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AN *IN SILICO* ANTISENSE RNA MOLECULES PREDICTION FOR SARS-COV-2 SPIKE PROTEIN INHIBITION

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Abstract
The present study was suggested as an in silico design of antisense RNA molecules as a preliminary suggestion for virus suppression, the spike protein DNA sequences of SARS-CoV-2 virus which isolates in Wuhan-Hu-1(MN908947.3) was used for prediction secondary structure, then RNA antisense molecules designs according to GC%, probabilities and energy, the predictions refer that the secondary structure of RNA is complex and have more than one regions for antisense creation, eight regions for antisense RNA , the first region (59-82)n, second (880-899), third (1141-1160), forth (1647-1668), fifth (1875-1895), sixth (2022-2052), seventh (2417-2445) and finally eighth (2804-2828). Each region has 1-4 target sites , The characteristics of antisense RNA also listed in the table, the GC% percentages were (45-50) %, the probabilities ranged (0.686-0.974) and energy (-0.1 – 12.8) kcal/mol

Keywords:*In silico Antisense, RNA molecules, prediction SARS-CoV-2, spike protein.*

Introduction

the SARS-CoV-2 is a new generation of corona virus family that become Since the emergence of the 2019 novel coronavirus (2019-nCoV) infection in Wuhan, China, in December 2019 (1), it has rapidly spread across China and many other countries during few months (2,3) , the disease is now called COVID-19.

The SARS-CoV-2 viron composed of a single-strand RNA belonging to the Betacorona virus (4,5,6) . The Phylogenetic investigations clarified that SARS-CoV-2 is closely related to two bat-derived SARS-like coronaviruses approximately 88–89% similarity, while it is approximately ~79% similarity to SARS-CoV and ~50% similarity to MERS-CoV (5,7,8).

To now, no specific medication is explored for COVID-19 treatment. However, the governments and pharmaceutical cooperated to find an effective drug. Different medication strategies have been proposed for COVID-19 therapy like antiviral drug Favilavir (9), antimalarial drugs Chloroquine and anti-arthritis drug hydroxychloroquine which recommended by the National Health Commission of

the People's Republic of China [10]. Currently, the Convalescent Plasma of COVID-19 used for treatment in several hospital centers [11].

On the other hand molecular therapy still have an interesting impacted in therapeutic strategies of viral infection treatment, the molecular therapy have been proved its efficiency in investigations and clinical trials, RNA therapy is one of the most molecular therapeutics types have an interesting's in scientific field, the potential RNA molecules for therapy belong to different classifications like siRNA, antisense RNA (ASOs), microRNA and *trans*-cleaving ribozymes[12]. The Antisense RNA molecules is a segment of DNA transcript consist of 19–23 nucleotides complementary to target mRNA, its playing important role in gene expression regulation at different levels, like at replication, transcription, and translation. Moreover, the artificial antisense RNAs have an effective activity to regulate related genes expression in host cells [13].

Two major mechanisms involved in antisense activity, first, it activates RNase H, which cleaves the RNA moiety of a DNA–RNA heteroduplex leads to target mRNA degradation in the nucleus and cytoplasm. Second the ASOs inhibit translation by steric blockade of the ribosome in the cytoplasm [14] . The ASOs targeted to the 5′-terminus thus the translation machinery binding and assembly can be prevented [15].

Some studies documented that The ASOs have been found that it can be utilized in Regulation of RNA processing, like destabilize pre-mRNA, and RNA splicing regulation [16,17] . These activities happened in cytoplasm or in the nucleus.

Researchers exhibit that using ASOs in viral infection treatment show promising results when its customized with chemical modification for more stability and best features, a Fomivirsen (Vitravene®, ISIS-2922) is ASOs antiviral drug consist of modified phosphodiester linkages by replacing a non-linking oxygen with a sulfur atom (phosphorothioate modification) [18,19]. There was no information documented about used RNA therapy for COVID-19 treatment, thus we aim to predict the potential antisense RNA of spike protein that have major role in SARS-CoV-2 pathogenicity especially when the SARS-CoV developed binding affinity to ACE2 by spike protein mutation [20].

Material and Methods

The present study aims to prediction potential antisense RNA molecules of SARS-CoV-2 spike protein using *in silico* bioinformatics. Severe acute respiratory syndrome SARS-CoV-2 virus isolates Wuhan-Hu-1, genome (MN908947.3) sequence from NCBI, secondary structure of spike protein gene creation using RNA structure https://rna.urmc.rochester.edu/RNAstructure.html[21] , the antisense RNA prediction using S fold software http://sfold.wadsworth.org/cgi-bin/index.pl, antisense molecules were filtered according to the unpaired probability ,the percentage of GC% 40% <= <= 60%; No GGGG in the target sequence and Antisense oligo binding energy [22].

Results and Discussion

The present study aims to prediction optimize antisense RNA molecules of spike protein gene of SARS-CoV-2. Spike protein gene was found to be 3821 n which it developed from the same gene of SARS-CoV by mutation in receptor -binding domain (S1 and S2) leads to high affinity of binding with ACE2 [20].

A complex secondary structures were observed through software's output and these structure may be differenced according to energy and self -sequence's complementary, in addition of numerous secondary structure of spike proteins that belong to large length one of these choose in present study that more according to

free energy . The secondary structure was created using RNA structure software 6.2 (figure 1).

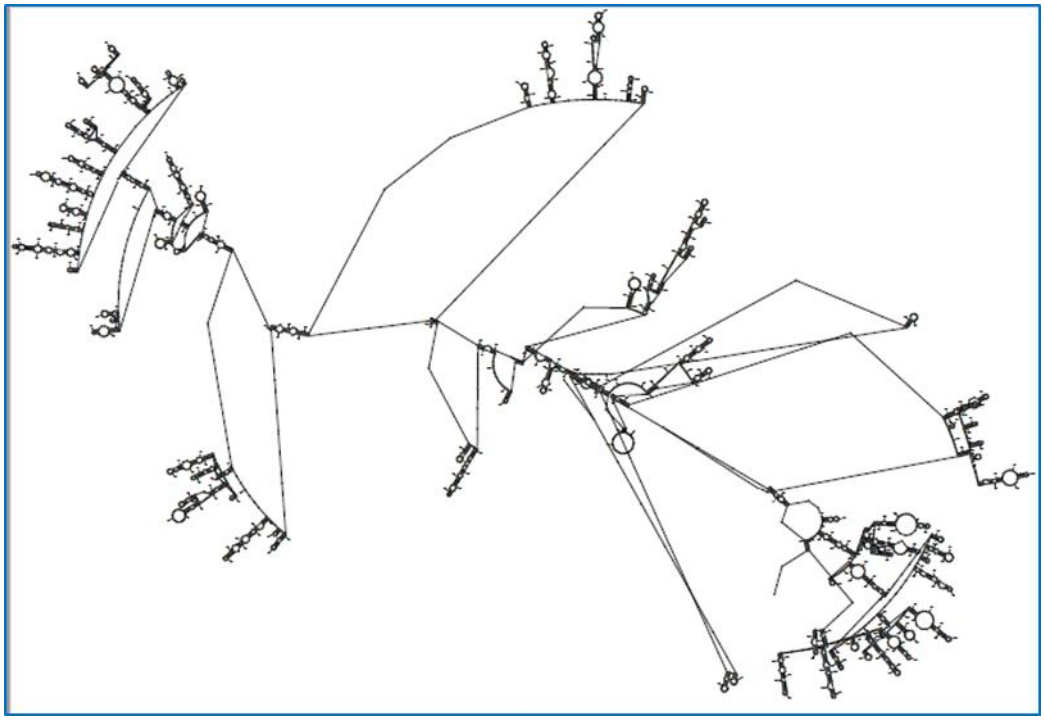


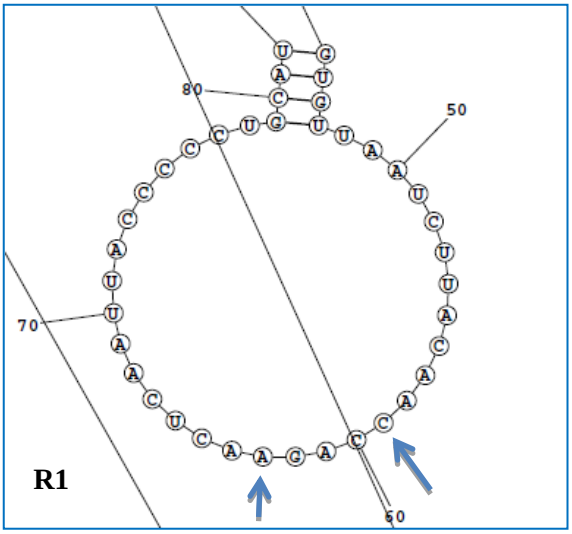
Figure (1) the secondary structure of RNA of spike protein gene created by RNA structure 6.2.

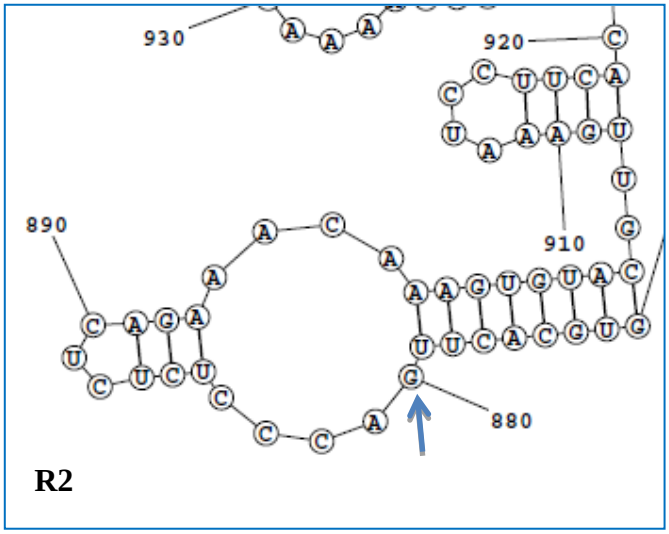
Spike protein gene divided to 8 regions for antisense RNA design according to the unpaired probability of nucleotides and target of antisense, the first region (59-82)n, second (880-899), third (1141-1160), forth (1647-1668), fifth (1875-1895), sixth (2022-2052), seventh (2417-2445) and finally eighth (2804-2828). Each region has 1-4 target sites (table 1). The characteristics of antisense RNA also listed in the table, the GC% percentages were (45-50) %, the probabilities ranged (0.686-0.974) and energy (-0.1 – 12.8) kcal/mol.

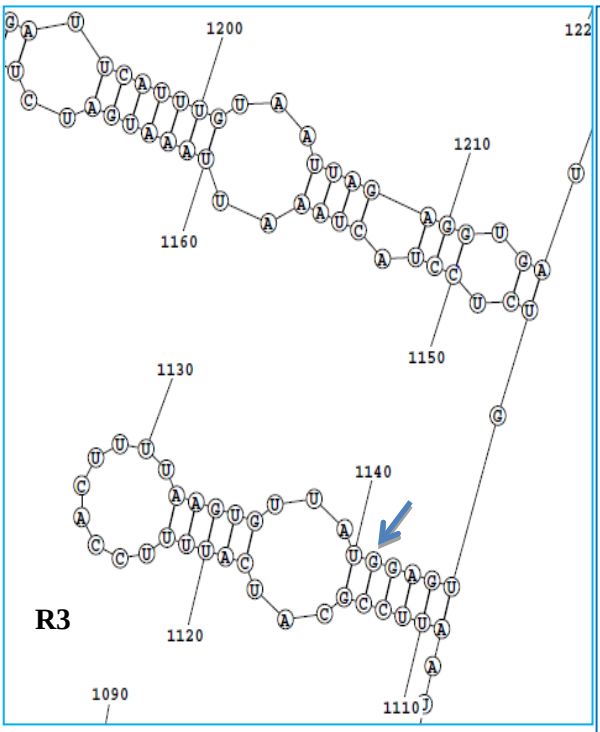
Table (1) features of antisense RNA predication molecules of spike protein gene

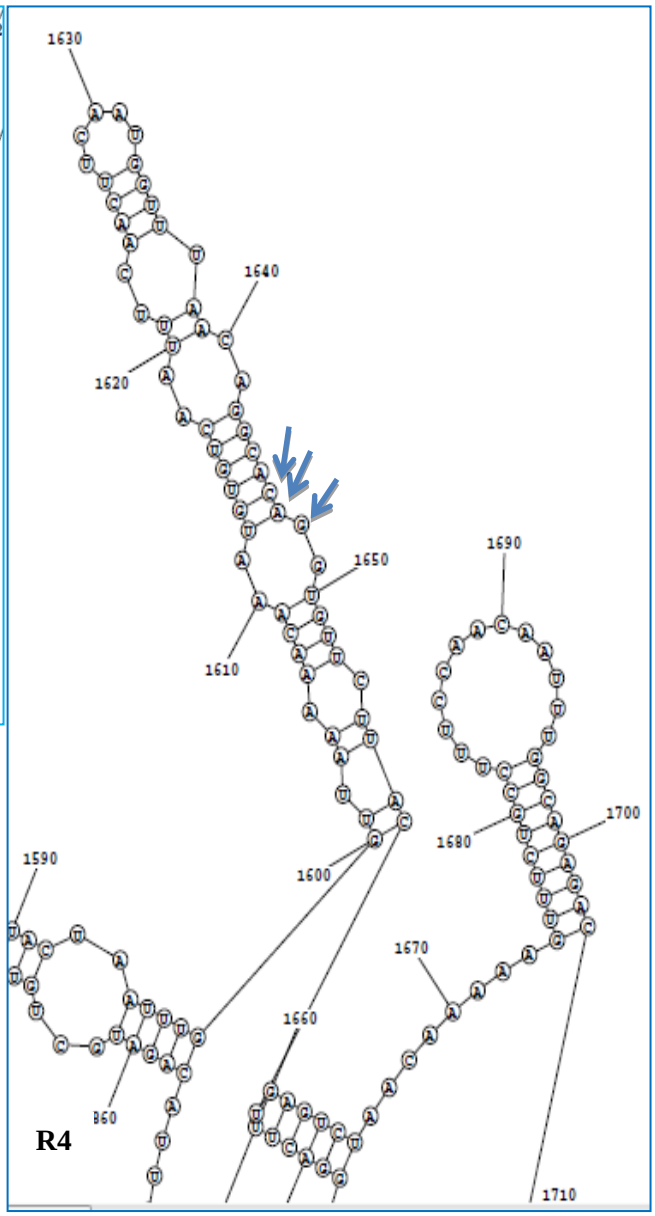
Region	Sequence sites	Target sequence	Antisense RNA	GC %	probability	energy (kcal/mol)
1	59 - 78	CCAGAACUCAAUUACCCCCU	AGGGGGTAATTGAGTTCTGG	50.0 %	0.924	-0.1
	63 - 82	AACUCAAUUACCCCCUGCAU	ATGCAGGGGGTAATTGAGTT	45.0 %	0.921	0.6
2	880- 899	GACCCUCUCUCAGAAACAAA	TTTGTTTCTGAGAGAGGGTC	45.0 %	0.686	3.2
3	1141-1160	GGAGUGUCUCCUACUAAAUU	TAGACTCAGTAAGAACACCT	40.0 %	0.699	7.0
4	1647-1666	AGGUGUUCUUACUGAGUCUA	AATTTAGTAGGAGACACTCC	40.0 %	0.737	4.7
	1648-1667	GGUGUUCUUACUGA	TTAGACTCAGTAAG	40.0	0.78	4.5

		GUCUAA	AACACC	%	6	
	1649-1668	GUGUUCUUACUGAGUCUAAC	GTTAGACTCAGTAAGAACAC	40.0%	0.770	4.3
5	1875-1894	UGCAGAUCAACUUACUCCUA	TAGGAGTAAGTTGATCTGCA	40.0%	0.740	5.3
	1876-1895	GCAGAUCAACUUACUCCUAC	GTAGGAGTAAGTTGATCTGC	45.0%	0.748	5.0
6	2022-2041	UCAGACUCAGACUAAUUCUC	GCCCGCCGAGGAGAATTAGT	40.0%	0.878	0.3
	2028-2047	UCAGACUAAUUCUCCUCGGC	GCCGAGGAGAATTAGTCTGA	50.0%	0.879	0.3
	2032-2051	ACUAAUUCUCCUCGGCGGGC	GAGAATTAGTCTGAGTCTGA	60.0%	0.804	0.7
	2033-2052	CUAAUUCUCCUCGGCGGGCA	TGCCCGCCGAGGAGAATTAG	60.0%	0.764	2.7
7	2417-2436	UACCAGAUCCAUCAAAACCA	TGGTTTTGATGGATCTGGTA	40.0%	0.974	0.3
	2418-2437	ACCAGAUCCAUCAAAACCAA	TTGGTTTTGATGGATCTGGT	40.0%	0.977	4.9
	2425-2444	CCAUCAAAACCAAGCAAGAG	CTCTTGCTTGGTTTTGATGG	45.0%	0.738	11.4
	2426-2445	CAUCAAAACCAAGCAAGAGG	TGTGGAAGAAAGTGAGTCTT	45.0%	0.689	12.8
8	2804-2823	AAGACUCACUUUCUUCCACA	TTGCTGTGGAAGAAAGTGAG	40.0%	0.831	5.9
	2808-2827	CUCACUUUCUUCCACAGCAA	CTTGCTGTGGAAGAAAGTGA	45.0%	0.827	5.9
	2809-2828	UCACUUUCUUCCACAGCAAG	CCTCTTGCTTGGTTTTGATG	45.0%	0.789	6.1


80
50
70
60
R1


930
920
890
910
880
R2


1200
1210
1160
1150
1130
1140
1120
1090
1110
R3


1630
1640
1620
1650
1610
1690
1700
1680
1590
1600
1670
1660
1710
R4

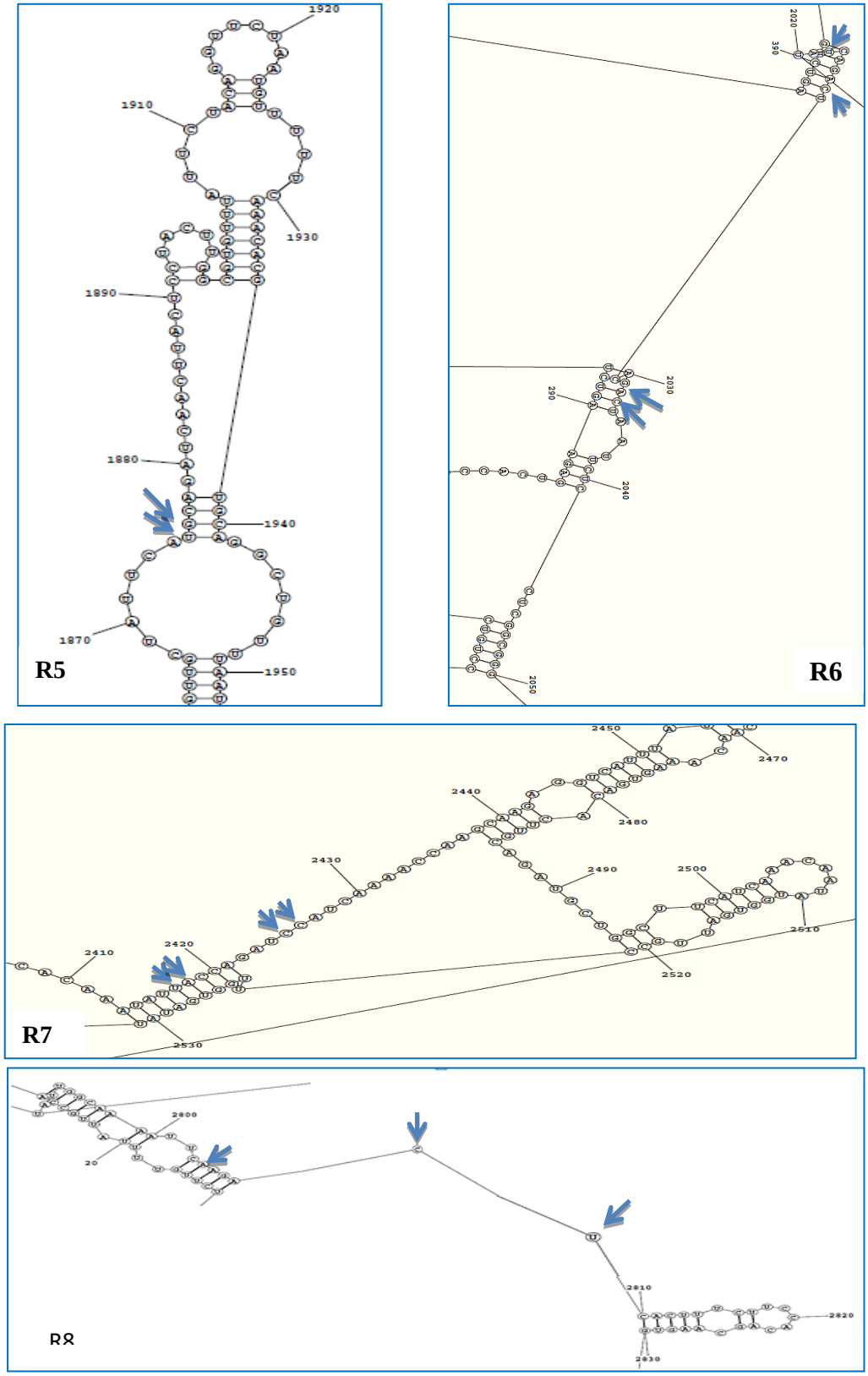


Figure (2) the antisense RNA molecules prediction in the RNA secondary structure of spike protein gene including 8 regions, R1-R8, the sings refer to the beginning of antisense molecules

These regions were candidate according to optimization sequences that can be complementary with synthetic antisense RNA, all RNA therapeutic design still in silico for COVID-19, to date all efforts in the world focused of vaccine developments, and no molecular therapeutic strategies have been suggested. Other viral infections were treated by antisense RNA for suppress viral genome replications like HIV (23) using a lentiviral vector created according to HIV-1 contains 937 bp of antisense sequence for HIV-1 envelope gene expression inhibitor. The HIV still treated with different antisense RNA strategies like using recombinant fluorescent lantiviral vector for distinguished the sense and antisense of viral genome transcription, results show efficiency of antisense RNA in viral inhibition (24).

Another study used antisense RNAs molecules for IV therapy, Ge et al. (25) proved that siRNAs molecules located with conserved regions of viral genomes can be robust suppresser viral replication.

The scientific efforts for utilized molecular therapy in COVID-19 treatment need more investigations and long time for proved its efficiency, to yet no information's about this kinds of researches were observed, in the other design of using RNA molecules deal with COVID-19 structural gene an in silico design for siRNA molecule of structural genes it was a preliminary opinion for COVID-19 inhibition show numerous of RNA molecules were prediction for viral inhibition (26).

We concluded that the viral genome have an potential target sequence for molecular therapy using RNA molecules at least the spike protein that used in present design.

References (SET)

1- Lu H., Stratton C.W., Tang Y.W. Outbreak of pneumonia of unknown etiology in Wuhan China: the mystery and the miracle. J Med Virol. 2020 Jan 16 [Epub ahead of print] [Google Scholar]
2- Li Q., Guan X., Wu P., Wang X., Zhou L., Tong Y. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. N Engl J Med. 2020 Jan 29 [Epub ahead of print] [Google Scholar]
3- Gorbalenya A.E., Baker S.C., Baric R.S., de Groot R.J., Drosten C., Gulyaeva A.A. Severe acute respiratory syndrome-related coronavirus: the species and its viruses—a statement of the Coronavirus Study Group. bioRxiv. 2020 Feb 11 [Google Scholar].
4- Chan J.F., Kok K.H., Zhu Z., Chu H., To K.K., Yuan S. Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. Emerg Microbes Infect. 2020;9:221–236. [PMC free article] [PubMed] [Google Scholar]
5- Lu R., Zhao X., Li J., Niu P., Yang B., Wu H. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet. 2020 Jan 30 [Epub ahead of print] [Google Scholar]
6- Zhu N., Zhang D., Wang W., Li X., Yang B., Song J. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020 Jan 24 [Epub ahead of print] [Google Scholar]
7- Jiang S., Du L., Shi Z. An emerging coronavirus causing pneumonia outbreak in Wuhan, China: calling for developing therapeutic and prophylactic strategies. Emerg Microbes Infect. 2020;9:275–277. [PMC free article] [PubMed] [Google Scholar]

8- Ren L.L., Wang Y.M., Wu Z.Q., Xiang Z.C., Guo L., Xu T. Identification of a novel coronavirus causing severe pneumonia in human: a descriptive study. Chin Med J (Engl) 2020 Jan 30 [Epub ahead of print] [Google Scholar]
9- Hongzhou Lu Drug treatment options for the 2019-new coronavirus (2019-nCoV) J-STAGE home BioScience Trends Volume 14 (2020) Issue 1Article overview
10- Singh AK, Singh A, Shaikh A, Singh R, Misra A. Chloroquine and hydroxychloroquine in the treatment of COVID-19 with or without diabetes: A systematic search and a narrative review with a special reference to India and other developing countries. *Diabetes Metab Syndr*. 2020;14(3):241–246. doi:10.1016/j.dsx.2020.03.011.
11- Kathleen Doheny Convalescent Plasma for the Treatment of COVID-19 Patients? Medscape Logo, 2020.
12- Burnett JC, Rossi JJ. RNA-based therapeutics: current progress and future prospects. *Chem Biol*. 2012;19(1):60–71. doi:10.1016/j.chembiol.2011.12.008.
13- Xu JZ, Zhang JL, Zhang WG. Antisense RNA: the new favorite in genetic research. *J Zhejiang Univ Sci B*. 2018;19(10):739–749. doi:10.1631/jzus.B1700594.
14- Bennett CF, Swayze EE. RNA targeting therapeutics: Molecular mechanisms of antisense oligonucleotides as a therapeutic platform. Annual Review of Pharmacology and Toxicology. 2010;**50**:259-293
15- Wu H, Lima WF, Zhang H, Fan A, Sun H, Crooke ST. Determination of the role of the human RNase H1 in the pharmacology of DNAlike antisense drugs. The Journal of Biological Chemistry. 2004 Apr;**279**(17):17181-17189.
16- Kole R, Krainer AR, Altman S. RNA therapeutics: Beyond RNA interference and antisense oligonucleotides. Nature Reviews. Drug Discovery. 2012 Feb;11(2):125-140.
17- Disterer P, Kryczka A, Liu Y, Badi YE, Wong JJ, Owen JS, et al. Development of therapeutic splice-switching oligonucleotides. Human Gene Therapy. 2014 Jul;**25**(7):587-598.
18- Orr R.M. Technology evaluation: fomivirsen, Isis Pharmaceuticals Inc/CIBA vision. Curr. Opin. Mol. Ther. 2001;3(3):288–294. [PubMed] [Google Scholar].
19- Geary R.S., Henry S.P., Grillone L.R. Fomivirsen: clinical pharmacology and potential drug interactions. Clin. Pharmacokinet. 2002;41(4):255–260. [PubMed] [Google Scholar].
20- Kristian G. Andersen ,Andrew Rambaut , W. Ian Lipkin, Edward C. Holmes and Robert F. Garry, The proximal origin of SARS-CoV-2 , correspondence Published: 2020, nature https://doi.org/10.1038/s41591-020-0820-9.
21- Mathews DH. RNA secondary structure analysis using RNAstructure. *Curr Protoc Bioinformatics*. 2006;Chapter 12:Unit-12.6. doi:10.1002/0471250953.bi1206s13
22- Ding Y, Chan CY, Lawrence CE. Sfold web server for statistical folding and rational design of nucleic acids. *Nucleic Acids Res*. 2004;32(Web Server issue):W135-W141. doi:10.1093/nar/gkh449.
23- Lu XB, Yu Q, Binder GK, et al. Antisense-mediated inhibition of human immunodeficiency virus (HIV) replication by use of an HIV type 1-based vector results in severely attenuated mutants incapable of developing resistance. J Virol. 2004;78(13):7079–7088. doi: 10.1128/Jvi.78.13.7079-7088.2004. [PMC free article] [PubMed] [CrossRef] [Google Scholar].
24- Kobayashi-Ishihara M, Terahara K, Martinez JP, Yamagishi M, Iwabuchi R, Brander C, Ato M, Watanabe T, Meyerhans A and Tsunetsugu-Yokota Y (2018)

HIV LTR-Driven Antisense RNA by Itself Has Regulatory Function and May Curtail Virus Reactivation From Latency. *Front. Microbiol.* 9:1066. doi: 10.3389/fmicb.2018.01066

25- Ge Q, McManus MT, Nguyen T, et al. RNA interference of influenza virus production by directly targeting mRNA for degradation and indirectly inhibiting all viral RNA transcription. Proc Natl Acad Sci USA. 2003;100(5):2718–2723. doi: 10.1073/pnas.0437841100. [PMC free article] [PubMed] [CrossRef] [Google Scholar].

26- Al-Terehi MN, Alkaim AF, Yaro A (2020): An in silico approach to design potential siRNA molecule of SARS-CoV-2 virus structural genes, a preliminary opinion for COVID-19 inhibition, Ann Trop Med & Public Health; 23(S11): SP23111. DOI: http://doi.org/10.36295/ASRO.2020.23111