Vaccines and Treatment Strategies for SARS-COV-2

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Abstract:

The 2019-novel coronavirus disease (COVID-19) outbreak caused by SARS-CoV-2 is now a pandemic as announced by WHO. It has crossed more than 34,41,767 (on 03.05.2020) infected patients around the world and death count also has gone above 2,43,922. Very recently, some improvements in therapeutic approaches have been found for treatment of this disease. This work aims to survey earlier approaches for vaccines, antiviral medicines, strategies and probable antigens experimented on SARS-COV, MERS and other coronaviruses, which may have potential application for SARS-CoV-2.

Keywords: Vaccine, anti-viral treatment, antigen, SARS-COV-2, SARS-COV, MERS, COVID-19

I. Introduction:

A novel pandemic on SARS-CoV-2 (2019-novel coronavirus) developing COVID-19 disease, a atypical pneumonia, was informed first in Wuhan, Hubei Province, China on mid-December, 2019 and already has invaded more than 212 countries around the world (3 May, 2020). WHO defines in January, 2020, new SARS-CoV-2 coronavirus outbreak as a Public Health Emergency of International Concern (PHEIC).

Coronaviruses are a family of enveloped and positive-strand RNA viruses, that are single-stranded and classified in *Nidovirales* order. The name "coronavirus," coined in 1968, is given for "corona" or "crown"-like morphology observed in these viruses in the electron microscope. There are pathogens in this coronavirus family which affect different animal species, like Rat coronaviruses (RCVs), Murine Hepatitis Virus (MHV) in mice, Porcine Respiratory Coronavirus (PRCv) in pigs, infectious bronchitis virus [IBV] in chickens, and Feline infectious peritonitis (FIP) in cats, as well as for humans, including severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002 (beta coronavirus), HCoV-229E (alpha coronavirus), NL63 (alpha coronavirus), HKU1 (beta coronavirus), OC43 (beta coronavirus), MERS (Middle East respiratory syndrome) in 2012 (beta coronavirus) and recently pandemic causing pathogen SARS-COV-2 in 2019.

Coronaviruses are analyzed for more than 70 years, since its first isolation from prototype murine coronavirus strain JHM in 1949. Porcine transmissible gastroenteritis virus (TGEV), porcine hemagglutinating encephalomyelitis virus, bovine coronavirus (BCoV), equine coronavirus and avian infectious bronchitis viruses (IBV) are of veterinary importance, while mouse hepatitis virus (MHV) is studied in the models for human diseases. Porcine epidemic diarrhea virus (PEDV), feline infectious

peritonitis virus (FIPV), Rat sialodacryoadenitis coronavirus and other avian coronaviruses, such as IBV, turkey coronavirus, and pheasant coronavirus, also belong to this family.

Some coronaviruses namely HCoV-229E, HKU1, OC43 and NL63 from this *Coronaviridae* family, are causing human respiratory infections and have caused outbreaks earlier. The CoVs that are first exposed are IBV that causes respiratory disease in chickens and human CoV-229E (HCoV-229E) and human CoV-OC43 (HCoV-OC43), which cause the common colds in humans. Since the emergence of HCoV-229E and HCoV-OC43, many other HCoVs are discovered, namely Severe Acute Respiratory Syndrome-CoV (SARS-CoV) in 2002, HCoV-NL63 in 2004, HCoV-HKU1 in 2005, Middle East Respiratory Syndrome-CoV (MERS-CoV) in 2012.

HCoV-229E can infect both human and bats, which binds to APN (aminopeptydase - N) receptor to enter its host cell [1]. It transmits via droplet-respiration and exposed inanimate objects (fomites). It is associated with asymptomatic or mild disease except one case. HCoV-229E likely emerges from a bat alphacoronavirus nearly two hundreds years ago [2] by molecular clock analysis.

With 4 genotypes, HCoV-OC43 is a member of Betacoronavirus I species, that infects humans as well as cattle [3]. Genotype B occurs in 1980s, while molecular clock analysis show its ancestor from 1950s. With HCoV-229E, it is a known virus causing 10–15% of common cold cases worldwide. HCoV-OC43 and bovine coronavirus (BCoV), both [2] betacoronaviruses, are very highly similar in sequences leading back to a nearby common origin. Molecular clock analysis of S protein of HCoV-OC43 BCoV shows that HCoV-OC43 is originated from BCoV ancestors around 1890.

The unexpected emergence of human SARS-COV [2] strain with severe pathogenicity, comes from zoonotic reservoirs in 2002. This novel human zoonotic coronavirus, Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) is first identified to be an atypical pneumonia in the isolated patients in Guangdong Province, China in 2002. This causes about 8000 infections for SARS-CoV and about 800 deaths around the world by July 2003. Although there is still absence of any effective therapeutics, only aggressive intervention approaches for human health contains this SARS-Cov epidemic. Lethality of SARS-CoV is sever largely for elderly (nearly 50%). Later, subsequent emergences of SARS-CoV are found in small carnivores like palm civets and raccoon dogs from Chinese wet markets and also causes SARS-like CoV in horseshoe bats (genus *Rhinolophus*) in 2013, 2018, predicted to be the reservoir host.

HCoV-NL63, called New Haven coronavirus, occurs in 2004 in a baby with bronchiolitis and conjunctivitis in Netherlands [4]. It causes from mild untill moderate Upper-Respiratory Tract infections, severe Lower Respiratory Tract infection, croup as well as bronchiolitis. It affects young children, elderly and immunocompromised patients with acute respiratory illness after originating from infected palm civets and also bats. It has a seasonal association with occurrence most frequently in the winter months in temperate climates, between 35 and 50 north and south latitudes. It spreads through direct person-to-person transmission in congested areas and likely through droplet expulsion from respiratory tract. Intravenous immunoglobulin is an FDA approved HCoV-NL63 inhibitor.

HCoV-HKU1 is an upper respiratory disease showing symptoms of common cold, which advances to pneumonia and bronchiolitis. It is originated from infected mice in 2005 in Hong Kong and

in same year in Australia and France [1]. It contains Hemagglutinin esterase (HE) gene and thus becomes a a member of the genus Betacoronavirus. HKU1 phylogenetically is found to be closely related to mouse hepatitis virus (MHV).

From its emergence on 2012 in Saudi Arabia, until December, 2019, WHO confirms 2,499 MERS-CoV cases and 861 deaths (or about 1 in 3) in twenty one countries. There is a Google map on MERS Coronavirus Cases and Sequences, showing details case history of each patient. Middle East respiratory syndrome-related coronavirus (MERS-CoV) or EMC/2012 (HCoV-EMC/2012)[5] can infect humans, bats as well as camels [5]. It is a Betacoronavirus with two phylogenetic clades. WHO defines it to be a likely cause of a future epidemic. Strong tropism of MERS-CoV exists on nonciliated bronchial epithelial cells, which finally evade innate-immune responses and antagonize interferon (IFN) production in those cells.

Recently the pandemic declared by WHO, named COVID-19, starts from Wuhan city, Hubei Province, China in mid-December, 2019. 34,41,767 cases with this *Coronaviridae* family SARS-CoV-2 infections is already confirmed up to date (03 May, 2020), and 2,43,922 people have already died in 212 countries around the world for it. SARS-COV-2 can infect humans, tigers and bats as far known till now. Recently, Tang et al describe that COVID-19 has evolved in 2 lineages— 'L' and 'S' types. The older 'S-type' is milder and less infectious, while 'L-type' is emerging later, spreading more quickly and aggressively [6].

SARS-COV-2 can spread by human-to-human transmission through small respiratory droplets and direct contact, with a common incubation period of 5 days (range, 1 to 14). The period between symptom onset and pneumonia development is 4 days. Fecal-to-oral transmission also have a role in SARS-COV-2 transmission. Infection with SARS-CoV-2, the virus that causes COVID-19, can cause a mild to severe respiratory illness and include symptoms of fever, cough and shortness of breath. Most patients have lymphopenia and bilateral ground-glass opacity changes on chest CT scans. The neuroinvasive propensity has been demonstrated as a common feature of CoVs. Some potential antiviral treatments are showing improvements very recently. Development of SARS-CoV-2-based vaccines is urgently required, while nearly 70 labs are trying to build its vaccine rapidly.

II. Human coronaviruses, their receptors and protein etiology

Table 1 Human coronaviruses groups, diseases and receptors

Group	Virus	Host	Disease(s) caused	Cellular receptor
I Alpha-coronavirus	229E	Human	Respiratory infection	Human APN
	NL-63	Human	Respiratory infection, croup	ACE2
II Beta-coronavirus	OC43	Human	Respiratory infection and possibly enteric infection	Neu5,9Ac2-containing moiety
	HKU1	Human	Respiratory infection	N-acetyl-9-O-acetylneuraminic acid
	MERS-CoV	Human	Middle East respiratory syndrome	DPP4
	SARS-CoV	Human	Severe acute respiratory syndrome	ACE2
	SARS-CoV-2	Human	COVID-19	ACE2

Two prototype human coronaviruses, OC43 and 229E in Table 1, are both etiologic agents of common cold. SARS-CoV causes a severe acute respiratory syndrome. HKU1 is a group II coronavirus which is isolated from an elderly patient with pneumonia. HCoV-NL63 is a group I coronavirus, which causes serious respiratory symptoms, croup and Kawasaki's disease in children.

The cellular receptor for Human coronavirus 229E, HCoV-229E, is a membrane protein human Aminopeptidase N (APN). It is the serves as receptor for HCoV-229E spike glycoprotein. The Spike protein type I glycoprotein that forms the peplomers on the virion surface. Hemagglutinin esterase (HE) is not encoded in the SARS-CoV genome. APN interacts with the S1 domain of human coronavirus 229E/HCoV-229E spike protein (Uniprot - P15144). Its region 288 – 295 is necessary and sufficient to mediate interaction with HCoV-229E. [7,8] APN is a Zinc ion binding protein, which a role in the final digestion of peptides generated from hydrolysis of proteins by gastric and pancreatic proteases.

Ezetimibe is a lipid-lowering compound and Cardiovascular agent, which targets Aminopeptidase N. Ubenimex is another natural product and immunemodulator, which targets APN and works as immunologic adjuvants, factors and Anti-HIV, anti-retroviral and anti-viral agents. [drugcentral card-2787]. Human calcitonin (DB06773) is constituted as a 32-amino acid single chain polypeptide structure that gets secreted as a regulatory agent in calcium-phosphorus metabolism. It targets APN. Icatibant (DB06196) (Firazyr) is a synthetic peptidomimetic drug. It acts as an effective antagonist of bradykinin B2 receptors with 10 amino acids. FDA approves Icatibant on August 25, 2011, which is an immunologic factor and inflammation mediator.

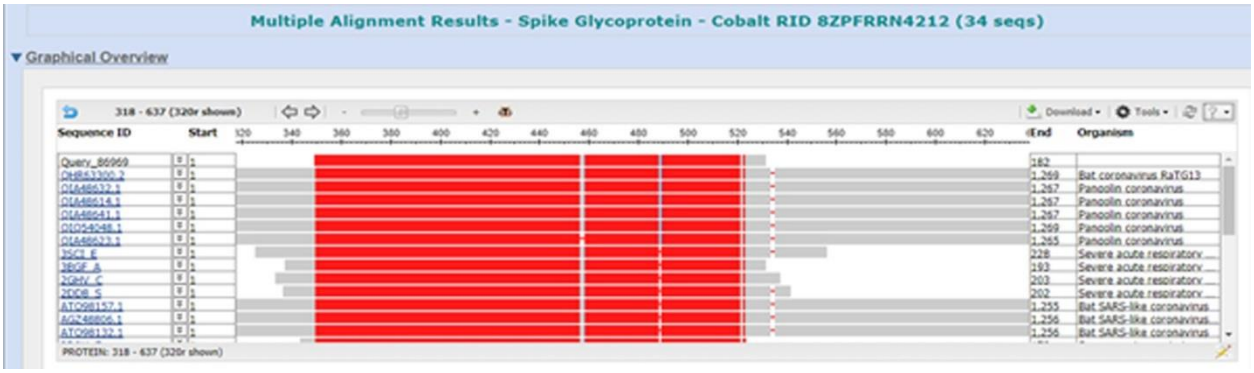

Figure 1 a) Multiple sequence alignments of SARS-COV-2 Spike Glycoprotein range 331-524 to betacoronaviruses [11].

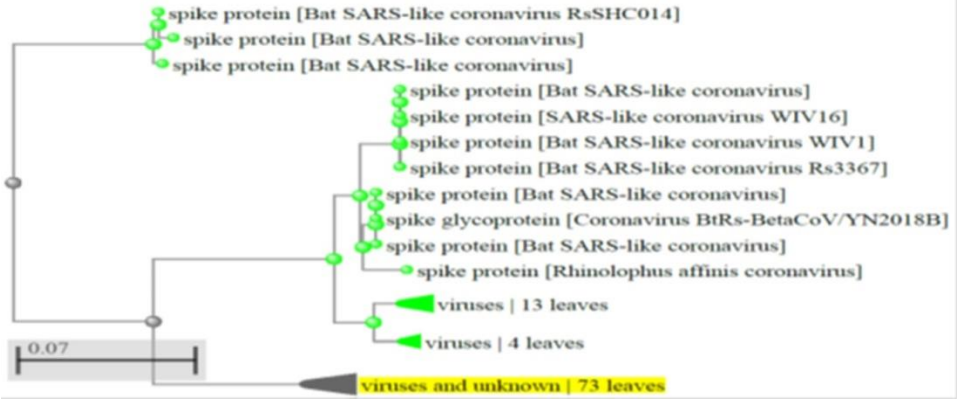

Figure 1 b) Blast tree view of SARS-COV-2 RBD with other Beta coronaviruses from NCBI [12].

HCoV-OC43 enters into host cell by binding to N-acetyl-9-O-acetylneuraminic acid (predominant sialic acid in human cells) receptor. HCoV-NL63 also enters its host cell by the ACE2 receptor (AGTR1 and AGTR2), like SARS-COV-2 [1]. Like OC-43, HCoV-HKU1 also enters host cell by binding to N-acetyl-9-O-acetylneuraminic acid recepter. MERS-CoV enters its host cell by binding to Dipeptydil peptidase-4 (DPP4) receptor. DPP4 encodes adenosine deaminase complexing protein 2 or CD26.

Carboxypeptidase, namely Angiotensin-converting enzyme 2 (ACE2)(UniProt- Q9BYF1) is a common cellular receptor for HCoV-NL63, SARS-CoV and SARS-CoV-2. It is important in amino acid transport to be partner in binding of amino acid transporter SL6A19 in intestine, which regulates trafficking, expression on the cell surface, and its catalytic activity (PubMed:18424768, PubMed:19185582). An N-linked (GlcNAc...) asparagine Glycosylation exists in position 90. This N-glycosylation on Asn-90 can be effective in SARS infectivity.

SARS-CoV and NL-63 also has same host receptor, Angiotensin Converting Enzyme 2 (ACE2), for binding and host cell entry. The viral attachment protein, surface Spike glycoprotein is an envelope-anchored trimeric protein, which determines host specificity and hence becomes a therapeutic target. For coronaviruses, there is a RNA proof-reading activity associated with the 30–50 exonuclease activity encoded within nsp14 [2]. Whether RNA proofreading fidelity of SARS-CoV alters with environmental changes or during stressed virus replication is still unknown, but for ecologic conditional changes, this may allow for rapid HCoV evolution [2]. SARS-CoV host cell entry also mainly depends on transmembrane protease/serine subfamily member 2 (TMPRSS2) S and ACE2 cleavage, specifically in airway and alveolar sites. Subsequently it leads to cathepsin L cleavage and thereafter to S2 fusion activation. The RBD (aa318–510) domain in S protein mostly determines host range for SARS-CoV.

Reverse genetics system is hard for corona viruses [9]. Substitution of 2 amino acids in RBD domain of human spike S protein with those of civet spike (N479K/T487S) almost abolishes the ability to infect (using the single-round infection assay) human cells expressing the SARS-CoV receptor [9]. SARS-CoV spike protein S is significant pathogenesis by inducing interleukin-8 (IL-8) in the lungs via activations of MAPK and AP-1. This activity is mapped to 324-688 amino acids of SARS-CoV spike S. Therefore, for similar binding method to host cells, Spike glycoprotein is also similarly significant for analysis for SARS-COV-2 pandemic in 2020.

II.1. Phylogenetic analysis and sequence alignments of Spike Protein of SARS-COV-2

Similar to the earlier results found by Dong et. Al [10], the results of Multiple sequence alignment of the region of SARS-CoV-2 RBD (receptor binding domain) at residues 331 to 524 of S protein (GenBank: QHR63250.2), has shown 100% similarity with SARS-CoV-2 Receptor binding domain of surface glycoprotein (GenBank: 6M17_E) in Supplimentary File S1. Next highest 88.46% Percent identity comes from spike glycoprotein [Bat coronavirus RaTG13] (Genbank: QHR63300.2). Second highest zoonotic similarity is found with spike protein [Pangolin coronavirus] (Genbank: QIA48632.1) with 85.71% identity. Chain E, Spike glycoprotein [Severe acute respiratory syndrome-related coronavirus] (Genbank: 3SCI_E) shows only 72.53% identity with this chosen S1 domain part of SARS-COV-2. Obtained BLAST Tree view from NCBI is shown in Figure 1a). The alignment is shown Figure 1b)

and detailed alignment results are shown in Supplementary materials with this article. This shows similar pattern of evolution of SARS-COV-2 from earlier SARS-CoV and intermediate other bat coronaviruses in 2014 and 2016, as bats are found to be the natural reservoir of SARS-CoV.

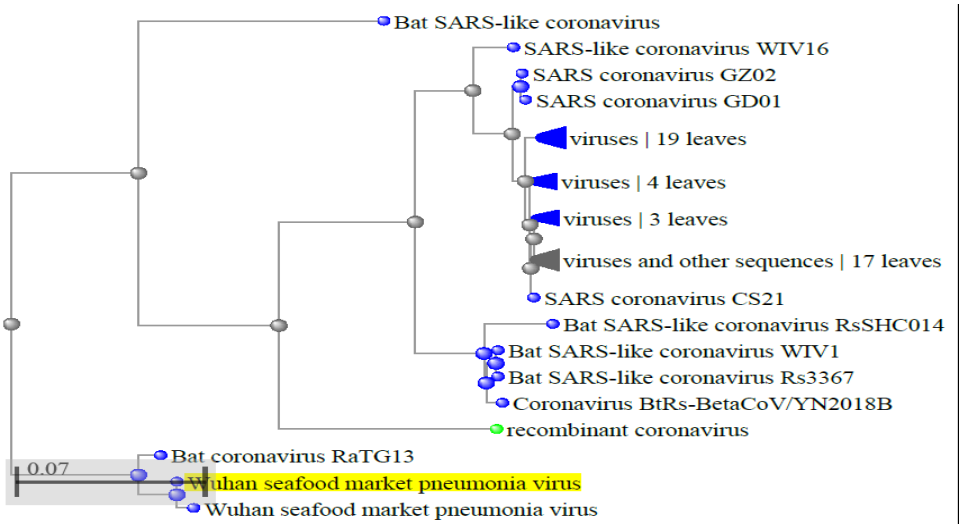

Figure 2 Blast Tree view of GenBank: QIA98583.1 Indian surface glycoprotein SARS-COV-2 [12].

Similar analysis is also being performed with surface glycoprotein [Severe acute respiratory syndrome coronavirus 2] (GenBank: QIA98583.1), submitted on 11-FEB-2020 at National Institute of Virology, India in Figure 2 (https://go.usa.gov/xv9YX). Deatiled results are shown in Supplimentary files S2 and S3. To study similarity and phylogenies of the surface glycoprotein, Spike protein (GenBank: QJC19491.1) (submitted: 2020-04-05) is compared with surface glycoprotein of SARS-COV-2 (GenBank: QIK50427.1) from USA (submitted: 2020-03-11), surface glycoprotein of SARS-COV-2 (GenBank: YP_009724390.1) from China (collection date: Dec-2019), spike glycoprotein [Bat coronavirus RaTG13] (GenBank:QHR63300.2) from China (collection date: 24-Jul-2013, isolation_source="fecal swab", host="Rhinolophus affinis") and spike glycoprotein [SARS coronavirus GZ02] (GenBank: AAS00003.1)(submitted: 15-SEP-2003) sequences and other spike sequences from HCoVs and Bat coronaviruses. The alignment is performed on chosen 9 similar sequences with high percent identity as shown in Supplimentary File S4.

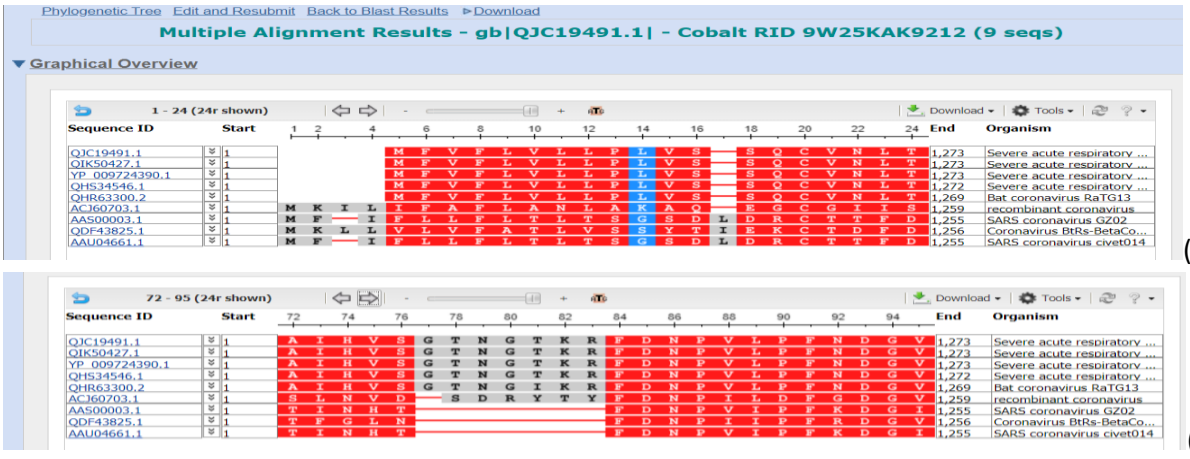

Figure 3 a)-g) Multiple Sequence Alignment differences of Indian SARS-COV-2 spike protein QJC19491.1 in comparison with WUHAN, USA, Bat RaTG13 and SARS-CoV spike proteins [11].

The multiple sequence alignment result shows that there is no difference in Spike protein among three chosen SARS-COV-2 sequences, which differs in geographic regions as well as in timeline of occurrence. However, there is an utmost similarity of QJC19491.1 with S protein of Bat coronavirus RaTG13 and no similarity among highest 100 sequences from Pangolin coronavirus, which may hint for intraspecies and interspecies evolution of SARS-COV-2, after its initial jump from any its zoonotic reservoir, pangolin/bat.

There are two mismatches of QJC19491.1 Indian Surface Glycoprotein SARS-COV-2 with QHR63300.2 spike glycoprotein [Bat coronavirus RaTG13] lies at position 688-691 PRRA residues and at 331 with D residue, as shown in Figure 3. However, all three S proteins of SARS-COV-2 differ from S proteins from Bat RaTG13, SARS-COV GZ02 and rest highest sequences in positions 1-4, 14, 17, 77-83, 150-152, 160-165, 253-258, 331, 470-486, 688-691 respectively. Among them 470-486 and 688-691 positions are significant for SARS-COV-2 host cell entry and specificity for host range.

Figure 4 Mutations found in multiple sequence alignments for three Spike glycoprotein sequences found in India. a) Multiple alignment view in NCBI alignment server [11]. b) MLA results from JALVIEW. c) Neighbor-Joining Tree on three Spike proteins.

Multiple sequence Alignment has been done on Surface glycoprotein [Severe acute respiratory syndrome coronavirus 2] (GenBank: QIA98583.1) submitted on 11-FEB-2020 from Kerala State, (GenBank: QJC19491.1) submitted on 2020-04-05 from Gujarat and (GenBank: QHS34546.1) submitted on 01-FEB-2020 from Kerala State, to find out how mutations are occurring in sequences within one country. Details results are shown in Supplimentray File S5 and S6. The MLA view from NCBI is shown in

Figure 4a). It shows 5 positions in Spike proteins which are mutated. There are differences at 5 positions – 144, 271, 408, 614 and 930 as shown in Figure 4b). Neighbor-Joining Tree from PID (Percent Identity) in Figure 4c) shows how mutations is changing the Spike sequences, where Spike protein sequences submitted on February are more nearby than the sequence submitted in April, 2020.

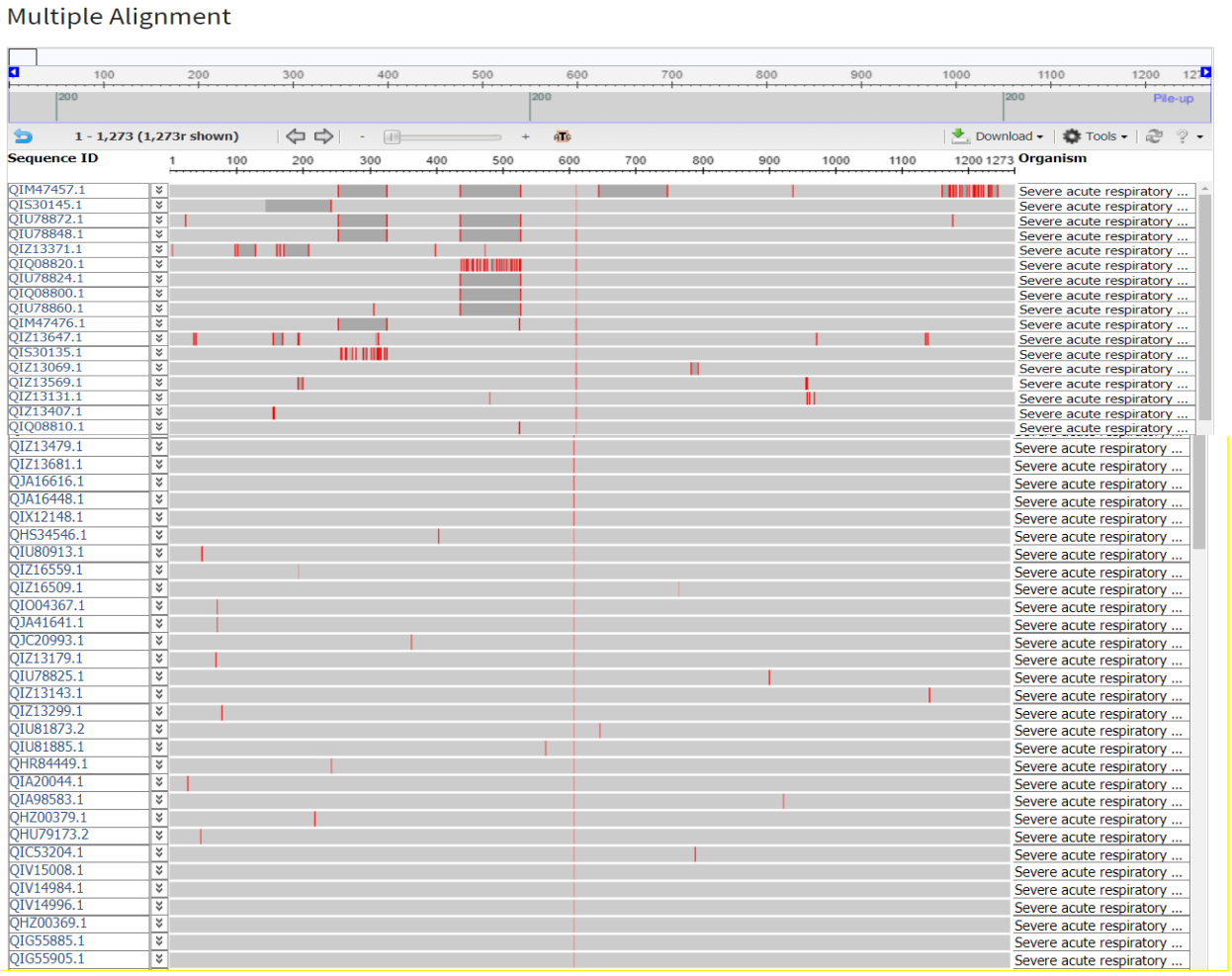

Figure 5 Mutations found in multiple sequence alignments for two hundred Spike glycoprotein sequences around the world [11].

There is a significant mutation at position 614 of chosen surface glycoproteins. It is D in sequences found in counties like China, Finland while it is G in countries like USA. Among chosen sequences in Figure 5, the surface glycoprotein sequence submitted on April, 2020 is found to have G in 614 position in 61 sequences. This shows that nearly 30% sequences within timeline March-April, 2020 around the world, have mutations at this position. Among 157 sequences world-wide from NCBI virus database with length 1273, only 4 sequences have shown same mutations within timeframe December,2019-Febraury,2020, among which 3 are from USA and one is from Spain. All MLA results are shown in details in Supplementary files. This position also is D in SARS-CoV sequence AAS00003.1, as we find in extended MLA results of Figure 5 in Supplementary file S7.

III. Vaccination strategies for HCoVs

There are several advantages and disadvantages of existing different vaccine strategies as are currently exhibited for virus outbreaks [13]. Advantages and disadvantages of some of the approaches are discussed here. First approach is called inactivated or killed virus vaccines, which are easy to prepare with safe high-titer neutralizing antibodies. But this approach is inapplicable for immunosuppressed individuals. Second approach is called live attenuated virus vaccines, which are developed very rapidly and induce high immune responses. However, the drawback of this approach is that it may cause genotypic or phenotypic inversions, with possibility of causing some diseases. Third approach is called Subunit vaccines, which are highly safe and consistent in production. These vaccines also can induce cellular and humoral immune responses with high-titer neutralizing antibodies. But these vaccine productions are very costly, with lower immunogenicity and require repeated doses and adjuvants to be effective.

Fourth approach is called viral vector vaccines, which is safe and induces high immune and humoral responses. But pre-existing immunity may be possible for these vaccines. Fifth approach is called DNA vaccines, which are highly safe and easier to design [13]. These vaccines can also create high-titer neutralizing antibodies. But these vaccines may cause lower immune responses in humans and also can make toxicity for repeated doses. Sixth approach is called mRNA vaccines, which are easier to design with higher degree of adaptability. However, they can induce strong immune responses. But they are highly unstable under certain physiological conditions.

Several current immunization methods target different Outer Membrane Proteins (OMPs) as antigens, as there will be no risk for pathogen to revert back to its virulent form [14]. This method also has fewer disadvantages when comparing with live attenuated or killed whole cell vaccination. The virus may acquire its resistance against multiple antibiotics, natural products or herbal products, like dengue or swine flu viruses.

In current researches, subtractive genomics study and reverse vaccinology approaches are becoming potential methods to sort antigenic and immunogenic proteins to be probable chimeric vaccine candidates [14]. Subtractive approach subtracts pathogen outer membrane proteins with virulence roles or containing essentiality factor for the survival of the pathogen but not present in the human host. Reverse vaccinology approach has the ability to identify antigenic and immunogenic MHC class I, MHC class II and B cell epitopes for certain OMPs, which enhances T cell and B cell mediated immunogenicity. Subsequently immunogenic peptides generates a multi-epitope vaccine combining with Immune-modulating adjuvants and Pan-epitopes. These are joined by linkers to increase immunogenicity. However, severe side effects observed in vaccinated individuals against severe pathogens in earlier cases, make it important to think about developing chimeric vaccines for coronavirus pathogenesis.

III.1. SARS-COV vaccines and treatments

Yang et. al [14] suggest a DNA vaccine to induce SARS, encoding the spike (S) glycoprotein of SARS-CoV. Viral replication is reduced by six times in lungs of vaccinated mice using these S plasmid DNA

expression vectors. They develop two sets of cDNAs encoding S glycoproteins of SARS-CoV by modified codons to optimize expression and to minimize recombination with CoVs. They develop 2 S carboxy-terminal mutants, in one they truncate cytoplasmic domain (SΔCD)(positions: 1-1242) and in the second mutant, they delete transmembrane and cytoplasmic regions (SΔTM) (positions: 1-1190). They create an intramuscular injection on mice. They applied these on ferrets and feline. It is shown for IBV (Avian coronavirus), these subunit vaccines with S1 subunit of S protein through baculovirus or from a fowlpox virus vector, shows inducive protection in apparently all vaccinated animals. Although small differences of 5% between among S1 sequences can cause poor cross protection as seen in earlier researches.

Earlier research works show that human monoclonal antibodies infer certain level of protection against SARS [9]. Traggiai et al. [16] develop an enhanced Epstein-Barr virus transformation method for human B cells. In both mouse and ferret models, administration of human monoclonal antibody with in vitro neutralization activity reduced SARS titers in the lungs (3- to 6-log10-unit decrease), also protecting from lung pathology in ferrets. Interestingly, DNA vaccination can induce humoral and cellular immunity against SARS-CoV in the mouse model. Yang et al. [17] show that a DNA vaccine from codon-optimized SARS spike glycoprotein induces neutralizing antibody as well as T-cell responses. Zeng et al. [18] have reported that mice immunized by plasmids encoding fragments of S1 developed a Th-1 antibody isotype switching. A prime-boost combination of DNA (SARS spike under control of the cytomegalovirus promoter and intron A) and whole killed SARS-CoV vaccines show higher antibody responses than single DNA or whole killed virus vaccines [19].

The highly attenuated modified vaccinia virus Ankara (MVA) has been used to express the spike glycoprotein of SARS-CoV in vaccination experiments using the mouse [20] and the ferret [21] models, with different results. Intranasal and intramuscular use of MVA encoding SARS-CoV S protein can lead to of humoral immune responses in mice and also reduces viral titers in respiratory tract. In ferrets, vaccination with MVA encoding the spike or nucleocapsid develops a strong antibody response, although, it fails to stop virus infection and spreading.

III.2. MERS-CoV vaccines and treatments

WHO lists all vaccines under trail for MERS-CoV till now [22]. The significant one is a DNA plasmid vaccine that encodes the MERS CoV spike (S) glycoprotein Electroporation device, developed by Inovio Pharmaceuticals. It is in Phase I for trials against MERS, but already in Phase II trials for other viruese like NIPA, ZIKA, HIV and HPV. IDT Biologika GmbH is also developing another Non-Replicating Viral Vector, MVA-MERS-S, which is already a licensed product against viruses like Influenza, Zika, Lassa or Ebola. Another Replicating Viral Vector, namely MV MERS-S (Measles vector) is being developed by Themis Bioscience GmbH, which is under PreClinical trial against MERS. IDRI is also developing a RNA vaccine by nanoparticle delivery with a replicating, VLP-secreting RNA (rvRNA) against MERS. Another significant development is a protein subunit vaccine, designed from full length S trimers/ nanoparticle by Novavax, and is in Pre-Clinical trial against MERS, although it is in Phase III trial against RSV, CCHF, HPV, VZV and EBOV.

Chan et. al [60] describe that codon 158 at the N-terminal domain and codon 509 at RBD of S gene may be significant for weaker positive selection. Chan et. Al[60] among other antiviral agents and immunomodulators against MERS-CoV , mentioned IFN, universal type 1 interferons, 3C-like protease inhibitor Lopinavir to inhibit a postentry step, TMPRSS2 inhibitor Camostat mesylate to decrease cell entry by ~15-fold, Broad-spectrum cathepsin inhibitor E-64-D , Cathepsin L inhibitor III to decrease entry of MERS-CoV pseudovirus by 97%, Chloroquine for inhibition of an early step in the replicative cycle, Hydroxychloroquine sulfate, ERK/MAPK signaling inhibitor Selumetinib with >95% inhibition and Ribavirin/lopinavir/IFN-α2a.

III.3. COVID-19 vaccines and treatment trials

McLellan and his colleagues [25] have shown that the spike S protein of SARS-COV-2 is quite similar to SARS-CoV. It also can bind to receptors of human cells more tightly than SARS-CoV, causing more easy spread of SARS-COV-2 from person to person, prominently by respiratory transmission.

Currently from February 25, 2020 [15], first NIH-sponsored randomized controlled clinical trial in USA at University of Nebraska Medical Center's(UNMC) biocontainment unit in Omaha [30] is evaluating safety and efficacy of the experimental broad-spectrum antiviral drug Remdesivir in hospitalized adults infected by SARS-COV-2. Remdesivir is shown to be successful to prevent MERS-CoV in rhesus macaques on February 13, 2020 [31]. The study is supported by Biomedical Advanced Research and Development Authority (BARDA), under U.S. Department of Health and Human Services. Gilead Sciences, Inc. develops Remdesivir (GS-5734) and participates in this trial research.

Remdesivir is tested on patients with Ebola virus earlier and has shown positive results in animal models to treat previous HCovs like MERS-CoV and SARS-CoV [11]. Clinical trials of Remdesivir are also ongoing in China. Participants in the experimental treatment group will be given 200 mg of Remdesivir intravenously on first day of enrollment and another 100 mg each day for the duration of hospitalization, up to 10 days total in this study.

NIAID is now experimenting with Moderna biotechnology company, Cambridge to develop a messenger RNA (mRNA) vaccine candidate, mRNA-1273, based on their prior studies of SARS and MERS. On March 16, 2020, a Phase 1 clinical trial evaluating mRNA-1273 against SARS-COV-2 has begun at Kaiser Permanente Washington Health Research Institute (KPWHRI) in Seattle [32]. 15 participants in each group will be given 25mg or 100 mg doses initially and then reviewed after 28 days. They will return to clinics for follow-up visits until one year.

Tian et al. [33] show recently that a SARS-CoV-specific human monoclonal antibody CR3022, may bind efficiently in SARS-COV-2 RBD (KD of 6.3 nM). The epitope of CR3022 does not overlap with ACE2 binding site within SARS-COV-2 RBD. These results implies CR3022 to be potential candidate therapeutics, alone or combinedly with other neutralizing antibodies, for SARS-COV-2.

IV. Leading Chemotherapeutic options for preventing SARS-COV-2

Significant numbers of investigational antivirals, immunotherapeutic strategies and host-directed therapies are currently used in clinical trials for Covid-19. CDC reports several clinical trials related to SARS-COV-2 very recently. Table 2 shows the intervention repurposing drugs or approaches and its country for these trials.

Table 2 List of vaccine and repurposing drug trials for Covid-19.

Index	Interventions	Country
1	Drug: Hydroxychloroquine	Canada, USA, France, Germany
2	Drug: Hydroxychloroquine 200 Mg Oral Tablet Drug: Azithromycin 500Mg Oral Tablet	Pakistan, USA, Spain
3	Drug: Hydroxychloroquine sulfate	Canada, Korea, USA(400mg,500mg,600mg)
4	Drug: chloroquine	Mild Symptomatic and mild asymptomatic
5	Drug: Remdesivir	USA (133,116), China, Czech Republic
6	Drug: Lopinavir/ritonavir	Canada, Korea
7	Drug: Favipiravir	Italy
8	Drug: BCG Vaccine	Netherlands
9	Drug: Recombinant human angiotensinconverting enzyme 2 (rhACE2), Drug: RhACE2 APN01	China, Austria, Denmark, Germany
10	Biological: COVID-19 convalescent plasma	Italy, India to be done, Mexico, USA
11	Drug: Sarilumab (anti-IL-6 receptor)	Canada
12	Drug: Baricitinib (janus kinase inhibitor)	Canada, USA
13	Drug: Tocilizumab (TCZ)	Italy, Spain, USA
14	Drug: Anakinra, Drug: Siltuximab, Drug: Tocilizumab	Belgium
15	Early Acute Coronary Syndrome Therapy	UK
16	Drug: DAS181	Wuhan, China
17	Drug: Best supportive Care (BSC) + IFX-1	Netherlands
18	Drug: Deferoxamine	Iran
19	Drug: Losartan	USA
20	Drug: Traditional Chinese Medicine Prescription	China
21	Drug: TCM prescriptions	China
22	Biological: NK cells,IL15-NK cells,NKG2D CAR-NK cells,ACE2 CAR-NK cells,NKG2D-ACE2 CAR-NK cells therapy	China
23	Drug: Ciclesonide Metered Dose Inhaler [Alvesco]	France
24	Drug: Peginterferon Lambda-1a	For mild Covid-19
25	Drug: Sildenafil citrate tablets	Wuhan,China
26	Drug: Sargramostim	Belgium
27	Drug: Nitric Oxide Gas	USA
28	Drug: Ruxolitinib 10 MG	Germany
29	Biological: Pathogen-specific aAPC	China
30	Dietary Supplement: vitamin C, Dietary Supplement:	USA

	Vitamin D, Dietary Supplement: Zinc	
31	Focused Lung Ultrasound	Denmark
32	Drug: Tranexamic acid tablets	USA
33	Drug: Antiviral treatment and prophylaxis	Spain
34	Drug: Atovaquone/Azithromycin	
35	Drug: PUL-042 Inhalation Solution	USA
36	Drug: Siltuximab, Drug: Methylprednisolone	Spain
36	Drug: Levamisole Pill + Budesonide +Formoterol inhaler	
37	Drug: Mavrilimumab	USA
38	Biological: bacTRL-Spike	Canada
39	Drug: Tetrandrine	China
40	Synthetic Minigene Vaccine	China
41	Drug: Bevacizumab	China
42	Drug: Chloroquine phosphate	Vietnam
43	Drug: thalidomide – Adjuvant	
44	Drug: Dexamethasone - Acute Respiratory Distress Syndrome treatment	Spain
45	Drug: Defibrotide Injection	

Hydroxychloroquinine, Hydroxychloroquine sulfate and Azithromycin are the drugs which are being used in trials for covid-19 in most countries. Hydroxychloroquine (HCQ) is a Disease-Modifying Antirheumatic Drugs (DMARDs), which is commonly used for diseases like Rheumatic Arthritis (RA) and Systemic Lupus Erythematosus (SLE). It also shows strong immunomodulatory capacity to prevent inflammation flare-ups and damaging of organs [27] . Shittu et. al [28] suggest that zinc additives may enhance efficinecy of of Chloroquine and Hydroxychloroquine for SARS-COV-2. Zinc is a diet which is found rich in fish, eggs, dairy products, shellfish (especially oysters), and red meat. Kaushik et al. [29] show that zinc salts can inhibit Hepatitis E virus replication through inhibiting RNA-dependent-RNA-polymerase (RdRp). RdRp is also active in SARS-COV-2 replication.

In India, Hydroxychloroquinine is one common medicine for treatment of both rheumatoid arthritis and Malaria, which are two very common diseases in this country. It is also seen that rate of SARS-COV-2 infections is low in India. Therefore, it may be analyzed how the rates of Infection is changing between rheumatoid arthritis and non- rheumatoid arthritis patients in India, considering medication of Hydroxichloroquine applied to them.

In some countries, these drugs combinedly with Dietary Supplements vitamin C, Vitamin D and Zinc, are also used to treat patients in Phase II trial with SARS-COV-2. According to ICMR, India, hydroxychloroquine is recommended only to asymptomatic healthcare workers and asymptomatic household contacts for confirmed SARS-COV-2 infected patients. Remdesivir (development code GS-5734) developed by Gilead Sciences was used as a treatment for Ebola virus and Marburg virus infections earlier. Remdesivir and Lopinavir/ritonavir are vaccines which single or combinedly are used in several trials of SARS-COV-2 pandemic among different countries.

Angiotensin II (Ang II) causes apoptosis in Henoch-Schonlein purpura (HSP), a hemorrhagic disease. Angiotensin converting enzyme 2 (ACE2) can antagonist the action of Ang II. ACE2 is a member of RAS - renin-angiotensin system, which regulates of cardiovascular, blood pressure and kidney function. PI3K/AKT signaling pathway has an important role in activity of recombinant human ACE2 (rhACE2) to promote activity of endothelial nitric oxide synthase (eNOS) [34]. Apeiron developed APN01 is rhACE2, which is approved for phase-II Clinical trials in April 2020 in Austria, Germany and Denmark and in February 2020 in China. It mitigates the harmful inflammatory reactions in the lungs and protects against acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) and pulmonary arterial hypertension (PAH) [34].

Camostat is shown to be MPRSS2 enzyme (Transmembrane protease, serine 2) inhibitor. It is utilized in Japan for chronic pancreatitis and postoperative reflux esophagitis patients [35]. Inhibition of TMPRSS2 partially blocked infection by SARS-CoV and HCoV-NL63 in HeLa cells [9]. In a separate in vitro study, Camostat prominently reduces SARS-CoV-2 infection in Calu-3 lung cells.

Leronlimab, one CCR5 antagonist is promising to reduce 'cytokine storm' in a small group of SARS-COV-2 severe patients in New York. Another interleukin-6 (IL-6) receptor antagonist, namely sarilumab (Kevzara used for rheumatoid arthritis treatment) is also tested for potential preventive stratey of acute respiratory distress syndrome (ARDS) in SARS-COV-2 severe patients.

FDA issues rules [36] for investigational convalescent plasma treatment with antibodies to SARS-CoV-2, obtained from recovered individuals from COVID-19. The study with convalescent plasma is also tried earlier in 2003 SARS-CoV-1 epidemic, 2009-2010 H1N1 influenza virus pandemic, and 2012 MERS-CoV epidemic.

This investigational convalescent plasma (CP) treatment is found to be effective in European trial recently after 3 days. There is no severe adverse reactions found after this CP transfusion. If donor of the convalescent plasma [37,38] has recovered from any pathogen very recently, then the plasma contains high levels of neutralising antibodies to that pathogen. Therefore, if their covalscent plasma is transfused into other patients, it develops an instant immune response.

Table 3 Vaccines used in trials against Covid-19.

Index	Vaccine	Effective against	Country
1	ChAdOx1 MERS	MERS	UK
2	Remdesivir	SARS-CoV, MERS-CoV	USA, China, Czech Republic
3	Lopinavir/ Ritonavir	HPV, HIV	Canada, Korea
4	Favipiravir	RNA viruses	Italy
5	BCG Vaccine	tuberculosis (TB)	Netherlands
6	Non-Replicating Viral Vector Adenovirus Type 5 Vector, CanSino	Ebola	China
7	DNA plasmid vaccine Electroporation device	Lassa, Nipah, HIV, Filovirus, HPV, Cancer indications, Zika, Hepatitis B	Inovio Pharmaceuticals

8	LNP-encapsulated mRNA	Multiple candidates	USA, Moderna/NIAID
9	Plasmid DNA, Needle-Free Delivery	SARS	Immunomic Therapeutics, Inc./EpiVax, Inc./PharmaJet, Inc.
10	Inactivated + alum Sinovac	SARS	China
11	Deoptimized live attenuated vaccines	HAV, InfA, ZIKV, FMD, SIV, RSV, DENV	Codagenix/Serum Institute of India
12	Adenovirus-based NasoVAX expressing SARS2-CoV spike protein	Influenza	Altimmune

Several vaccines are currently being tested and developed in several countries to find out their effectivity on SARS-COV-2, as shown in Table 3. Sanders et al.[39] show how the potential vaccines may target SARS-COV-2 in its lifecycle. Liu et al. [40] describe different type of vaccines relevant to SARS-COV-2, including attenuated virus vaccines, DNA-based vaccines, protein-based vaccines, virus-like particle vaccines and mRNA based vaccines.

Remdesivir is an investigational intravenous drug with broad antiviral activity that inhibits viral replication through premature termination of RNA transcription. In 2015, GS-5734 compound had blocked the Ebola virus in Rhesus monkeys. It is effective against SARS-CoV and MERS-CoV. In January, USA and China found Remdesivir "fairly good inhibitory effects" on SARS-CoV-2. WHO has announced pragmatic clinical trial (SOLIDARITY trial) to treat patients with Remdesivir. FDA also has approved its usage.

Lopinavir is another antiretroviral in protease inhibitor class. In 1995 Lopinavir is created and approved for use against HIV infections in a fixed-dose combination with another protease inhibitor, Ritonavir. This Lopinavir/Ritonavir combination is called "Thai cocktail". In 2014, Lopinavir is found effective against Human Papilloma Virus (HPV). WHO is also searching ability of Lopinavir/Ritonavir with Interferon-beta through SOLIDARITY study against COVID-19.

Favipiravir (T-705, Avigan, or “Favipira’’) in Japan is a pyrazinecarboxamide derivative, which is previously effectively tested for RNA viruses like influenza viruses, West Nile virus, yellow fever virus, some flaviviruses, arenaviruses and alphaviruses. It is related to the selective inhibition of viral RNA-dependent RNA polymerase. In a trial in China, it significantly reduced viral clearance time to four days. It is in Phase-III trial for covid-19 now.

Bacillus Calmette–Guérin (BCG) vaccine is an effective vaccine used against Tuberculosis (TB) in many countries in the world, like India, Japan, South Africa since 1921. BCG vaccine protection remains for up to 15 years by stimulating human immune system. It is a weakened live vaccine, developed from Mycobacterium bovis (cows). When BCG vaccine is inoculated, macrophages and dendritic cells (DCs) phagocytose the bacterium starting an innate immune response by secreting immunomodulatory components like cytokines and chemokines. For generating adaptive immune responses, in lymph nodes, DCs start to stimulate CD4+, CD8+, CD1+-restricted T cells, TFH, T regulatory cells, and B cells

[41]. Then CD4+ and CD8+ T cells migrate out of lymph nodes and move towards inoculation site. They provide the necessary stimulation to innate cells. Subsequently, B cells differentiate into antibody producing plasma cells or memory B cells. Together, all these cells of human adaptive immune systems develops the immune response against *Mycobacterium tuberculosis* [41].

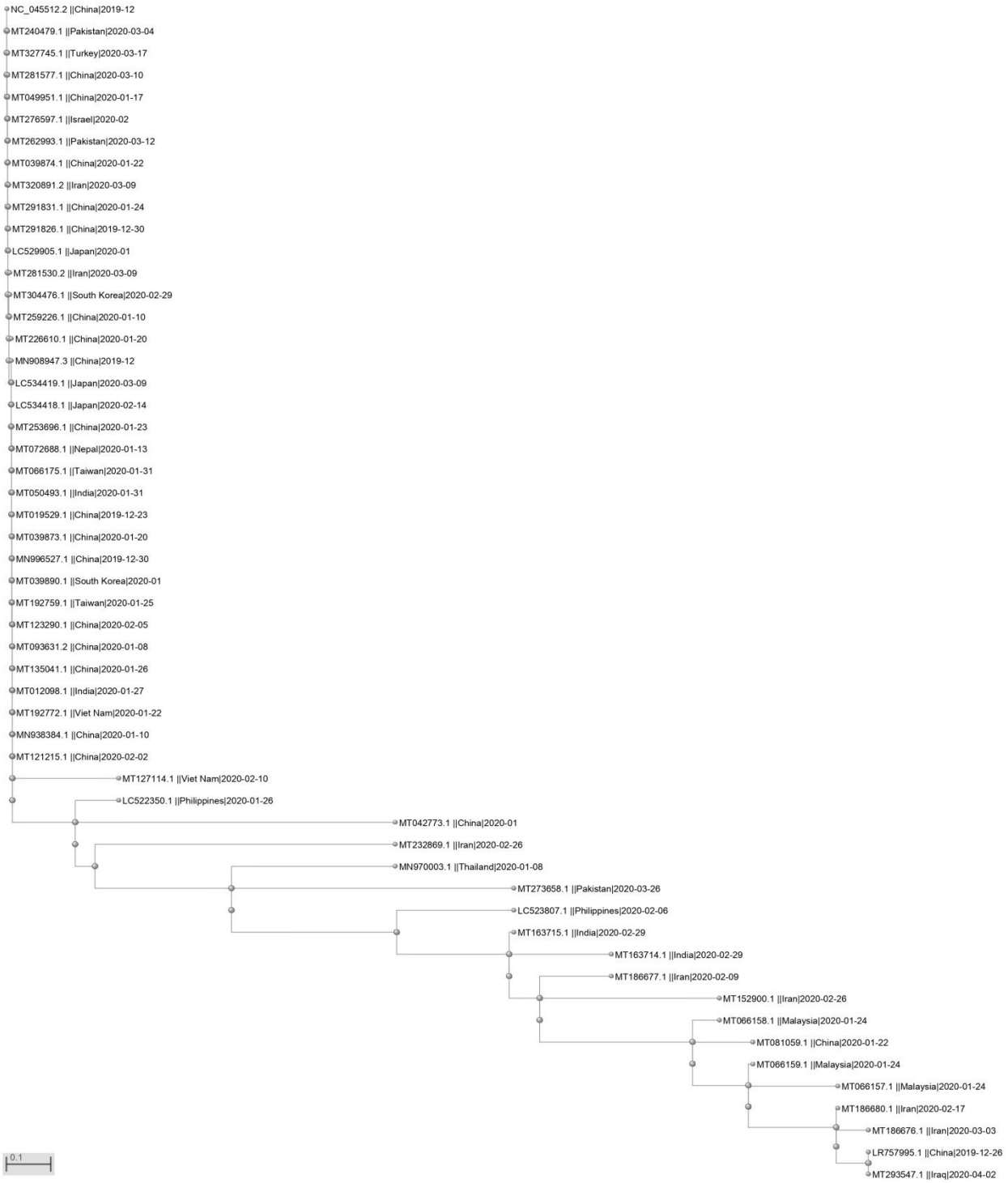

Figure 6 Phylogenetic tree of Asian SARS-COV-2 genomes showing collection dates [42].

BCG vaccine should not be given to immunosuppressed patients and during preganancy. Earlier BCG is found to be protective on leprosy, Buruli ulcer and bladder cancer. BCG vaccine is currently be used in Phase 3 trials (as of March 2020) on COVID-19 in Australia and Netherlands [43]. A study in April 2020 shows that Countries with a BCG vaccine could have a death toll 20 times less [44]. In April 13, 2020, WHO says they will evaluate evidence from two clinical trials on BCG vaccine, until then WHO does not recommend it.

V. Therapeutic potential approaches for preventing SARS-COV-2

The phylogenetic tree in Figure 6 developed from all Asian SARS-COV-2 genomic sequences (sorted collection date wise) in NCBI GeneBank, clearly show how earlier collected genomes of SARS-COV-2 are differing from recent ones. 4 Indian genomes are collected between 27/01/2020 and 29/02/2020, which show significant distances among themselves in the phylogenetic tree. This tree clearly indicates how SARS-COV-2 virus is phylogenetically differing for its original mutations in Asia through timeline January-April, 2020.

Spike glycoprotein P59594 (SPIKE_CVHSA) of HCoV SARS-CoV, is a single-pass type-I membrane protein with1255 amino acids. It has three participative domains to fuse SARS-CoV virus membrane with its host (human and masked palm civet) membrane.

Tor2 is the name of a virus prototype, isolated during SARS outbreak in 2002-2003. GD03prototype is isolated from next second mild SARS outbreak in winter 2003-2004. Tor2 spike protein efficiently binds human ACE2. This may explain the high pathogenicity of Tor2 virus, whose spike is highly adapted to the human host. Therefore, the cause for lack of severity of SARS in 2003-2004 may be for incomplete adaptation of GD03 binding to human ACE2. Mutations at Asn-479 and Thr-487 in palm civet coronavirus are shown to be necessary and sufficient for SARS-CoV to gain ability to infect humans.

This Spike surface glycoprotein of SARS-CoV cleaves into 3 chains - spike protein S1, S2 and S2' (PRO_0000444082 position 798 – 1255). Spike protein S1 (PRO_0000037209 position 14 – 667) works to attach SARS virion to human cell membrane by interacting with host receptor (human,tiger,bat) ACE2, and also starts the infections in host body. Spike protein S2 (PRO_0000037210 position 668 – 1255) mediates cell-to-cell fusions of SARS virion and cellular membranes (Class I viral fusion protein). During SARS-CoV viral and host cell membrane fusions, the heptad repeats in coiled coil regions, develops a trimer-of-hairpins structure. This positions the fusion peptide in close proximity to C-terminal region of ectodomain. The structure formation starts apposition and causes subsequent fusions between viral and host cell membranes. Spike protein S in SARS-CoV accumulates in endoplasmic reticulum, Golgi intermediate compartments to participate in virus particle assembly.

Biological properties of Spike protein precursor of SARS-CoV (P59594) shows that mutagenesis at positions 348:C → A, 454:D → A, 467:C → A and 474:C → A cause complete loss of human ACE2 binding in vitro, as shown in evidences. Mutagenesis at positions 452: E → A cause 90% loss and 463:D → A cause partial loss of human ACE2 binding in vitro, as shown in publications. Mutagenesis at position 667:R → S causes 40% loss of cell-cell fusion and at position 797:R → N causes complete loss of trypsin-induced membrane fusion. Mutagenesis at positions 1251:K → A and 1253:H → A associateively cause

decrease in Golgi localization, and complete loss of COPI binding. Region 306 – 527 is the Receptor-binding domain and region 424 – 494 is the receptor-binding motif, which binds to human ACE2 for SARS-CoV.

Considering similarity with binding mechanism of SARS-CoV, Andersen et al. in their work on SARS-COV-2 [45] show that 6 RBD amino acids are critical for binding to ACE2 receptors and for choosing host ranges for SARS-CoV-like viruses. On SARS-CoV coordinates, these amino acids are Y442, L472, N479, D480, T487 and Y491. These positions correspond to L455, F486, Q493, S494, N501 and Y505 amino acids in SARS-CoV-2. 5 among of these 6 residues are different between SARSCoV-2 and SARS-CoV. Andersen et al [45] also show that there is a polybasic cleavage site (RRAR) at S1-S2 subunit junction of spike protein S. This site allows effective cleavage by furin and other proteases. Therefore it has significant role for viral infectivity and determining host ranges. Moreover, a leading proline is also inserted at this site in SARS-CoV-2, making the sequence to be PRRA. The turn developed for proline is shown to result in adding of O-linked glycans to S673, T678 and S686. These glycans are unique to SARS-CoV-2. These polybasic cleavage sites are not present in similar "lineage B" Betacoronaviruses, as also shown in our MLA results in Figure 4. However, other HCoVs, like HKU1 (lineage A) have these sites and predicted O-linked glycans.

Procko [46] recently shows that soluble ACE2 can inhibit entry of both SARS-CoV and SARS-COV-2 in the role of a decoy in S binding sites. Therefore, it becomes an important candidate for prophylaxis and therapeutic development. Some researchers have shown that S binding does not dependent on ACE2 catalytic activity. S binding occurs only on outer surface of ACE2, while angiotensin substrates bind within a deep cleft which contains the active site (27). Substitutions in this substrate-binding cleft of ACE2 may act to make controls. This substitutions may have minimal impact on S binding interactions, while may be significant to reduce substrate affinity, which may lead to enhance in vivo safety. This is why this cleft is so important to prevent SARS-COV-2 infections. Some recent works have shown that catalytically active recombinant ACE2 protein also may replenish lost ACE2 activity caused by SARS-COV-2 in respiratory distress, similar to improvement in SARS-CoV symptoms of acute lung injury in mice [47].

Porcko [46] also shows that residues which reside in spike surface interface of RBD-bound ACE2 tend to be conserved, while residues nearby interface periphery or in substrate-binding cleft shows mutation tolerance for "nCoV-S-High sorts". 2 ACE2 residues at N90 and T92 jointly form a consensus N-glycosylation motif and they are important hotspots for enriched mutations. He shows that all substitutions of N90 and T92 prone to be highly favorable for RBD binding. Single exception is T92S which remains N-glycan. Therefore, all these substitutions indicate that N90-glycan may partially hinder S/ACE2 interaction [46]. Interacting amino acids of SARS CoV2 for binding with ACE2 , that is, 413GLY, 414 GLN, 415 THR and 416 GLY are totally conserved between two spike protein structures. This result is shown also in the following Figure 7.

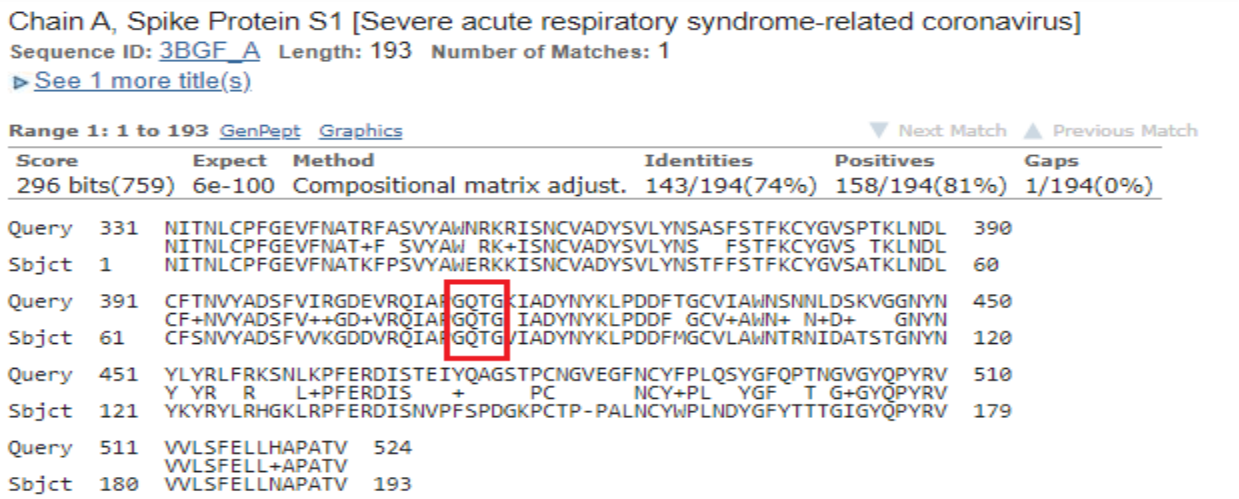

Figure 7 Protein sequence alignment of 2019-nCoV and SARS-CoV RBD, showing the predominant residues that contribute to interactions with ACE2 or SARSCoV-specific antibodies [48].

In Figure 7, an alignment between SARS-CoV-2 and SARS-CoV, shows that positions 348(C), 454(D), 467(C) and 474(C) in SARS-CoV are preserved in SARS-CoV-2 in 361(C), 468(D), 480(C) and 488(C) positions respectively. Therefore, as earlier mutagenesis in these positions 348:C → A, 454:D → A, 467:C → A and 474:C → A in SARS-CoV have caused loss of human ACE2 binding, similar mutagenesis effects in SARS-CoV-2 at positions 361: C → A, 468:D → A, 480:C → A and 488:C → A may also cause some loss of ACE2 binding.

Earlier, Wong et. al [49] show that an 193-amino acid fragment (residues 318-510) of spike glycoprotein S protein in SARS-CoV, can create more effective bound to ACE2 than the wholel S1 domain (residues 12-672). There are significant between 318 to 326 and between 491 to 509, which works either directly to S1 domain-ACE2 binding or causes in proper folding of RBD [49]. One single point mutation at glutamic acid 452 or aspartic acid 454 of SARS-CoV, interferes or abolishes interactions to ACE2. Wong et. al [49] show that this 193-residue fragment can block S protein-mediated infection with an IC(50) of less than 10 nm, whereas IC(50) of S1 domain is apparently 50 nm. This information show an independently folded RBD in SARS-CoV Spike protein. Therefore, due to alignment similarities between SARS-Cov and SARS-COV-2, these residues R426SARS-CoVN439SARS-CoV-2, Y442SARS-CoVL455SARS-CoV-2, L472SARS-CoVF486SARS-CoV-2, N479SARS-CoVQ493SARS-CoV-2, Y484SARS-CoVQ498SARS-CoV-2, and T487SARS-CoVN501SARS-CoV-2 become significant key contact residues to consider for prevention [50]. As no mutations among these residues have been found among SARS-COV-2 sequences, the differences among lineages for severity of COVID-19 are certainly not caused by these mutations and may come from cell-cell fusion mutations.

To find out the effective agents against SARS-CoV-2, WHO has announced SOLIDARITY study considering the potential utility of four different classes of clinically known drugs or drug combinations. The four arms of this SOLIDARITY study contain a) viral polymerase inhibitor remdesivir, b) anti-malarial agents chloroquine and hydroxychloroquine, c) HIV protease inhibitors lopinavir and ritonavir and d)

lopinavir/ritonavir with interferon-beta. Significant results also indicate that serine protease inhibitor camostat mesylate is also effective as repurposing drug for preventing SARS-CoV-2.

Zhou et. al [51] show a phylogenetic analysis of coronaviruses and analyze 135 drugs for network-based drug repurposing for preventing SARS-COV-2, considering network proximities among SARS-CoV, MERS-CoV, IBV, and MHV. They select 16 drugs among different Selective estrogen receptor modulators, Angiotensin receptor blockers, Immunosuppressant or antineoplastic agents and Anti-inflammatory agents. Finally they suggest three network-predicted candidate drug combinations for SARS-CoV-2, namely Sirolimus plus Dactinomycin, Toremifene plus Emodin and Mercaptopurine plus Melatonin [51].

ACE2 activity has a strong pH dependence under acidic conditions [52], such that the enzyme is almost inactive at pH 5.0 and has optimal activity at pH 6.5. ACE2 proteolytic activity is greatly enhanced by high concentrations of chloride or fluoride. A characteristic of ACE that is unique among metalloproteases is its activation by monovalent anions, including Cl-, Br- and Fl- (10-12). ACE2 proteolytic activity is also activated by monovalent anions, with a ten-fold enhancement in the presence of 1.0 M NaCl. Therefore, NH4Cl may be tried as to enhance proteolytic activity of ACE2 in covid-19 cases also.

Simmons et. al show that SARS-CoV uses spike (S) glycoprotein for receptor binding and its entry to host cells. SARS-CoV infection is also sensitive to lysosomotropic agents, which can perturb endosomal pH [53]. They show that subsequent infection is sensitive to inhibitors of endosomal acidification such as ammonium chloride [53], suggesting that SARS-CoV requires a low-pH milieu for infection. Ammonium chloride is a lysosomotropic amine and raises pH inside acidic vesicles by acting as a proton trap.

Methylamine [54] and chloroquine [55], which are also lysosomotropic amines, earlier are reported to inhibit the cytotoxic activity of Furin-cleaved ETA by blocking endocytic uptake, rather than by blocking a later step dependent on a low pH within an intracellular compartment [56]. Furin (a serine endoprotease) also cleaves off S1/S2 domain of Spike protein of SARS-COV-2 and therefore also is a suitable antiviral agent. Inhibitors of endosomal cysteine protease and transmembrane protease serine can partially block viral entry into the cell. Earlier work [47] shows that NH4Cl neutralizes acidic endo-lysosome compartments, thus suggesting that pH was responsible for PV capsid conformational changes leading endosome escape for Human Papillomavirus Type 16 (HPV16). They show that Ammonium chloride prevents HPV16 trafficking to caveosomes.

Vincent et al. [56] suggest that choloroquine as well as Ammonium chloride has significant effects on SARS-CoV infection. In their report, they dentify chloroquine as an effective pre- and post-infection antiviral agent for SARS-CoV. Choloroquine and hydroxycholoroquine are already established for treatment of SARS-COV-2 by WHO in 2020.

Another common lysosomotropic agent, NH4Cl, Ammonium chloride, also has been widely used in studies addressing endosome-mediated virus entry earlier. Coincidently, NH4Cl was earlier shown to reduce the transduction of pseudotype viruses decorated with SARS-CoV S protein [56]. They observe a

93–99% inhibition with NH4Cl at ≥ 5 mM on SARS-CoV [56]. Their research shows that both NH4Cl (≥ 5 mM) and chloroquine (≥ 10 μM) are very efficient for reductions of SARS-CoV infections.

They [56] suggest that similar mechanisms may mediate spreading and infections of SARS-CoV with effects of chloroquine and NH4Cl. They also inform that NH4Cl treatment affects trimming and/or terminal modifications of N-glycosylated chains of ACE2. Subsequently, at 10 mM NH4Cl, the ER form of ACE2 migrates with slightly faster mobility. That is an indication that NH4Cl at this particular concentration may also affect core glycosylation. They also treat cells with chloroquinine to examine terminal glycosylation status of ACE2 [56].

Vincent et al. [56] find a stepwise increase in electrophoretic mobility of ACE2 in SARS-CoV with increasing concentrations of chloroquine, which is similar in cases of NH4Cl also. On the basis of flow cytometry and immunoprecipitation analysis results, they infer that both NH4Cl and chloroquine impair ACE2-terminal glycosylation, where NH4Cl can show a more dramatic effect. Therefore for SARS-COV-2 also NH4Cl may show significant effects in virus entry, which needs to be proven in laboratories now. They also state that biosynthesis and processing of S protein is also not negatively affected by NH4Cl [56]. Although Carboxypeptidase ACE2 is expressed in similar quantities at the cell surface, variations in glycosylation status of ACE2 may cause loss of efficiency in ACE2-SARS-CoV interactions and thereby may lead to inhibit virus entry with both NH4Cl and chloroquine treated cells [56].

Chan et. al [23] also suggest that membrane fusion reaction in endosomal cell entry can be overcome by low PH and cathepsins. They also suggest lysomorphic agent NH4Cl and cathepsin inhibitors in cell-type dependent manner to prevent proteolytic cleavage by changing PH level. Similar low PH proteolytic cleavage [24] may happen for SARS-COV-2 infections as well, which needs to be checked.

Endosomal proteolysis of cathepsins is one of the cause for entry of murine coronavirus MHV, type 2 mediated by spike in host cells [57]. Mizzen et. al [58] show that in mouse model, NH4Cl (concentration of 20 mM) can make 4 to 5 hour delay in producing virus progeny among highly MHV infected mouse L-2 cells. For Feline Enteric Coronavirus(FECV) and Feline Infectious Peritonitis Virus(FIPV) infections, cathepsin B and cathepsin L can induce a certain cleavage event at low pH, similar to endosomal proteolysis of SARS-CoV [59]. NH4Cl and bafilomycin A1 can significantly inhibit TGEV (transmissible gastroenteritis virus) infection found in protein biosynthesis analysis, but it is effective if only when added early in TGEV infection timeline - about 1 hour after start of endocytosis as is found in the experiment [60]. Similar study to check effectiveness of NH4Cl is also suggested for SARS-COV-2 also by Yang et. Al [61].

Ammonium chloride (NH4Cl) is one inexpensive as well as readily available catalyst. Therefore, as Porcko [46] suggest that catalytically active protein may have effects for replenishing lost ACE2 activity in COVID-19 patients, NH4Cl may be tried also. Ammonium Chloride (NH4Cl) or *sal ammoniac* (salmiak), is sometimes available as an expectorant in cough medicines. The expectorant action of NH4Cl comes from its irritative action on bronchial mucosa. This effect causes the production of respiratory tract fluid which in order facilitates the effective cough. NH4Cl is also used as systemic acidifying agent

to treat severe metabolic alkalosis. It is also sometimes used in oral acid loading test to detect distal renal tubular acidosis. It also helps to maintain urine at an acid pH for treatment of some urinary-tract disorders.

Ammonium Chloride can be found in European candies (such as salt licorice), dough conditioner for baked goods, or in condiments, in margarine or dried foods. Salty liquorice or salmiak liquorice denoteis a seriesty of liquorice flavoured with "salmiak salt" (sal ammoniac – NH4Cl). It is a common confectionery found in Nordic countries, Benelux and northern Germany. Salmiakki candies are very popular in Finland. Salty liquorice or salmiak is also used as a flavouring in other products, such as ice creams, syrups, chewing gum and alcoholic beverages. In 1930s, it worked as pastilles in Finland, Norway, Denmark, Sweden and Netherlands. The Dutch calls it as "Zoute Drop" or "Dubbel Zoute Drop" (double salted liquorice). In Germany, it is commonly known as salt liquorice (Salzlakritz) candy and salmiak pastilles (Salmiakpastillen). In Finnish, it is called salmiakki, pastilles like a black diamond-shaped lozenge. In Sweden, most popular salty liquorice may contain an average of 7% of NH4Cl. To 7.99% level NH4Cl, salmiak pastilles are considered as "traditionally-applied medicine to assist expectoration in the airways".

An antibacterial effect is produced for the event of neutralization of slightly acidic NH4Cl (pH about 5.5) by relatively alkaline saliva (pH about 7) [9, 62] when ammonia is released with a disinfecting effect. In addition to be in candies, salmiak is also found to flavour vodka, chocolate, distilled rye brandy, ice cream, cola drinks, snus, and meat. [https://en.wikipedia.org/wiki/Salty_liquorice] In Iran, Tajikistan, India, Pakistan and Arab countries, it is also found as "Noshader", which is used to make snacks like samosas and jalebi more crisper.

Nowadays food manufacturers use NH4Cl instead of Sodium Chloride as a flavoring agent in bread, biscuits. Because eating too much NH4Cl certainly harms human body, some studies show that long-term excessive salt can cause high blood pressure and kidney diseases, aggravate diabetes, exacerbate asthma, make prone to osteoporosis and even fractures. Exposure to NH4Cl is considered to be moderately hazardous, which may cause irritation, shortness of breath, cough, nausea and headache.

Ammonium Chloride is used as an Expectorant in cough syrups. Many caugh syrups has NH4Cl as its content like – Benadryl, Amcal Expectorant mixture, Bronchial Cough Mixture, UNICOUGH 14mg / 135mg / 1.1mg in 5ml Oral Solution, COF-RYL etc. It should be used with caution in patients with high total CO2 and buffer base secondary to primary respiratory acidosis. Therefore, in case of SARS-COV-2, Ammonium Chloride may be thought to be used as an expectorant for only mild patients and asymptomatic people in first 7 days, when SARS-COV-2 stays at respiratory tract causing caughs and spreading.

SARS-CoV binds to ACE2 as an entry receptor [26] and thereafter uses TMPRSS2 cellular serine protease for S protein priming. SARS-CoV also uses cathepsin B and L (CatB/L) endosomal cysteine proteases [53] for S protein priming in cell lines along with TMPRSS2. Therefore, inhibition of both proteases is needed to proper blockade of virus-host cell entry [63]. It has been found that, later on only

TMPRSS2 activity becomes essential for cell-to-cell fusions and SARS-CoV spreading and infections. CatB/L activity is not indispensable for this spreading.

To find out if SARS-CoV-2 uses CatB/L for cell entry, Hoffman et. Al [64,25] initially use NH4Cl [65] to elevate endosomal pH, which subsequently blocks CatB/L activity. 293T cells (TMPRSS2−, transfected to express ACE2 for S protein-initiated entry) and Caco-2 cells (TMPRSS2+) are used as targets. They show that NH4Cl blocks VSV-G-dependent entry in these two cell lines. NH4Cl treatment is shown to strongly inhibit SARS-COV-2-S- and SARS-CoV-S-driven entry into TMPRSS2− 293T cells. This suggests CatB/L dependence in both cases. Inhibition of entry TMPRSS2+ Caco-2 cells is found to be less efficient by them, which suggests SARS-COV-2-S priming by TMPRSS2 in Caco-2 cells.

Clinically proven serine protease inhibitor, Camostat Mesylate, is active against TMPRSS2. Kawase et. Al [63] show that Camostat Mesylate partially blocks SARS-COV-2-S-driven entry into Caco-2 and Vero-TMPRSS2 cells. E-64d is another inhibitor of CatB/L . Kawase et. Al [63] find full inhibition adding both Camostat Mesylate and E-64d. This indicates that SARS-COV-2-S uses both CatB/L and TMPRSS2 for priming in the cell lines. On the contrary, Camostat Mesylate is not found to interfere with SARS-COV-2-S-driven entry into 293T TMPRSS2− cell lines [52] and Vero, where E-64d effectively blocks, displaying CatB/L dependency. Therefore,instead of E-64d, commonly available NH4Cl may be experimented with to block CalB/L in SARS-COV-2 entry following previous work of Vincent et. al [56]. They suggest that antibody responses developed against SARS-CoV may partially protect against SARS-CoV-2 infections. Therefore following Vincent et al [56] NH4Cl should be tested to find its effectiveness in entry of SARS-COV-2 on mild symptoms and asymptomatic people.

Cao et al. [66] show that for 3 patients in China received high-dose intravenous immunoglobulin (IVIg) and show satisfactory recovery. One patient was released only after 9 days of treatment. A high-dose IVIg at 0.3–0.5 g per kg weight each day for 5 consecutive days is prescribed on the patients for a potent and safe immune modulator. This approach followed well-established practices in immune modulation therapy earlier found in other diseases, like neuromuscular disorders. Intravenous Immunoglobulin was also earlier FDA-approved for HCov-NL63. Therefore, it is also an experimented possible application in this SARS-COV-2 pandemic applicable before the acceleration phase of patient.

Boulos et al. [67] show that GIS systems can also help online real-or near real-time mapping of disease cases and of social media reactions to disease spread, predictive risk mapping using travel data from population. It traces and maps super-spreader trajectories and contacts among space and time, to find out SARS-COV-2 spreading. Johns Hopkins University, WHO and HealthMap crate their dashboards for Covid-19. China creates their own ‘close contact detector’ app using big data for proximity detection with infected person and published it with three most popular social and payment apps. Global risk assessment and misinformation spreading can also be analyzed with GIS.

WHO informs that Ralph Baric from University of North Carolina, Chapel Hill and Miles Carroll from Public Health England with University of Liverpool are progressing to produce recombinant virus expressing nCoV Spike protein, which may help in viral vaccines. Barney Graham from NIAID, Vasan

Vasan from CSIRO and Bart Haagmans from Erasmus Medical Center are working on recombinant Spike protein and its validation on ELISA assay.

VI. Conclusions

In this article, we discuss about the etimology, survey vaccines, antiviral strategies and candidate antigens applied on earlier HCoVs, which may have potential to be applicable against SARS-CoV-2. Several vaccine strategies are recently tested for SARS-COV-2. However effective vaccine design after all phase trails becomes a very long process. Therefore, drug repurposing strategies should be effective in current scenario of SARS-COV-2 pandemic. Some earlier drugs like chloroquinine has been shown to be effective for SARS-COV-2 also. Therefore, following the previous work of Vincent et al, testing effectivity of lysosomotropic agent Ammonium Chloride and Camostat Mesylate along with Hydroxychloroquinine may be effective for SARS-COV-2 as suggested in this article.

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SUPPLIMENTARY FILES LIST

1. QHR63250.2 Blast sequence alignment results - NCBI Blast_Spike Glycoprotein.pdf
2. QIA98583.1 and other 57 sequences – Multiple Sequence Alignment results - 98583and57Aln1.png
3. QIA98583.1 and other 57 sequences – Phylogenetic tree view - 98583and57NJ2.png
4. QJC19491.1 and other 8 sequences Multiple Sequence Alignment results - S4_COBALT_ - Cobalt RID (seqs).pdf
5. QJC19491.1 and other 2 sequences – Multiple Sequence Alignment results – S5_19491andothertwoAln3.png
6. QJC19491.1 and other 2 sequences – Phylogenetic tree view - S6_19491andothertwoNJ2.pdf
7. QIM47457.1 and other 199 sequence - Multiple Sequence Alignment results – S7_47457and199Anl1.png