Anti-SARS-coronavirus treatment based on molecular interaction

Gazmend Temaj
University for Business and Technology - UBT, gazmend.temaj@ubt-uni.net

Anti-SARS-coronavirus treatment based on molecular interaction

Gazmend Temaj[1], Kumrije Sopi-Xharra[2], Shefki Xharra[2]

[1]College UBT, Medical Faculty, Lagjja Kalabira 56, Prishtinë, 10000 Kosova
[2] Regional Hospital Prizren, Sheh Emini nn, Prizren, 20000 Kosovo

{gazmend.temaj@ubt-uni.net, kumrijensopi@gmail.com, saxharra@gmail.com}

Abstract.
SARS-CoV-2 belongs the family betacoronavirus in Coronoviridae; it is known to have single strand RNA which is enveloped. The first case is reported late2019 in China. From there it is circulate around the world, causing the COVID-19 pandemic situation with higher fatality rates. At the beginner of April 2021 SARS-CoV-2 has infected more than 130 million people and led to 2.84 million death. There are several strategies for cure of SARS-CoV-2 infection, to date the number of drugs who are used for treatment is increased depends of these drugs are used alone or in combination form. FDA has approved remedesivir who have the ability to neutralized antibodies, although clinical effects were controversial. Here we discuss for development of new strategies for therapeutic reason in patients infected by SARS-CoV-2.

Keywords: SARS-CoV-2, replication, human proteins, small and large molecules

1 Introduction

The term coronavirus came from crown-like spikes on the surface of the virus. The coronavirus are known to envelop positive strand RNA viruses; the coronavirus have ability to infect various vertebrates, including humans. In the 1960s, two kind of human coronavirus are discovered; alpha and beta. They are known to cause disorders such as respiratory illness of mild to moderate severity [1, 2]. The whole world is currently in a pandemic situation since the first case of patients infected with SARS virus appeared in late of December 2019 in Wuhan, China. The patients were exhibit common symptoms like fever, dry cough, sore throat, breathlessness, and tiredness. Before the spread of COVI-19 [3, 4], SARS appear as an epidemic in 2003, followed by Middle East respiratory syndrome (MERS) 2012, both caused by a novel coronaviruses assigned to the genus Beta coronavirus [5]. Coronavirus contain positive single strand RNA as a genetic inherited material, which is 30kb in length. This single strand RNA is protected from double fatty layer and help virus to evade host immune system inside host cell [6, 7]. The subfamily of Coronavirinae is divided into fourth genera: alpha, beta, gama, and delta [8]. The SARS –CoV-2 genome consist from 12 open reading frame (ORF). In 5' end ORF 1a is overlapped by ORF1b, which encodes the RNA polymerase and other non-structural proteins. Genes encoding non-structural proteins such as S (spike), M (membrane), E (enveloped), and N (nucleocapside) are in the remaining part of the genome (one-third) spanning from the 5' to the 3' terminal along with several gene encoding non-structural proteins (NSP). Besides they belong the same serogroup, there is a slight differences in the nucleotide number, sequences, gene order and expression [9-12]. Last time is

reported that some nucleotide substitution has occurred in the gene which encode the S protein, such as NSP15, NSP12, NSP16, NSP9, NSP10, NSP8, NSP2, NSP3, NSP1 [13-15]. The NPS3 is the most strongly enriched protein, NPS6 play pivotal role in formation of double membrane vesicles [16]. Mutation of NPS2 and NPS3 believed to enhance infection of coronavirus [17, 18]. The genetic mutation in RNA help perhaps virus to escape the host immune system and develop drug resistance. The research has found in many infection patients minor mutation in genotype SARS-CoV-2 [19].

SARS-CoV-2, use ACE2 (angiotensine-converting enzyme 2) receptors to have access inside human cells; the MERS-CoV is shown to bind with specific DPP4 (dipeptidyl peptidase 4) receptors [20, 21].

1.1 Replication of SARS-CoV-2

The coronavirus start to login inside cell by connection with host receptor of the S glycoprotein present on their surface. The S protein contain the RBD (receptor-binding domain). In some type of coronavirus, the RBD is found to be present at the N terminus region of S protein, whereas in SARS-CoV-2, it is placed at the C-terminus region [22, 23]. After the SARS-CoV-2 is inside of host cell via membrane fusion, it shown to release +ve ssRNA genome into the cytoplasm, where start the translation of ORF1a and ORF1b from ribosome, and came to formation of two large polyproteins (pp1a and pp1ab). After that came to activation of proteases which interact with polyproteins to cleavage them into 16 non-structural proteins (NSP1-16), which eventual have the possibilities to create the viral RNA polymerase and other accessory proteins for virus assembly [24-26].

The glycoprotein E is shown to be incorporated into rough endoplasmatic reticulum or Golgi membrane. The single strand RNA have possibilities to interact with capsid and to form nucleocapsid, followed by budding of assembled virus particles in the ER-Golgi intermediate compartment [27].

1.2 Human proteins who bind SARS-CoV-2 RNA

The expanded SARS-CoV-2 RNA encompassed include 104 human proteins and included 13 SARS-VoV-2 encoded proteins. The majority of human RNA interactome proteins (100 proteins), have been identify previously-during the study of attract proteins that crosslink to RNA [26]. Only 10 of the 332 proteins that is found to link during the recombination of SARS-CoV-2 proteins in uninfected cells, also bound directly to viral RNA in cells which are infected with virus [28].

1.3 Pathogenesis of SARS-CoV-2

SARS-CoV-2 arrived to lung via the naso-oral cavity. After the virus came to lung, it is shown to interact with ACE receptors and initiate its replication [29-31]. This is a first phase which lasts 1-2 days, during this time the virus have possibilities to multiply in the upper respiratory tract. After that came second phase who begin from

day 2 and lasts till day 14 which is manifested with different symptoms (see above). In this phase patients start to exhibit enhance activity of pro-inflammatory cytokines response that lead to viral sepsis accompanied by other complication. The infect patients rarely are manifested by intestinal symptoms like diarrhea [32]. The patients who are sick from other diseases such as asthma, obesity, hypertension, diabetic, heart, and kidney, liver are also in higher risk to acquire the disease [33]. The autopsy of patients who die by SARS-CoV-2 infection show multi-organ dysfunction, higher viral titers in lung and immune cells in circulation, thus damaging immune system and lung [34, 35]. In children affected with SARS-CoV-2 show severe symptoms but with rare death and better prognosis [36]. The researcher unsuspected for two ways. One is ACE2 receptor activity is higher in children aged 4-13 years; after that ACE2 receptors start to decline until adolescences. The second way is differential CD4+ and CD8+ T cell population are found in children compared with adults [37, 38].

1.4 Structure and function of the -1 PRF (programmed -1 ribosomal frameshift) signal of SARS-CoV-2

SARS-CoV-2 use a molecular mechanism called programmed -1 ribosomal frameshift (-1PRF) to control gene expression and protein synthesis. The -1PRF signal is very important for SARS-CoV-2: the frameshift gene product including RNA polymerase is required for replication. Many research paper show on SARS-CoV show that mutation suppressing -1 PRF significantly in cell culture [39-41]. Frameshift –stimulatory structure are shown to be a potential target for anti-viral drugs [42].

The pseudoknot stimulating –1 PRF is shown to have three stem architecture; this is characteristic just for coronaviruses, in contrast many viruses have two stem pseudoknots [43]. The pseudoknot sequences are much conserved between coronaviruses [44], several mutation in this part are identify in SARS-CoV-2 during the COVID-19 pandemic [45]. Some of these mutation are found in one patients; others are found in different patients from different countries of word (Figure 1).

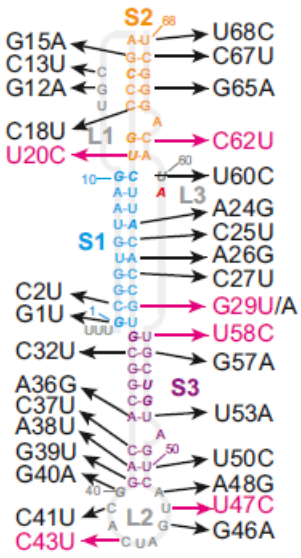

Fig 1. Natural of mutation in SARS-CoV-2 pseudoknot. The mutation are found in patients affected by SARS-CoV-2 in structure of pseudoknots (figure adapted from Neupane et al, 2020) [42].

How these mutation affect -1 PRF in SARS-CoV-2; it is not known well, but understanding these mutation is a good issue for future therapies [42]. The major mutation in these position are transition (purine-purine or pyrimidine-pyrimidine conservation) rather than transversions. The majority of these mutation approximately 62% in the stem keep it in original state the wild –type base pairing by conserving pairs G:C to G:U, A:U to G:U to G:C [42].

The -1PRF of SARS-CoV-2 begins with U UUA AAC slippery site, continue by a 6-nt spacer region and then three-stemmed mRNA pseudoknot that stimulates -1PRF. A second regulatory element is attenuator hairpin, which located at 5’ at the slippery site; this part is much conserved sequences (Figure 2) [39].

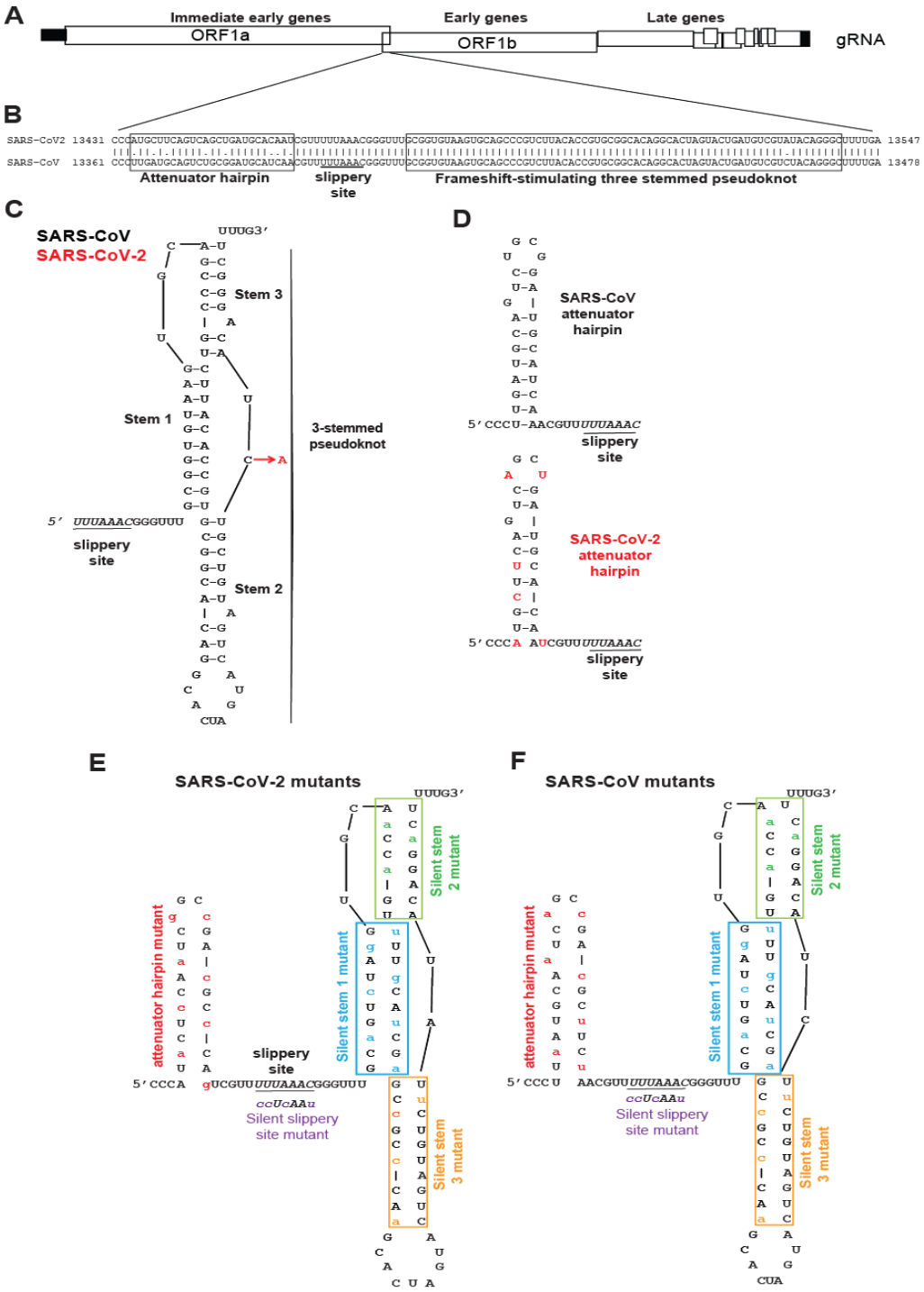
A
Immediate early genes
ORF1a
Early genes
ORF1b
Late genes
gRNA
B
SARS-CoV2 13431
SARS-CoV 13361
13547
13478
Attenuator hairpin
slippery site
Frameshift-stimulating three stemmed pseudoknot
C
SARS-CoV
SARS-CoV-2
Stem 3
Stem 1
Stem 2
3-stemmed pseudoknot
slippery site
D
SARS-CoV attenuator hairpin
slippery site
SARS-CoV-2 attenuator hairpin
slippery site
E
SARS-CoV-2 mutants
attenuator hairpin mutant
slippery site
Silent slippery site mutant
Silent stem 1 mutant
Silent stem 2 mutant
Silent stem 3 mutant
F
SARS-CoV mutants
attenuator hairpin mutant
Silent slippery site mutant
Silent stem 1 mutant
Silent stem 2 mutant
Silent stem 3 mutant

Fig 2. The structure of SARS-CoV-2. A) Cartoon who describe genome organization of SARS-CoV-2 including a -1 PRF. B) pairwise analysis of the –PRF signals. C) structur of the SARS-CoV -1PRF. D) comparison of SARS-CoV and SARS-CoV-2-1PRF elements. E and F) silent mutations in slippery side in the SARS-CoV2 and SARS-CoV-1PRF (figure adapted from Kelly et al, 2020) [47].

1.5 Small and large molecules who have possibilities to inhibit SARS-CoV-1PRF

Based on conservative sequence of the frameshift SARS-CoV-2, the possibilities to inhibit –1 PRF is only by small molecules. This is reported by Kelly et al, [47] when they tested the small molecule who have possibilities to bind with pseudoknot and suppress -1 PRF, 2-{[4-(2-methyl-thiazol-4-methyl)-[1,4]diazepane-1-carbonyl]-amino}-benzoic acid ethyl ester, hereafter denoted as MTDB [48, 49]. This provide concrete evidence for small-molecule frameshifting inhibitors in SARS-CoV-2 and support the hypothesis that the frameshift – stimulatory pseudoknot may be an attractive therapeutic target [42, 47]. The small compound FR6 referred as 1.3-dimethyl-6H-pyrrolo [3.4-d] pyrimidine-2.4-dione, have the affinity to complex with NSP9 COVID-19 and to accommodate orientation of motifs GxxxG [50].

Large molecule such as merafloxacin, belongs antibacterial compound known as fluoroquinolones [51]. Fluoroquinolones is tested for inhibition of -1 PRF, and it is shown the ability to inhibit the -1 PRF [52].

The modification of RNA is known to play pivotal role in different process in our organism. One form of modification is N6-methyladenosine (m6A) who control gene expression. The m6A modification is performed by a nuclear methyltransferase complex (METTL3/METTL14/WTAP) that include the essential methyltransferase-like enzyme 3 (METTL3) catalytic subunit [53]. m6A modification of RNA are recognized by a number of RNA-binding proteins, so called “the readers”, including nuclear YTHDC1 and three other paralogs who are placed in cytoplasm such as YTHDF1, YTHDF2, and YTHDF3 as well as other RNA binding proteins [54, 55]. Understanding of modification in this case m6A may have influence in future therapy opportunities to interfere in virus replication and spread of SARS-CoV-2 [56].

Oxidation is another type of modification which is shown to be involved in replication of genetic inheritance material in virus. Oxidation of iron-sulfur cluster by the stable nitroxide TEMPOL caused their disassembly, and inhibited the RdRp, and blocked SARS-CoV-2 replication in cell culture. These iron-sulfur cluster it is possibilities to serve as a target therapy of COVID-19 [57].

1.6 Non-structural protein as a target for cure of SARS-CoV-2

Non-structural protein gene is known to encode S protein. As we mention above there are a huge number of NSP, which play pivotal role during the infection of host cell. Some of NSP are involved in replication/transcription process, such as NSP7 and NSP8 [58-60]. In vitro is shown that NSP7, NSP8 and NSP12 are involved in in the RNA-dependent RNA polymerase (RdRp) activity [61]. But transcription of full genome is facilitated by NSP9 and NSP13 [24, 62, 63]. The coronavirus NSP9 is single strand RNA binding proteins, who can be repurposed to direct interface with nucleotidyltransferase or NiRNA, domain of NSP12 [64]. The NSP9 is reported to

have ability to insert into enzymatic site and to recruit NSP10 and NSP14 [65]. The NSP8 may bind to 7SL RNA discrete region and to involve in modification of host protein trafficking [66].

1.7 Treatment strategies for SARS-CoV-2

Till today they are not approved any specific drug for SARS-CoV-2 treatment. FDA- has approved some antiviral drugs, vaccines and immunotherapy already have being used to treat COVID-19. The molecular, structural, and functional relationships SARS-CoV-2 with SARS-CoV might define the use of existing anti-viral drugs against COVID-19 [67, 68]. The increasing knowledge of the genetic, immunological, and pathologic pathway might help in developing specific treatment approaches for COVID-19 in the future.

1.7.1 Vaccine design strategies

The number of researcher who are working hard to develop vaccine and to stop the pandemic situation caused by SARS-CoV-2. Development of vaccine is not easy task, as a huge number of clinical trials are required before approval for patients. The common strategies are used for develop of vaccine, such as monoclonal antibodies, virus vectors, protein vaccine, and DNA/RNA-vaccine [69-72].

An adenovirus vector-based vaccine candidate, is developed by Oxford University (AstraZeneca) for used against SARS-CoV-2, and has been reported both humoral and cell mediated immune response when tested in rhesus monkey [73]. Another group have used a recombinant adenovirus type 5 (Ad5-nCoV) and has been very effective in generating humoral and T-cell response post immunization [74]. Another type of vaccine is neutralization of specific regions such S1-NTD, or the S2 region and in this form came to blocking the interaction of virus with the receptors [75]. The monoclonal antibody has been identify, who target the conserved region S-RBD and came to effective neutralization of SARS-CoV-2 [76]. The new technologies of the microneedle array has been approved for delivering SARS-CoV-2 S1 subunit vaccine, which is very helpful in the treatment of patients infected with SARS-CoV-2 [77].

2 Conclusion

Considering the current situation more than 8 million of people has been infected, and number of death every day is going to be higher and higher. There is an urgent need to control the SARS-CoV-2 pandemic. The government authorities in every country have approved guidelines and take action of quarantines for infected people, to break the spread infection in the community. Different antibodies, vaccines, and potential drugs could be used for treatment of infected people. Combination of different drugs can be used for neutralization of -1 PRF or S protein. Among different antiviral compound has been approved by FDA, such as chloroquine/ hydroxychloroquine has shown to have good outcomes in infected patients.

References

1. Tyrrell, DA., Bynoe, ML.: Cultivation of viruses from a high proportion of patients with colds. Lancet (1966), 108, 257. [CrossRef]
2. Hamre, D., Procknow, JJ.: A new virus isolated from the human respiratory tract. Proc. Soc. Exp. Biol. Med. (1966), 121, 190. [CrossRef]
3. Temaj, G., Xharra, K., Xharra, Sh., Moder, A., et al.: "Pharmacogenetics-gene and SARS-COVID 19 Medication" (2020). UBT International Conference. (2020) 250, 25-32.
4. Spahiu, L., Berisha, E., Behluli, E., Nefic, H., Hadziselimovic, R., Temaj, G.: CoVID-19 Implications at the Clinical University Center in Kosovo-Department of Pediatrics. Arch Med Sci Civil Dis (2021), 6, e61–e67
5. de Wit, E., van Doremalen, N., Falzarano, D., Munster, VJ.: SARS and MERS: recent insights into emerging coronaviruses. Nat Rev Microbiol. (2016) 14:523–34.
6. Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., et al.: A novel coronavirus from patients with pneumonia in China, 2019. N Engl JMed. (2020) 382:727–33.
7. Schoeman, D., Fielding, BC.: Coronavirus envelope protein: current knowledge. Virol J. (2019) 16:1–22. doi: 10.1186/s12985-019-1182-0
8. Lim, Y., Ng, Y., Tam, J., Liu, D.: Human coronaviruses: a review of virus–host interactions. Diseases. (2016) 4:26.
9. Zhou, P., Yang, XL., Wang, XG., Hu, B., Zhang, L., Zhang, W., et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. (2020) 579:270–3.
10. Cui, J., Li, F., Shi, ZL.: Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol. (2019) 17:181–92.
11. Song, Z., Xu, Y., Bao, L., Zhang, L., Yu, P., Qu, Y., et al.: From SARS to MERS, thrusting coronaviruses into the spotlight. Viruses. (2019) 11:59.
12. Temaj, G., Xharra, K., Xharra, Sh., Moder, A., et al.: "Investigational Anti SARS-COVID 19 Medication" (2020). *UBT International Conference*. (2020) 295, 9-24.
13. Schmidt, N., Lareau, CA., Keshishian, H., Ganskih, S., et al.: The SARS CoV2 RNA protein interactome in infected human cells. Nat Microbiol. (2021) 6(3), 339-353
14. Wu, A., Peng, Y., Huang, B., Ding, X., Wang, X., Niu, P., et al.: Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China. Cell Host Microbe. (2020) 27:325–8.

15. Angeletti, S., Benvenuto, D., Bianchi, M., Giovanetti, M., Pascarella, S., Ciccozzi, M.: COVID-2019: The role of the nsp2 and nsp3 in its pathogenesis. JMed Virol. (2020) 92:1–5.
16. Simmons, G., Reeves, JD., Rennekamp, AJ., Amberg, SM., Piefer, AJ., Bates, P.: Characterization of severe acute respiratory syndrome-associated coronavirus (SARS-CoV) spike glycoprotein-mediated viral entry. Proc Natl Acad Sci USA. (2004) 101:4240–5.
17. Xu, J., Zhao, S., Teng, T., Abdalla, AE., Zhu, W., Xie, L., Wang, Y., Guo, X.: Systematic comparison of two animal-to-human transmitted human coronaviruses: SARS-CoV-2 and SARS-CoV. Viruses (2020), 12, 244. [CrossRef]
18. Li, W., Moore, MJ., Vasilieva, N., Sui, J., Wong, SK., Berne, MA., Somasundaran, M., Sullivan, JL., Luzuriage, K., Greenough, TC., et al.: Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature (2003), 426, 450. [CrossRef]
19. Anand, K., Ziebuhr, J., Wadhwani, P., Mesters, JR., Hilgenfeld, R.: Coronavirus main proteinase (3CLpro) structure: Basis for design of anti-SARS drugs. Science (2003), 300, 1763. [CrossRef]
20. Pillaiyar, T., Manickam, M., Namasivayam, V., Hayashi, Y., Jung, SH.: An overview of severe acute respiratory syndrome-coronavirus (SARS-CoV) 3CL protease inhibitors: Peptidomimetics and small molecule chemotherapy. J. Med. Chem. (2016), 59, 6595. [CrossRef]
21. Rota, PA., Oberste, MS., Monroe, SS., Nix, WA., Campagnoli, R., Icenogle, JP., Penaranda, S., Bankamp, B., Maher, K., Chem, MH., et al.: Characterization of a novel coronavirus associated with severe acute respiratory syndrome. Science (2003), 300, 1394. [CrossRef] [PubMed]
22. Taguchi, F., Kubo, H., Suzuki, H., Yamada, YK.: Localization of neutralizing epitopes and receptor-binding site in murine coronavirus spike protein. Adv Exp Med Biol. (1995) 380:359–65.
23. Cheng, PKC., Wong, DA., Tong, LKL., Ip, SM., Lo, ACT., Lau, CS., et al.: Viral shedding patterns of coronavirus in patients with probable severe acute respiratory syndrome. Lancet. (2004) 363:1699–700.
24. Brockway, SM., Clay, CT., Lu, XT., Denison, MR.: Characterization of the expression, intracellular localization, and replication complex association of the putative mouse hepatitis virus RNA-dependent RNA polymerase. J Virol. (2003) 77:10515–27.
25. Sawicki, SG., Sawicki, DL.: Coronavirus transcription: a perspective. Curr Top Microbiol Immunol. (2005) 287:31–55.
26. Adriaan, H., de Wilde, Eri,c J., Snijder, MK., van HMJ.: Host factors in coronavirus replication. Curr Top Microbiol Immunol. (2018) 419:1–42.
27. Perrier, A., Bonnin, A., Desmarets, L., Danneels, A., Goffard, A., Rouillé, Y., et al.: The C-terminal domain of the MERS coronavirus M protein contains a trans-Golgi network localization signal. J Biol Chem. (2019) 294:14406–21.

28. Yan, R., Zhang, Y., Li, Y., Xia, L., Guo, Y., Zhou, Q.: Structural basis for the recognition of the SARS-CoV-2 by full-length human ACE2. Science. (2020) 2:1444–8.
29. Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., et al.: SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell. (2020) 181:1–10.
30. Walls, AC., Park, YJ., Tortorici, MA., Wall, A., McGuire, AT., Veesler, D.: Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell. (2020) 180:281–92.
31. Sungnak, W., Huang, N., Bécavin, C., Berg, M., Queen, R., Litvinukova, M., et al.: SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. Nat Med. (2020) 26:681–7.
32. Prompetchara, E., Ketloy, C., Palaga, T.: Immune responses in COVID-19 and potential vaccines: lessons learned from SARS and MERS epidemic. Asian Pacific J allergy Immunol. (2020) 38:1–9.
33. CDC. People Who Are at Higher Risk for Severe Illness | Coronavirus | COVID-19. (2020). Available online at: https://www.cdc.gov/coronavirus/2019-ncov/need-extra-precautions/groups-at-higher-risk.html (accessed April 7, 2020).
34. Mazzulli, T., Farcas, GA., Poutanen, SM., Willey, BM., Low, DE., Butany, J., et al.: Severe acute respiratory syndrome-associated coronavirus in lung tissue. Emerg Infect Dis. (2004) 10:20–4.
35. Gu, J., Gong, E., Zhang, B., Zheng, J., Gao, Z., Zhong, Y., et al. Multiple organ infection and the pathogenesis of SARS. J Exp Med. (2005) 202:415–24.
36. Centis, R., Sc, M.: SARS-CoV-2 Infection in Children. N Engl J Med. (2020) 382:4–6.
37. Zhang, Z., Guo, L., Lu, X., Zhang, C., Wang, X., Huang, L., et al.: Vulnerability of children with COVID-19 infection and ACE2 profiles in lungs. SSRN. (2020) 1:1–18.
38. Zhu, L., Lu, X., Chen, L.: Possible causes for decreased susceptibility of children to coronavirus. Pediatr Res. (2020) 145:2020.
39. Plant, EP., Pérez-Alvarado, GC., Jacobs, JL., Mukhopadhyay, B., Hennig, M., Dinman, J.D.: A three-stemmed mRNA pseudoknot in the SARS coronavirus frameshift signal. PLoS Biol., (2005), 3, e172.
40. Plant, EP., Rakauskaitė, R., Taylor, DR., Dinman, JD.: Achieving a golden mean: mechanisms by which coronaviruses ensure synthesis of the correct stoichiometric ratios of viral proteins. J. Virol., (2010), 84, 4330–4340.
41. Plant, EP., Sims, AC., Baric, RS., Dinman, JD., Taylor, DR.: Altering SARS coronavirus frameshift efficiency affects genomic and subgenomic RNA production. Viruses, (2013), 5, 279–294.
42. Neupane, K., Munshi, S., Zhao, M., Ritchie, DB., Ileperuma, SM., Woodside, MT.:
Anti-Frameshifting Ligand Active against SARS Coronavirus-2 Is Resistant

to Natural Mutations of the Frameshift-Stimulatory Pseudoknot. J Mol Biol. (2020), 432(21), 5843-5847.

43. Ahn, DG., Lee, W., Choi, JK., Kim, SJ., Plant, EP., Almazán, F., Taylor, DR., Enjuanes, L., et al.: Interference of ribosomal frameshifting by antisense peptide nucleic acids suppresses SARS coronavirus replication. Antivir. Res., (2011), 91, 1–10.
44. Rangan, R., Zheludev, I.N., Das, R.: RNA genome conservation and secondary structure in SARS-CoV-2 and SARS-related viruses: a first look. RNA, (2020), 26, 937–959.
45. Elbe, S., Buckland-Merrett, G.: Data, disease and diplomacy: GISAID's innovative contribution to global health. Global Chall., (2017), 1, 33–46.
46. Plant, EP., Pérez-Alvarado, GC., Jacobs JL., Mukhopadhyay, B., Hennig, M., and Dinman JD.: A three-stemmed mRNA pseudoknot in the SARS coronavirus frameshift signal. *PLoS Biol*. (2005), 3, e172 10.1371/journal.pbio.0030172
47. Kelly, JA., Olson, AN., Neupane, K., Munshi, S., San Emeterio, J., Pollack, L., Woodside, MT., Dinman, JD.: Structural and functional conservation of the programmed -1 ribosomal frameshift signal of SARS coronavirus 2 (SARS-CoV-2). J Biol Chem. (2020), 295(31), 10741-10748
48. Park, SJ., Kim, YG., Park, HJ.: Identification of RNA pseudoknot-binding ligand that inhibits the -1 ribosomal frameshifting of SARS-coronavirus by structure-based virtual screening. *J.Am.Chem.Soc. (2011)*, **133**, 10094–10100
49. Ritchie, DB., Soong, J., Sikkema, WKA., Woodside, MT.: Anti-frameshifting Ligand Reduces the Conformational Plasticity of the SARS Virus Pseudoknot. *J. Am. Chem. Soc. (2014),* **136**, 2196–2199.
50. Littler, DR., Gully, BS., Colson, RN., Rossjohn, J.: Crystal Structure of the SARS-CoV-2 Nonstructural Protein 9, Nsp9. *iScience, (2020), 23, 101258*
51. Hooper, DC., Jacoby, GA.: Topoisomerase Inhibitors: Fluoroquinolone Mechanisms of Action and Resistance. Cold Spring Harb Perspect Med (2016), *6*.
52. Sun, Y., Abriola, L., Surovtseva, YV., Lindenbach, BD., Guo, JU.: Restriction of SARS-CoV-2 Replication by Targeting Programmed -1 Ribosomal Frameshifting In Vitro. bioRxiv. (2020), 21, 2020.10.21.349225.
53. Schöller, E., Weichmann, F., Treiber, T., Ringle, S., Treiber, N., Flatley, A., Feederle, R., Bruckmann, A., Meister, G.: Interactions, localization, and phosphorylation of the m6 A generating METTL3-METTL14-WTAP complex. RNA (2018), 24, 499–512. doi:10.1261/rna.064063.117
54. Lasman, L., Krupalnik, V., Viukov, S., Mor, N., Aguilera-Castrejon, A., Schneir, D., Bayerl, J., Mizrahi, O., Peles, S., Tawil, S, et al.: Context-dependent functional compensation between Ythdf m6 A reader proteins. Genes Dev (2020), 34: 1373–1391.

55. Zaccara, S., Jaffrey, SR.: A unified model for the function of YTHDF proteins in regulating m6 A-modified mRNA. Cell (2020), 181: 1582–1595.e18. doi:10.1016/j.cell.2020.05.012

56. Burgess, HM., Depledge, DP., Thompson, L., Srinivas, KP., Grande, RC., Vink, EI., Abebe, JS., Blackaby, WP., Hendrick, A., Albertella, MR., Kouzarides, T., Stapleford, KA., Wilson, AC., Mohr, I. Targeting the m^6A RNA modification pathway blocks SARS-CoV-2 and HCoV-OC43 replication. Genes Dev. (2021),35(13-14), 1005-1019.

57. Maio, N., Lafont, BAP., Sil ,D., Li, Y., Bollinger, JM Jr., Krebs, C., Pierson, TC., Linehan, WM., Rouault, TA.: Fe-S cofactors in the SARS-CoV-2 RNA-dependent RNA polymerase are potential antiviral targets. Science. (2021), 373(6551), 236-241.

58. Sola, I., Almazan, F., Zuniga, S., and Enjuanes, L.: Continuous and Discontinuous RNA Synthesis in Coronaviruses. *Annu Rev Virol (2015)*, **2**, 265-288

59. Snijder, E. J., Decroly, E., Ziebuhr, J.: The Nonstructural Proteins Directing Coronavirus RNA Synthesis and Processing. *Adv Virus Res (2016)*, **96**, 59-126

60. Kirchdoerfer, R. N., Ward, A. B.: Structure of the SARS-CoV nsp12 polymerase bound to nsp7 and nsp8 co-factors. *Nat Commun (2019)*, **10**, 2342

61. Yin, W., Mao, C., Luan, X., Shen, D. D., Shen, Q., Su, H., Wang, X., Zhou, F., Zhao, W., Gao, M., Chang, S., Xie, YC., Tian, G., Jiang, H. W., Tao, SC., Shen, J., Jiang, Y., Jiang, H., Xu, Y., Zhang, S., Zhang, Y., and Xu, HE.: Structural basis for inhibition of the RNA-dependent RNA polymerase from SARS-CoV-2 by rem remdesivir. *Science (2020)*, **368**, 1499- 1504

62. Chen, J., Malone, B., Llewellyn, E., Grasso, M., Shelton, PMM., Olinares, PDB., Maruthi, K., Eng, ET., Vatandaslar, H., Chait, BT., Kapoor, TM., Darst, SA., and Campbell, EA.: Structural Basis for Helicase-Polymerase Coupling in the SARS-CoV-2 Replication-Transcription Complex. *Cell (2020)*, **182**, 1560-1573 e1513

63. Ivanov, KA., Thiel, V., Dobbe, JC., van der Meer, Y., Snijder, EJ., and Ziebuhr, J.: Multiple enzymatic activities associated with severe acute respiratory syndrome coronavirus helicase. *J Virol (2004)*, **78**, 5619-5632

64. Yan, L., Ge, J., Zheng, L., Zhang, Y., Gao, Y., Wang, T., Huang, Y., Yang, Y., Gao, S., Li, M., Liu, Z., Wang, H., Li, Y., Chen, Y., Guddat, L. W., Wang, Q., Rao, Z., Lou, Z.: Cryo-EM Structure of an Extended SARS-CoV-2 Replication and Transcription Complex Reveals an Intermediate State in Cap Synthesis. *Cell (2021)*, **184**, 184-193 e110

65. Yan, L., Yang, Y., Li, M., Zhang, Y., Zheng, L., Ge, J., Huang, Y. C., Liu, Z., Wang, T., Gao, S., Zhang, R., Huang, YY., Guddat, LW., Gao, Y., Rao, Z., Lou, Z.: Coupling of N7- methyltransferase and 3'-5' exoribonuclease

with SARS-CoV-2 polymerase reveals mechanisms for capping and proofreading. *Cell (2021)*, **184**, 3474-3485 e3411

66. Banerjee, AK., Blanco, MR., Bruce, EA., Honson, DD., Chen, LM., Chow, A., Bhat, P., Ollikainen, N., Quinodoz, SA., Loney, C., Thai, J., Miller, ZD., Lin, A. E., Schmidt, MM., Stewart, DG., Goldfarb, D., De Lorenzo, G., Rihn, SJ., Voorhees, RM., Botten, JW., Majumdar, D., Guttman, M.: SARS-CoV-2 Disrupts Splicing, Translation, and Protein Trafficking to Suppress Host Defenses. *Cell (2020)*, **183**, 1325-1339 e1321
67. Groneberg, DA., Hilgenfeld, R., Zabel, P.: Molecular mechanisms of severe acute respiratory syndrome (SARS). Respir Res. (2005) 6:1–16.
68. Wan, Y., Shang, J., Graham, R, Baric, RS., Li F.: Receptor recognition by the novel coronavirus fromWuhan: an analysis based on decade-long structural studies of SARS coronavirus. J Virol. (2020) 94:1–9.
69. Roper, RL., Rehm, KE.: SARS vaccines: where are we? Expert Rev Vaccines. (2009) 8:887–98.
70. Ishii, K., Hasegawa, H., Nagata, N., Ami, Y., Fukushi, S., Taguchi, F., et al.: Neutralizing antibody against severe acute respiratory syndrome (SARS)-coronavirus spike is highly effective for the protection of mice in the murine SARS model. Microbiol Immunol. (2009), 53,75–82.
71. Guo, JP., Petric, M., Campbell, W., McGeer, PL.: SARS corona virus peptides recognized by antibodies in the sera of convalescent cases. Virology. (2004), 324, 251–6.
72. Tsunetsugu-Yokota, Y., Ohnishi, K., Takemori, T.: Severe acute respiratory syndrome (SARS) coronavirus: application of monoclonal antibodies and development of an effective vaccine. Rev Med Virol. (2006), 16, 117–31.
73. van Doremalen, N., Lambe, T., Spencer, A., Belij-Rammerstorfer, S., Purushotham, JN., Port, JR., et al.: ChAdOx1 nCoV-19 vaccination prevents SARS-CoV-2 pneumonia in rhesus macaques. bioRxiv. (2020), 2020, 05.13.093195.
74. Zhu, FC., Li, YH., Guan, XH., Hou, LH., Wang, WJ., Li, JX., et al.: Safety, tolerability, and immunogenicity of a recombinant adenovirus type-5 vectored COVID-19 vaccine: a dose-escalation, open-label, non-randomised, first-in-human trial. Lancet. (2020), 6736, 1–10.
75. Jiang, S., Hillyer, C., Du, L.: Neutralizing antibodies against SARS-CoV-2 and other human coronaviruses. Trends Immunol. (2020), 41, 355 -9.
76. Wang, C., Li, W., Drabek, D., Okba, NMA., Haperen, R Van., Osterhaus, ADME., et al.: A human monoclonal antibody blocking SARS-CoV-2 infection. Nat Commun. (2020), 11, 1–6.
77. Kim, E., Erdos, G., Huang, S., Kenniston, TW., Balmert, SC., Donahue Carey, C., et al.: Microneedle array delivered recombinant coronavirus vaccines: immunogenicity and rapid translational development. EBioMedicine. (2020), 55, 102743.