the non-protonated part of the chloroquine invades the cell and it turns cell into a protonated and acidic [32].

CONCLUSIONS

The mortality rate of the SARS-CoV-2 might be not high risk but the factor which made all of us be isolated is its rate and speed of getting spread and pandemic. By seeing these reports, we can conclude that yet there are no such effective and potential approved drugs for the SARS-CoV-2 treatment. But the best way to stay far from the Covid-19/ SARS-CoV-2 is to maintain the physical distance and following the WHO's (World Health Organization) safety guidelines [33] and we hope that more and more clinical trials would increase to find out the potential drugs as early as possible and get the world back to its normalcy.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

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