How realistic is it to develop a small molecule therapy for Covid-19? Could such a therapy be rolled out in a timeframe that it could have an impact on the current pandemic?

A cruel irony. The fact that something so invisible, so lifeless has eliminated millions of lives around the world in the space of a couple of years is nothing but a cruel irony. But is this so-called 'invisible' enemy invincible too? Or can this virus that brought unparalleled misery, death, social isolation and economic distress to millions if not billions of people around the world be overcome by something even more 'invisible' than it?

Small molecules, big potential

For nearly a century, small molecule drugs – defined as artificially-synthesised compounds comprised of just 20-100 atoms (1) – have been the pillar of the pharmaceutical industry to the extent that in the present day and age, practically all traditional drugs and more than 90% of therapeutic agents are derived from small molecules. (2) Due to their particularly low molecular weight, small molecules possess an advantage over other medicines, as they can penetrate cell membranes, target intracellular enzymes and receptors and inhibit their activity through the formation of complexes. (3) For this reason, small molecules could potentially be employed as medication for Covid-19, by inhibiting the action of the SARS-CoV-2 virus.

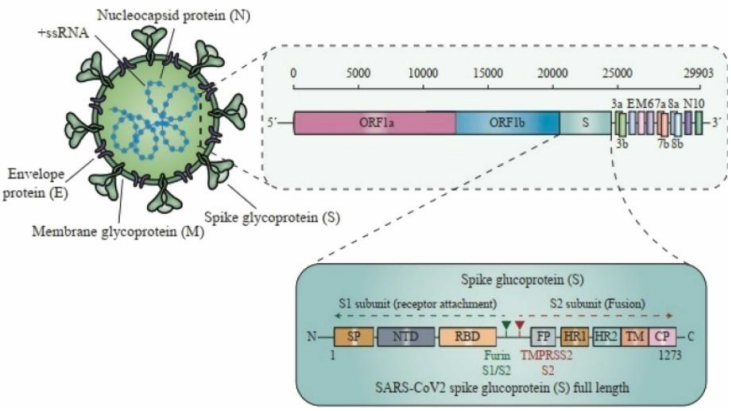

Figure 1: Diagram showing structure and genome of SARS-CoV-2 *(25)*

How SARS-CoV-2 hijacks host: entry and replication

Before discussing the possible methods for Covid-19 treatment through small molecule therapy, the pathogenesis of the disease must be understood.

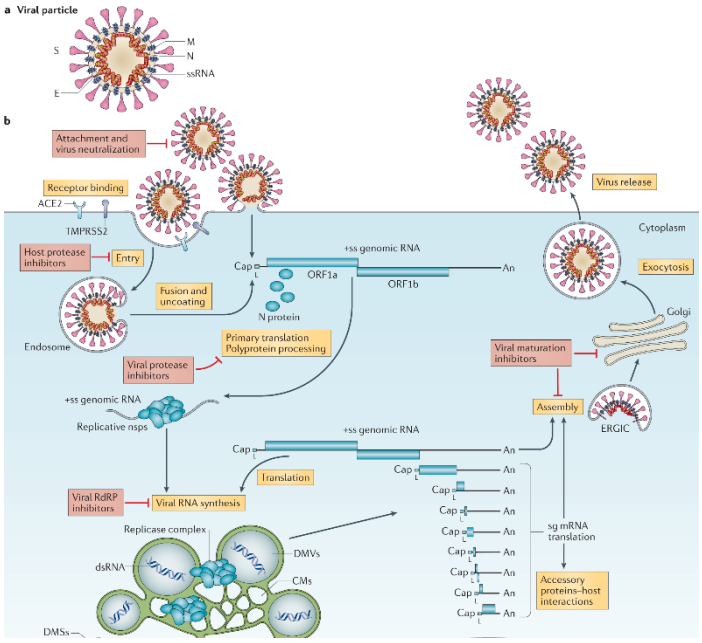

Figure 2: Entry and replication of the virus *(7)*

First discovered in Wuhan, China in December 2019, SARS-CoV-2 is a virus that belongs to the highly diverse *Coronaviridae* family of positive-sense ssRNA viruses (4). It causes the disease Covid-19, which is still ravaging across the world causing severe respiratory illness, multi-system organ failure and in many unfortunate cases even death. On February 19, 2022, there were just over 416.6 million cases, with almost 5.9 million confirmed deaths globally. (5)

Four major structural proteins make up the SARS-CoV-2 virion: the envelope (E), nucleocapsid (N), membrane (M), and spike (S). The S protein is arguably the most crucial determinant in viral infection and is composed of 2 subunits, shown in Fig.1 as S1 and S2, of which S1 facilitates receptor-binding to ACE2 receptors –prevalent in

pneumocytes in the respiratory tract– and S2 aids fusion with the cell membrane. As well as ACE2 receptors, the host cell contains TMPRSS2 receptors – a serine protease that can cleave (or split) the spike protein (6) at the S1/S2 cleavage site, allowing the virus to infect the host cell, as well as effectively proliferate throughout the host organism. Once inside the cell, viral genomic RNA (gRNA) is released from the virion into the cytosol, in preparation for transcription and translation at host ribosomes. Translation of ORF1a and ORF1b genetic code (illustrated in Fig.1) into the replicase polyproteins pp1a and pp1ab, is crucial for the assembly of new viral genomes, as these are further cleaved into 16 non-structural proteins (nsps) (7) for the construction of replication–transcription complexes (RTCs). These platforms are responsible for producing new gRNAs and sg-RNAs, including ORFs 2–9b which code for the S, E, M and N proteins of the new virions. (8) After coating the new gRNAs with N proteins, the structures 'bud' into the ERGIC (endoplasmic reticulum Golgi intermediate compartment), where it gains its final components: M proteins and the lipid envelope with S proteins (7), which the virus will use to infect more and more cells, making us progressively more and more sick.

Though this process seems highly formidable, there is some room for error, which can be targeted.

Targeting the enemy: the feasibility of small molecule therapy for Covid-19

Small molecules can certainly be rolled out as treatments for Covid-19 – in fact, this had already happened in 2020. The primary concern is not so much being able to develop and administer these in time, but rather to do so without compromising on efficacy. At present, at least three distinct categories of inhibitory drugs– RdRp, non-structural protein and host-targeting inhibitors –are valid to act as a treatment for this respiratory disease.

RdRp inhibitors: RdRp enzyme

As previously seen, after infecting a host cell, SARS-CoV-2 must replicate its gRNAs and consequently, transcribe and translate essential viral proteins. For members of the coronavirus family to successfully do so, they rely on an abnormally extensive set of RNA-synthesising replicase enzymes, RdRp (RNA-dependent RNA polymerase) being the primary enzyme responsible for this. (6) These enzymes are made up of three nsps: nsp12, the core catalytic unit and the cofactors nsp8 and nsp7 (9) and are solely responsible for the catalysis of phosphodiester bond formation between ribonucleotides and in so doing, synthesising new RNA strands. Since RdRp plays such a crucial role in the production of new virus particles, it is viable to be a target for inhibition through small molecule drugs.

RdRp inhibitors: mechanism

Two classes of inhibitors are currently known: nucleoside (NA) and non-nucleoside analogue (NNA) drugs. The difference between the two lies in the location where they act upon: NAs bind to RdRp active sites, whereas NNAs do so at allosteric sites. NAs are generally preferred over their non-nucleoside counterparts, as they act directly on the active sites of the polymerase and are not as prone to drug resistance as NNAs are.

A couple of the leading RdRp NAs are remdesivir and molnupiravir, both of which are prodrugs that metabolise and go under phosphorylation to form a triphosphate, which brilliantly mimics the natural nucleoside triphosphate (NTP) molecules that the polymerase binds to during replication. The two

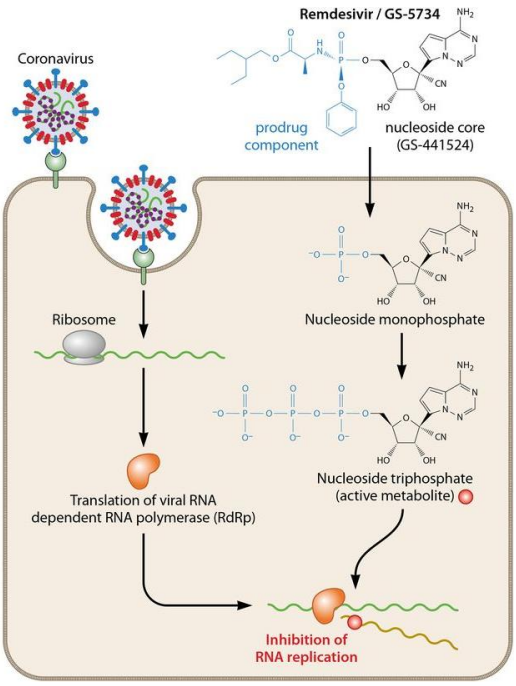

Figure 3: action of remdesivir on viral replication *(24)*

molecules share so much similarity that the difference between them seems to pass by unnoticed by the proofreading nsp14 in this highly error-prone enzyme (10). In essence, a weakness in the virus makes our ability to synthesise small molecule drugs for Covid treatment stronger. The nucleoside analogue drug targets the enzyme at its active site and is incorporated into the nascent RNA chain (11), resulting in chain termination or deadly mutations, thereby stalling further replication. (12)

Remdesivir

Remdesivir (or G5-5734) is the single FDA-approved drug fit for the treatment of Covid-19. After being initially introduced for the Ebola epidemic, this product was shown to decrease viral load and pulmonary damage in rhesus macaques with Covid-19 (13) and was therefore considered suitable for human treatment. The active form of remdesivir (RTP) acts as a nucleoside analogue inhibitor and is incorporated into the emerging RNA product. As a result of its highly similar structure, this small molecule evades the proofreading action of viral exonuclease (nsp14), allowing it to add just three more ribonucleotides before RNA synthesis is terminated.

The approval of remdesivir, however, was rushed: it was based on data from small samples consisting of just 1,000 participants. After a larger scale study – the Solidarity trial - conducted by the WHO, it was confirmed that in fact, remdesivir had little to no effect on hospitalised patients with Covid-19, proving it to be ineffective. (14)

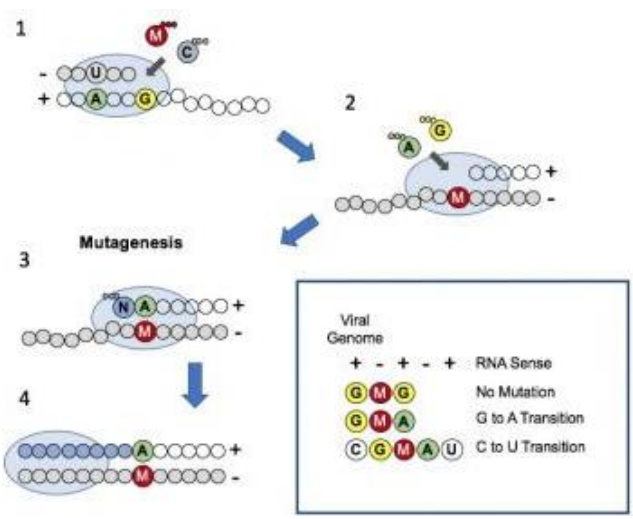

Figure 4: How molnupiravir causes mutagenesis *(26)*

Molnupiravir

Molnupiravir (or EIDD-2801) is another NA inhibitor drug, which works in a slightly different way to remdesivir: instead of cutting the RNA sequence short, it mimics the natural analogue cytidine and causes base-pair misinterpretation, thereby increasing the frequency of 'lethal mutagenesis' and impairing viral replication in mammals. (15) Similarly to remdesivir, molnupiravir evades the coronavirus nsp14 exonuclease proofreading, meaning that it can easily be incorporated into the RNA strand and severely impede the viral replication mechanism. Fig.4 depicts molnupiravir competing with cytidine for incorporation during RNA synthesis and once incorporated, the drug forms complementary base pairs with either ATP or GTP: ATP-pairing results in mutagenesis – the emergence of (fatal) mutations.

A recent study by Hetero has discovered that, unlike remdesivir, molnupiravir "reduced hospitalisation by 65% in patients with mild cases of Covid-19" (16), which suggests that there is still hope for this drug and RdRp inhibitors.

Non-structural protein inhibitors

The fate of small molecule drugs as potential anti-SARS-CoV-2 agents does not rest upon RdRp inhibitors alone, though: the use of nsp inhibitors might also be achievable.

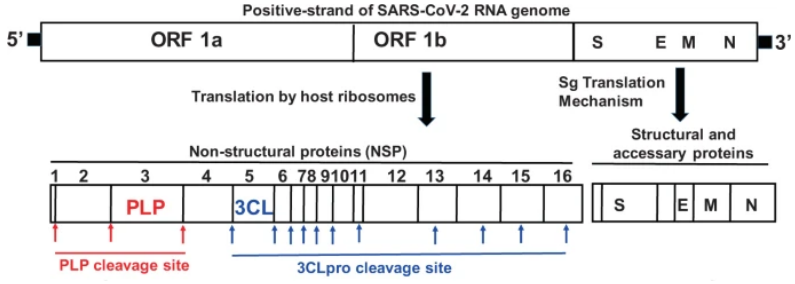

Figure 5: SARS-CoV-2 genome (arrows represent protease cleavage sites) *(27)*

As explored earlier, once the coronavirus enters and takes over protein synthesis mechanisms in the host cell, it creates the polyproteins pp1a and pp1ab, which are then cleaved at 16 cleavage sites to create 16 nsps by 2 cysteine proteases: a papain-like protease (nsp3 or PL^{pro}) and 3-chymotrypsin-like proteases (nsp5, M^{pro} or $3CL^{pro}$). Out of these 16 nsps, utilised by the virus as enzymes or RTCs, 11 are created by $3CL^{pro}$ (17), therefore this protease is critical for replicase processing and is an attractive target for Covid treatment.

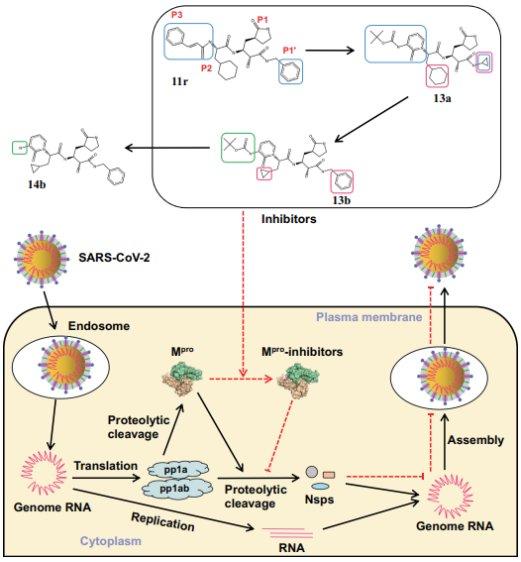

Figure 6: M^{pro} inhibitory mechanism *(18)*

Non-structural protein inhibitors: α-ketoamide inhibitors

Small molecule drugs that specifically bind to and inhibit SARS-CoV-2 M^{pro} are promising potential treatments for Covid. The main protease of the virus has a crystalline structure, which allows for easy molecular docking of modified α-ketoamide inhibitors (shown as 13a in Fig.6) – these are peptidomimetic inhibitors: peptide analogues that can imitate the functionality of natural peptide substrates of the virus (19). They form a non-covalent complex with the peptide binding site to competitively inhibit nsp5 activity, hence preventing the cleavage of the polyproteins, the formation of the RTC and essential enzymes for gRNA replication.

HTA inhibitors and mechanism

Direct-acting antiviral agents (DAAs), the category in which previously discussed NAs lie, and host-targeting antivirals (HTAs) are the main two types of broad-spectrum antiviral drugs in modern medicine. Although DAAs do have their benefits, they usually take a long time to develop and can cause cytotoxicity and drug resistance; consequently, HTAs are generally seen as more effective against the effects of viruses, as they enhance the immune response to pathogens by targeting virulence factors and reduce organ damage and mortality.

Corticosteroids are a significant type of HTA inhibitor and include medication like dexamethasone: a drug that was trialled in the UK during the RECOVERY study (2020) and was proven to “cut deaths by about one-third in patients who were on ventilators because of coronavirus infection” (20). Drugs like this work by activating ACE2 expression, which is rapidly diminished when SARS-CoV-2 invades the body, affecting the respiratory and cardiovascular system; when corticosteroids are taken, ACE2 expression is restored, thus minimising organ failure.

<u>Ignorance is *not* bliss: challenges of small molecule drugs</u>

As the synthesis and mechanism of action seem to be known, one may expect the administration of small molecule drugs to be straightforward, but the truth is that much remains unknown.

Firstly, the drug must be tailormade to its target; a slight error in specificity could render the drug ineffective. This is particularly the case when considering RdRp inhibitors, for instance, that must be so alike natural nucleosides that they can fool the proofreading system of the virus, but still be different enough so that these modified NAs don’t accomplish the same in human enzymes, also dependent on nucleosides. With regards to the development of protease inhibitors (like M^{pro}), this is also a difficult ordeal, as several proteases exist in human cells and it would be fearfully detrimental to our health if the drugs targeted these instead of the virus.

Moreover, since Covid is still a new disease, a lot of information, such as the dose and dosing regimen, of upcoming small molecule therapies is unknown; this would usually be established in phase 2 clinical

trials by observing the PK and PD of the drug or skipped entirely if the optimal dosing regimen is known. This is not possible for COVID-19. Furthermore, as we do not know how SARS-CoV-2 behaves in terms of its viral dynamics, it is difficult to determine the optimal treatment windows, possibly leading to inaccurate conclusions.

Ironically, another reason why the drug development process is taking so much time is time itself (or the lack of it). Conventionally, it takes a matter of decades for drug research, discovery and testing, however, with the unprecedented arrival of Covid, this lengthy but critical process is a drawback. For a drug to be approved by associations like the FDA, it must pass several trials, the 'gold standard' of them all being Randomised Controlled Trials (RCTs), which require vast sample sizes and must run for a long time. The drug remdesivir had not undergone this type of trial before gaining approval from the FDA in October 2020: instead, it had passed test-tube and mice studies and then larger-scale placebo studies, but it did not show any effect on patients in the much larger trial conducted by the WHO. This trial involved 405 hospitals from 30 countries of different socio-economic statuses (14) and revealed that in fact, remdesivir did not affect the duration of hospitalisation and was confirmed to be impractical.

Small molecule drug development in a short timescale is certainly not an issue, as we have seen. The issue is being able to deliver these drugs in this short period while also ensuring that they would have a beneficial impact. The best way to do this is to first put the drugs through valid, representative studies and trials, and then to ensure key medical institutions (such as the FDA) are transparent with their data and decisions about the distribution of the medication. For these minute molecules to have an immense impact on the ever-evolving dynamics of the pandemic, taking these steps is simply imperative.

Bibliography

All accessed: 19/02/2022

1. **AZBio.** Small Molecules, Large Biologics and the Biosimilar Debate. *AZBio.* [Online] AZBio, February 18, 2020. https://www.azbio.org/small-molecules-large-biologics-and-the-biosimilar-debate.
2. *The druggable genome.* **Hopkins AL, Groom CR.** s.l.: Nature Reviews Drug Discovery, 2002, Vol. 1. https://doi.org/10.1038/nrd892.
3. *Small molecules, big targets: Drug discovery faces the protein-protein interaction challenge.* **Scott D.E., Bayly A.R., Abell C., Skidmore J.** s.l.: Nature Reviews Drug Discovery, 2016, Vol. 15.
4. *The species severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2.* **Coronaviridae Study Group of the International Committee on Taxonomy of Viruses.** s.l.: Nature Microbiology, 2020, Vol. 5. https://doi.org/10.1038/s41564-020-0695-z.
5. **World Health Organisation.** WHO Coronavirus (COVID-19) Dashboard: Overview. *WHO Coronavirus (COVID-19) Dashboard.* [Online] WHO. https://covid19.who.int/.
6. *RNA-dependent RNA polymerase: Structure, mechanism, and drug discovery for COVID-19.* **Yi Jiang, Wanchao Yin, H. Eric Xu.** s.l.: Biochemical and Biophysical Research Communications, 2021, Vol. 538. https://doi.org/10.1016/j.bbrc.2020.08.116.
7. *Coronavirus biology and replication: implications for SARS-CoV-2.* **V'kovski, P., Kratzel, A., Steiner, S. et al.** s.l.: Nat Rev Microbiology, 2021, Vol. 19. https://doi.org/10.1038/s41579-020-00468-6.
8. *Coronavirus envelope protein: current knowledge.* **Schoeman, D., Fielding, B.C.** 69, s.l.: Virol J, 2019, Vol. 16. https://doi.org/10.1186/s12985-019-1182-0.
9. *Structure of the RNA-dependent RNA polymerase from COVID-19 virus.* **Gao Y, Yan L, Huang Y, et al.** 6492, s.l.: Science (New York, N.Y.), 2020, Vol. 368. https://doi.org/10.1126/science.abb7498.

10. *Structural and Functional Basis of the Fidelity of Nucleotide Selection by Flavivirus RNA-Dependent RNA Polymerases.* **Selisko, B., et al.** 59, s.l.: Viruses, 2018, Vol. 10. https://doi.org/10.3390/v10020059.
11. *Nucleoside inhibitors of human immunodeficiency virus type 1 reverse transcriptase.* **Sharma P.L., Nurpeisov V., Hernandez-Santiago B., Beltran T., Schinazi R.F.** 9, s.l.: Curr. Top. Med. Chem, 2004, Vol. 4.
https://doi.org/10.2174/1568026043388484.
12. *Mechanism of SARS-CoV-2 polymerase stalling by remdesivir.* **Kokic, G., Hillen, H.S., Tegunov, D. et al.** 279, s.l.: Nat Comm, 2021, Vol. 12.
https://doi.org/10.1038/s41467-020-20542-0.
13. *Clinical benefit of remdesivir in rhesus macaques infected with SARS-CoV-2.* **Williamson, B.N., Feldmann, F., Schwarz, B. et al.** 7824, s.l.: Nature, 2020, Vol. 585.
https://doi.org/10.1038/s41586-020-2423-5.
14. *Repurposed Antiviral Drugs for Covid-19 — Interim WHO Solidarity Trial Results.* **WHO** 6, s.l.: New England Journal of Medicine, 2020, Vol. 384. https://www.nejm.org/doi/10.1056/NEJMoa2023184.
15. *Mechanism of molnupiravir-induced SARS-CoV-2 mutagenesis.* **Kabinger, F., Stiller, C. et al.** s.l.: Nature Structure Molecular Biology, 2021, Vol. 28.
https://doi.org/10.1038/s41594-021-00651-0.
16. **Thacker, Teena.** Molnupiravir reduced Covid-linked hospitalisation by over 65%: Study. *Healthcare/Biotech.* [Online] February 19, 2022.
https://economictimes.indiatimes.com/industry/healthcare/biotech/pharmaceuticals/molnupiravir-reduced-covid-linked-hospitalisation-by-over-65-study/articleshow/89692187.cms.
17. *Structures of the MERS coronavirus 3C-like protease reveal insights into substrate specificity.* **Needle, D., Lountos, G. Tet al.** s.l.: Biol. Crystallogr., 2015, Vol. 71.
https://doi.org/10.1107/S1399004715003521.
18. *Designing of improved drugs for COVID-19: Crystal structure.* **Xiaojiao Fan, Tengchuan Jin.** 67, s.l.: Signal Transduction and Targeted Therapy, 2020, Vol. 5.
https://doi.org/10.1038/s41392-020-0178-y.
19. *Peptidomimetics: Synthetic Tools for Drug Discovery and Development.* **Del Gatto Annarita, Cobb Steven L., Zhang Jinqiang, Zaccaro Laura**, s.l.: Frontiers in Chemistry, 2021, Vol. 9.
https://doi.org/10.3389/fchem.2021.802120
20. *Coronavirus breakthrough: dexamethasone is the first drug shown to save lives.* **Ledford, Heidi.** s.l.: Nature, 2020.
https://www.nature.com/articles/d41586-020-01824-5.
21. *ACE2, Much More Than Just a Receptor for SARS-COV-2.* **Lobelia Samavati, Bruce D. Uhal.** s.l.: Frontiers in Cellular and Infection Microbiology, 2020, Vol. 10. ISSN 2235-2988.
22. **Brandon Malone, Nadya Urakova et al.** *The SARS-CoV-2 infection cycle.* Nat Rev Mol Cell Biol, s.l.: 2022.
https://doi.org/10.1038/s41580-021-00432-z
23. *COVID-19: Current understanding of its Pathophysiology, Clinical presentation and Treatment.* **Parasher, Anant.** 1147, New Delhi: Postgraduate Medical Journal, 2021, Vol. 97.
24. **Jakob J. Malin, Isabelle SuÃ¡rez et al.** *Remdesivir against COVID-19 and Other Viral Diseases.* Clinical Microbiology Reviews, 2020.
https://journals.asm.org/doi/abs/10.1128/CMR.00162-20
25. *RdRp inhibitors and COVID-19: Is molnupiravir a good option?* **Seyed M.R. Hashemian, Mohammad H. Pourhanifeh, Michael R. Hamblin.** s.l.: Biomedicine & Pharmacotherapy, 2022, Vol. 146.
https://doi.org/10.1016/j.biopha.2021.112517.
26. **Gotte, Matthias.** *Mutagenesis Model of Molnupiravir*. University of Alberta Faculty of Medicine and Dentistry,
https://doi.org/10.1016/j.jbc.2021.100770: 2021.
27. *Identification of 3-chymotrypsin like protease (3CLPro) inhibitors as potential anti-SARS-CoV-2 agents.* **Mody, V., Ho, J., Wills, S. et al.** 93, s.l.: Commun Biol, 2021, Vol. 4.
https://doi.org/10.1038/s42003-020-01577-x.