Study of Genome, Vaccine and Probable Anti Viral Drugs to Save the World From the Capture of SARS-CoV-2: A Mini-Review

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Abstract- The coronavirus disease 2019 (COVID-19) is a single-stranded RNA (+) virus and causes infectious disease by the viral strain "severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2). Now, COVID-19 has become pandemic, and there are neither potential vaccines nor drugs discovered. Its RNA contains genes for structural (S, E, M, N) and non-structural proteins (PLpro, 3CLpro, RdRp, Hel). Interaction between the S protein of SARS-CoV-2 and the ACE 2 receptor of the host cell plays a vital role in the entry of the virus into the cell. Favipiravir, ribavirin, remdesivir, galidesivir, lopinavir, ritonavir, chloroquine, and hydroxychloroquine are the few effective drugs against SARS-CoV-2. Live attenuated virus (mutant MERS-CoV and SARS-CoV or recombination with another live attenuated virus) can act as vaccine platforms against SARS CoV-2 along with DNA vaccine and subunit vaccine.

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Introduction

The history of the corona virus says that it was first detected in 1960 as a cause of the common cold. As reported, more than 500 patients were detected with symptoms like flue in Canada in 2001; out of them, 3.6% were found positive for HCoV-NL63 strain (1). Surprisingly, the coronavirus was reported as a simple, nonfatal until 2002; however, in 2002-2003, an outbreak of this virus in Guangdong province in China resulted in spread to many other countries like Taiwan, Hong Kong, Singapore, Thailand, Vietnam, and the United States of America. This virus also caused severe acute respiratory syndrome (SARS), leading to a mortality rate of 1000 patients (1). This high mortality drew the attention of many microbiologists and infectious disease experts to understand the pathogenesis of this disease and found a new form of causative virus known as Coronavirus. In 2004, World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC) declared a state of emergency due to 774 deaths out of 8096 infections (2). Similarly, in 2012, the outbreak of the corona virus in Arabia resulted in many deaths and spread to many other countries, such as first in the Middle East followed by worldwide. Some previous reports suggested that the corona virus is unstable, becomes more virulent, and finally lethal to humans (1).

In 2019, the first outbreak of coronavirus was detected in Wuhan, Hubei province, China, which became spread rapidly across China and other countries in the world. So far, the coronavirus has affected more than 375498 patients in 195 countries, as reported by World Health Organisation on 24th March 2020 (https://www.who.int/westernpacific/emergencies/covid-19) (Figure 1). The World Health Organisation, on 11th Feb 2020, announced a new name for this epidemic disease as coronavirus disease (COVID-19) (3). Finally, due to this outbreak of COVID-19, on 11th March 2020, the WHO director-general reported this COVID-19 as a pandemic(https://www.who.int/westernpacific/emergencies/covid-19). Various studies reported about the COVID-19 transmission from human-t-human, hence

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high risk of the wide spread of this disease (3). Figure1 shows the rapid spread of the corona virus to different countries, as reported by WHO.

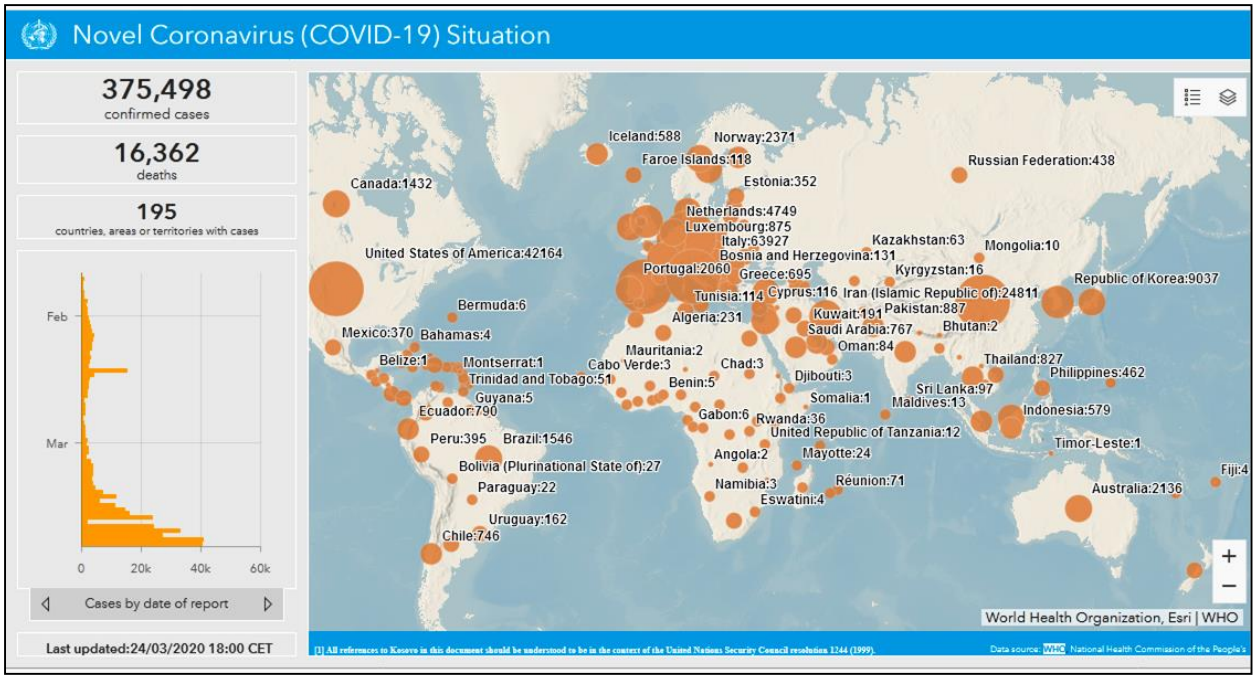

Figure 1. The confirmed cases of infection and death as reported by the World Health Organisation on 24th, March 2020 (https://experience.arcgis.com/experience/685d0ace521648f8a5beeeee1b9125cd)

The most common symptoms are related to the respiratory system, such as dry cough, shortness of breathing, sputum production, and sore throat. The death is mainly occurred due to pneumonia and multi-organ failure. The modes of transmission of COVID-19 are droplets and direct contact. Animal to human and human to human transmissions are previously reported (4). Wang Wet *et al*., reported that the mortality rate is more in males and individuals of more than 70 years (5).

In this present study, the genetic makeup of SARS CoV-2 (causal agent of COVID-19) is well described. The mode of transmission and replication of coronavirus in the host cell is focused. This study also focused on the synthetic vaccine for SARS CoV-2. A number of potential antidrugs against SARS CoV-2 are described here.

The genome of SARS-CoV-2 (COVID 19 virus)

It is a very small spherical virus (65-125nm in diameter) with spike protein (S), membrane glycoprotein (M), and envelope glycoprotein (E) are present in its lipid bilayer (6,7). S proteins of the surface look like crown. So, this virus is named as coronavirus. Kannan S *et al*., reported that COVID 19 virus encodes an extra glycoprotein that has acetyl esterase and hemagglutination properties. These properties make the COVID 19 virus different from other coronaviruses (6).

SARS-CoV-2 contains one positive single-stranded RNA (26 to 32 kbs) as its genetic material. This RNA is embedded with nucleocapsid proteins (N) (Figure 2).

This N protein plays an important role during infection by countering the host immune response. The reason is that N protein inhibits the viral suppressor protein of RNAi (VSR) (6).

RNA of a typical SARS-CoV contains both 5' methylated cap and 3' polyadenylated tail. The genes are arranged from 5' end to 3' end in the following orders: replicase genes, S gene, E gene, M gene, and N gene. All the non-structural genes (replicase genes) are present towards 5' end within two open reading frames (ORF 1a and ORF 1b). ORF 1a contains papain-like cysteine protease (PLpro) and 3C like serine protease (3CLpro), which are treated as proteolytic enzymes. ORF 1b contains RNA-dependent RNA polymerase (RdRp) and helicase (Hel), which are helpful for replication of viral genome (Figure 3) (8,9).

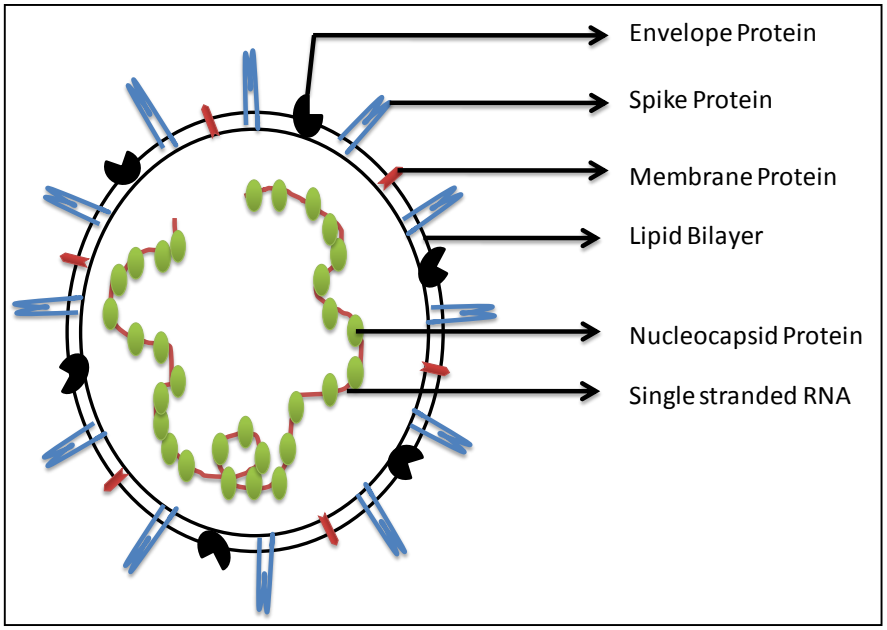

Figure 2. Schematic diagram of COVID-19 virus

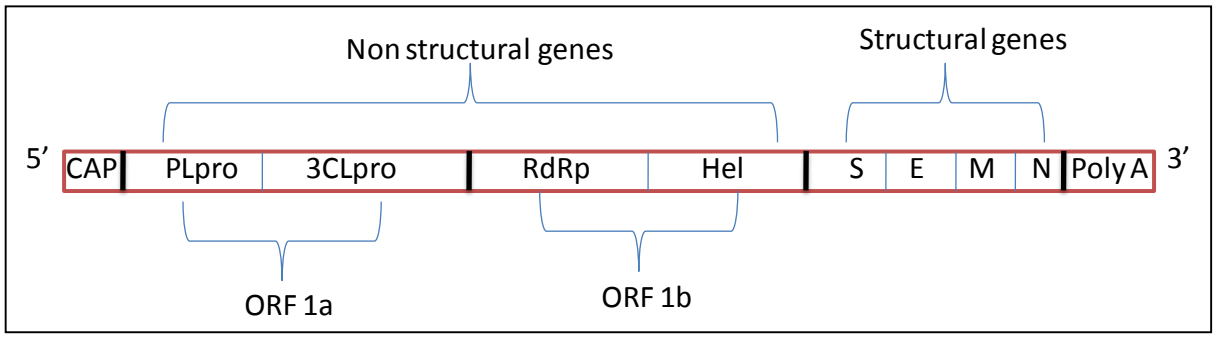

Figure 3. The genome of the COVID-19 virus

Ren LL *et al*., isolated a bat-borne human CoV from a patient in Jin Yin-tan Hospital of Wuhan, China (10). The virus was subjected to next-generation sequencing (Illumina, San Diego, CA, USA) followed by phylogenetic analysis with MEGA software. Finally, 5'-ORF 1a-ORF 1b-S-E-M-N-3' sequence was similar with SARS-like (SL)-CoV. Clustal W program using MEGA revealed that the isolated virus was 87.6% to 87.7% similar with those of bat SL-CoV ZC45 and ZXC21 (GenBank MG772933, MG772934).

ORF 1b gene and N gene are important for the identification of SARS-CoV in patients. Researchers try to amplify these marker genes with very specific primers to confirm the COVID-19 positive. It is tested the positiveness of SARS-CoV-2 in an oropharyngeal swab specimen of a 32-year Nepalese student at the Wuhan University of Technology in Wuhan, China (10). The primers and probe used to amplify ORF 1b were 5'-TGGGGYTTTACRGGTAACCT-3' (F), 5'-AACRCGCTTAACAAAGCACTC-3' (R), and 5'-TAGTTGTGATGCWATCATGACTAG-3' (probe). Similarly, 5'-TAATCAGACAAGGAACTGATTA-3' (F), 5'-CGAAGGTGTGACTTCCATG-3' (R), and 5'-GCAAATTGTGCAATTTGCGG-3' (probe) were used amplify N gene through real-time reverse transcriptase-polymerase chain reaction (rt-RT-PCR) (11).

Mechanism of infection of SARS CoV-2

The reservoir of SARS CoV-2

Shereen MA, 2020 reported that the primary reservoirs of SARS CoV are raccoon dogs and palm civets (7). In 2001 it was found that the human body (persons of Hongkong) contained antibodies against SARS CoV. This suggested that SARS coronavirus may be circulating in human (11). Sequence analysis of SARS CoV in palm civets and humans indicated that it

is highly homologous with each other (99.6%). Rhinolophus bats (horseshoe bats) were found in the reservoir of SARS CoV in 2005 (12). Shereen MA also reported that only α and β coronavirus infected humans from the animal through consumption and contact with infected persons (Figure 4) (7).

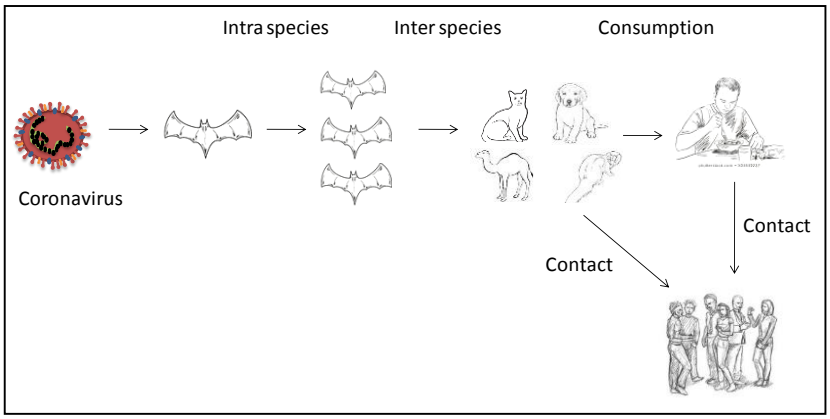

Figure 4. Transmission of coronavirus from the reservoir to a human

Mode of transmission among humans

SARS CoV-2 is a highly contagious virus that can spread among human beings easily. It can transmit from patient to healthy person through droplets that are coming out through cough or sneeze. It can be transferred to an unaffected person through contact with contaminated surfaces or objects. It enters the body mainly throughthe nose, eyes, and mouth. Doremalen NV (2020) reported the life span of SARS CoV-2 on different surfaces given in Table 1 (13).

Table 1. Longevity of SARS CoV-2 on different surfaces

Surface	Life Time of SARS CoV-2
In Aerosols	3 hrs
Copper	4 hrs
Stainless steel	48 hrs
Plastic	72 hrs

Prevention method

The transmission of SARS CoV-2 can be prevented by the following methods (14):

- Prevent contact with SARS CoV-2 patients or suspected persons
- Use mask during the outgoing
- Regularly wash hands with soap
- Use sanitizer
- Proper cough etiquette should be followed
- Avoid contact with animals
- Avoid touching to metallic bars or supports
- Maintain social distance

Entry of SARS CoV-2 in the host cell

S protein is the essential glycoprotein which is most important to interact with host because it acts as ligand for host cell surface receptor angiotensin-converting enzyme 2 (ACE 2) as shown in the Figure 5. (7,9). 394 glutamine residue of receptor binding domain (RBD) of S protein recognizes and binds with lysine 31 residue of ACE 2 receptor of host cell. Then through endosomal pathway, SARS CoV-2 enters into the cytosol of host. Jumla A *et al.*, (9) reported that in addition to endosomal pathway, there is a direct fusion occurs between the envelope of SARS CoV-2 and the plasma membrane of the host cell (non-endosomal pathway) (9). Uncoating releases RNA from the virus to cytosol. RNA translates to produce non-structural proteins like replicase (PLpro, 3CLpro, RdRp, and Hel) and structural viral proteins like S proteins, E proteins, M proteins, and N proteins. These viral proteins and genome RNA assemble in the endoplasmic reticulum (ER) and Golgi bodies to form a number of virions. Finally, these newly formed virions are transported via vesicles and released out from the cell by exocytosis (Figure 5).

Vaccine for COVID-19 virus

There is no approved vaccine or treatment for this emerging infection; therefore, every researcher tries to design a vaccine against 2019-nCoV using various approaches. Abdelmageed MI *et al.*, had designed a multi-epitope peptide vaccine against 2019-nCoV using an immunoinformatics approach (T cell epitopes-based peptide vaccine) (15). This study also concluded that 10 MHC1 and MHC2 related peptides were promising

candidates for vaccine design against 2019-nCoV. Ahmed SF *et al*., identified a set of B cell and T cell epitopes derived from the spike (S) and nucleocapsid (N) proteins that map identically to SARS-CoV-2 proteins. This study provided a screened set of T cell epitopes that can help guide experimental efforts towards the development of vaccines against SARS-CoV-2 (16).

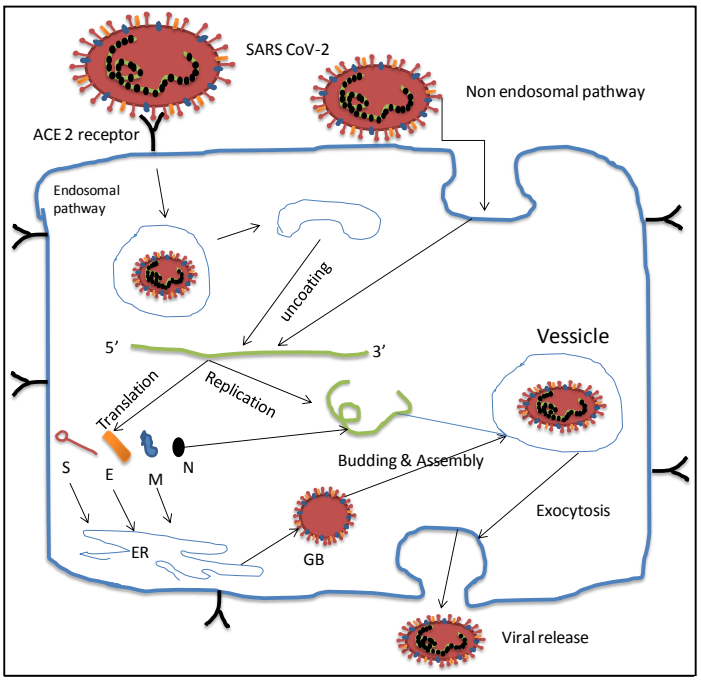

Figure 5. Replication of SARS CoV-2 in the host cell

Dhama K *et al*., suggested that S protein could be a key viral antigen for the designing of the vaccine against SARS CoV-2 in the future (17). It is reported that DNA (full length of spikes or S1), viral vector (full length of spikes or S1), subunit (Full-length Spike, S1, RDB, nucleocapsid), virus-like particles (RDB, S or Co-expressing of S1, M, and E), inactivated virus (whole virus inactivated by formaldehyde or gamma irradiation), live attenuated virus (mutant MERS-CoV and SARS-CoV or recombination with another live attenuated virus) can act as vaccine platforms against SARS CoV-2 (18). Robson B *et al*., identified a well-conserved oligopeptide "KRSFIEDLLFNKV" using molecular modeling and docking, which can be a synthetic vaccine for SARS CoV-2 (19).

Anti-COVID-19 virus drugs

Although currently there is no availability of approved treatment options for COVID-19, there are several drugs being investigated. The similarity of SARS-CoV-2 with SARS-CoV and MERS-CoV makes drug repurposing a primary option for the treatment of COVID-19. There are several approved drugs available to effectively inhibit SARS-CoV and MERS-CoV, which are being investigated to treat COVID-19. In addition, toSARS-CoV and MERS-CoV inhibitors, HIV inhibitors and anti-malarial drugs are being investigated to treat COVID-19. Here are some examples explored.

Favipiravir and ribavirin

Favipiravir, an approved influenza drug in Japan (20), and Ribavirin, an approved HCV (hepatitis C virus) and RSV (Respiratory syncytial virus) drug (21) are RNA-dependent RNA polymerase (RdRp) inhibitors which are under randomized clinical trials. Favipiravir, a guanine analog, effectively improved 7 days clinical recovery rate in the clinical trial (ChiCTR200030254) (22). Fapilavir is the first approved clinical drug to treat COVID-19 in China, which includes favipiravir as the most active ingredient. The drug is yet to be approved by FDA (U.S. Food and Drug Administration), USA. Ribavirin, a guanine derivative, is also being investigated in randomized clinical trials combined with other drugs (ChiCTR2000029387) (23).

Remdesivir and galidesivir

Remdesivir (GS-5734) is a nucleotide prodrug that has shown effectiveness in inhibiting both SARS-CoV

and MERS-CoV (24). Remdesivir also effectively inhibits SARS CoV-2 with EC50 =0.77 μM, CC50 > 100 μM, and SI > 129.87 (Wang *et al*., 2020c). The efficacy and the safety of the drug made it enter the clinical trial phase 3 (NCT04292730) and could be an effective solution to the treatment of COVID-19. Galidesivir (BCX4430) an adenosineanalogue is being investigated to treat yellow fever (NCT03891420) has inhibitory effect against several pathogens including coronavirus (25).

Lopinavir and ritonavir

Lopinavir and ritonavir are protease inhibitors originally approved to inhibit HIV. The two drugs inhibit 3CLpro protease that process the polypeptide translation product of RNA genome in to functional component of virus. The drugs have been used combinedly in clinical trial to treat COVID-19. The outcome of clinical trial shows it could not effectively inhibit SARS CoV-2 in adult patients with severe disease symptoms (26) although it has the potential to treat less severe patients.

Chloroquine

An anti-malarial drug chloroquine has shown to be effectively inhibiting SARS CoV-2 growth in vitro (EC50 = 1.13 μM; CC50 > 100 μM, SI > 88.50) (23). There are several clinical trials registered to propose chloroquine as treatment option for COVID-19. The clinical trial result shows that chloroquine is effective in safety and treatment of COVID-19 in China (27). Chloroquine sequesters protons in lysosomes to increase the intracellular pH (endosomal acidification inhibitor) and becomes one of the effective drugs against SARS CoV-2 (9).

Apart from chloroquine a derivative of it, hydroxychloroquine shows promising effect in inhibiting SARS-CoV-2 in vitro (28). Hydroxychloroquine is a safer option than chloroquine as it is less toxic than the later and also has been registered for clinical trials to treat COVID-19.

In addition to above drugs, there are several anti-viral molecules that are being studied to treat COVID-19. Griffithsin, an antiviral lectin from red algae which is being studied to inhibit HIV glycoprotein (29), could be a potential option for the treatment of COVID-19. Interferon-alpha2b, which is being studied to treat MERS (21), is also under randomized clinical trial to treat COVID-19 (NCT04251871). Moreover, monoclonal antibodies derived from patients recovered from Ebola Virus Disease (EVD) have proven to be effective in inhibitingthe Ebola virus in other patients (30). Similarly, monoclonal antibodies could be derived from COVID-19 recovered patients and studied to treat the disease. Although above mentioned therapeutic molecule (small molecule or antibody) has the potential to inhibit SARS CoV-2, combinational use of these molecules could enhance their efficacy. Besides these known anti-viral drugs, there are studies using virtual screening to reveal novel probable anti-viral molecules (31-33). These novel small molecules have shown potential anti-viral properties in silico, which could be used in the future as COVID-19 therapeutic options.

It is confirmed that the main reservoirs of SARS CoV-2 are rhinolophus bat, snake, raccoon dogs, palm civets, camels, etc. It becomes pandemic, and the death rate becomes more in various countries like Italy, Spain, China, etc. Its single RNA contains many genes for its replications as well as viral proteins. Its RNA contains 5'cap and 3' poly-A tail-like eukaryotic mRNA. During the entry into the host cell, spike protein interacts with the ACE 2 receptor. Few probable antiviral drugs are prescribed to suppress its spreading, and these are favipiravir, ribavirin, remdesivir, galidesivir, lopinavir, and ritonavir. An anti-malarial drug, chloroquine, is also used to treat COVID-19 along with other drugs. Still, it needs huge research for the innovation of the best vaccines and anti-viral drugs for 100% curing from COVID-19.

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