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RNAi-mediated siRNA sequences to combat the COVID-19 pandemic with the inhibition of SARS-CoV2

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ABSTRACT

The outbreak of the COVID-19 pandemic has cost five million lives to date, and was caused by a positive-sense RNA virus named SARS-CoV2. The lack of drugs specific to SARS-CoV2, leads us to search for an effective and specific therapeutic approach. Small interfering RNA (siRNA) is able to activate the RNA interference (RNAi) pathway to silence the specific targeted gene and inhibit the viral replication, and it has not yet attracted enough attention as a SARS-CoV2 antiviral agent. It could be a potential weapon to combat this pandemic until the completion of full scale, effective mass vaccination. For this study, specific siRNAs were designed using a web-based bioinformatics tool (siDirect2.0) against 14 target sequences. These might have a high probability of silencing the essential proteins of SARS-CoV2, such as: 3CLpro/Mpro/nsp5, nsp7, Rd-Rp/nsp12, ZD, NTPase/HEL or nsp13, PLpro/nsp3, envelope protein (E), spike glycoprotein (S), nucleocapsid phosphoprotein (N), membrane glycoprotein (M), ORF8, ORF3a, nsp2, and its respective 5′ and 3′-UTR. Among these potential drug targets, the majority of them contain highly conserved sequences; the rest are chosen on the basis of their role in viral replication and survival. The traditional vaccine development technology using SARS-CoV2 protein takes 6–8 months; meanwhile the virus undergoes several mutations in the candidate protein chosen for vaccine development. By the time the protein-based vaccine reaches the market, the virus would have undergone several mutations, such that the antibodies against the viral sequence may not be effective in restricting the newly mutated viruses. However, siRNA technology can make sequences based on real time viral mutation status. This has the potential for suppressing SARS-CoV2 viral replication, through RNAi technology.

1. Introduction

In December 2019, the World Health Organization (WHO) announced a new type of virus called Severe Acute Respiratory Syndrome Coronavirus 2 or briefly, SARS-CoV2. SARS-CoV2 gives rise to violent damage to the world as a pandemic (called COVID-19 disease) affecting more than 222 countries and territories (Worldometer) with 253,982,410 confirmed cases, including 5,114,571 fatalities (WHO) until November 2021. The world is in great need of effective measures to prevent or treat this pandemic. Many different types of therapeutic agents of other targets (antiviral, anti-malarial, anti-cancer, etc.) have been tested to determine their potential effectiveness against SARS-CoV2, but their efficacy has not yet been confirmed (Ghosh et al., 2020). Likewise, drugs used against SARS-CoV and MERS-CoV were initially found to be ineffective against SARS-CoV2 (Naqvi et al., 2020). The tendency of potential adaptive mutations of the SARS-CoV2 genome possibly made it extremely pathogenic, causing problems in the development of drugs and vaccines (Xu et al., 2020). The challenges for the treatment require a novel dimension, especially when we are in need of an effective antiviral agent.

RNAi is a specific post-transcriptional gene-silencing mechanism that can be activated via siRNA (Saadat, 2013) and has the potential to block pathogenic viral replication and further infection in animal cells (Ge et al., 2003). siRNA-silencing technology was used to restrict HCV, HIV (Wilson et al., 2003), SARS and MERS viral replication (Li et al., 2005; Wu et al., 2005; Yi et al., 2005).

Abbreviations: SARS, severe acute respiratory syndrome; MERS, Middle East Respiratory Syndrome; SARS-CoV2, severe acute respiratory syndrome coronavirus 2; COVID-19, Coronavirus Disease of 2019; RNAi, RNA interference; siRNA, small interfering RNA; ORF, open reading frame; PLpro, papain like proteases; Mpro, main proteases; 3CLpro, 3-chymotrypsin like proteases; Rd-Rp, RNA dependent-RNA polymerases; nsp, non-structural protein; UTR, untranslated region.

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 Genetic variance analyses of the complete genome in 48,635 SARS-CoV2 samples, comparing it with the reference genome (Wuhan genome) NC_045512.2, revealed a fair average of 7.23 mutations per sample (Mercatelli and Giorgi, 2020). Genetic variances of SARS-CoV2 even within the same country are an obstacle to finding a universally applicable therapeutic agent (Biswas and Mudi, 2020; Toyoshima et al., 2020). This reason leads us to think about specific siRNA-based universal therapeutics by focusing on conserved and various potential targets in SARS-CoV2 genome reference sequences. This effort may pave the way for precision/personalized medicine to treat individuals

Table 1
List of siRNAs with the specifications of nsp2 gene.

| Target gene      | Target position | Target sequence 21 nt target + 2 nt overhang | RNA oligo sequences 21 nt guide (5′ → 3′) | Seed duplex stability (Tm) | Guide Passenger |
|------------------|----------------|---------------------------------------------|-------------------------------------------|-----------------------------|----------------|
| nsp2 (NC_045512.2:806-2719) | 285-307       | TCTCTAAAATCTCAATATACAA                     | UUGAUAAGUCAUUUUGUGGAA                   | 8.7 °C                     | −4.3 °C        |
|                  | 541-563       | CCCCAAAAATGCTGTGTTAAAAAT                 | UUAAACACGACUACUGUGG                      | 7.2 °C                     | 4.2 °C         |
|                  | 812-834       | TGGAATATCCCTTTAAAAGGAA                   | UCUUUUGGAGUUAUCAA                       | 10.3 °C                    | 4.6 °C         |
|                  | 1397-1419     | GGGAAATGTGATAATTCTTA                      | GAAAUUGUUAACUAAACUCUC                   | 1.8 °C                     | 5.3 °C         |
|                  | 1567-1589     | TTGGAATTAGTGAAAACCTTG                   | GAAAUUGUUAACUAAACUCUC                   | 5.3 °C                     | −4.3 °C        |

Table 2
List of siRNAs with the specifications of nsp3 gene.

| Target gene      | Target position | Target sequence 21 nt target + 2 nt overhang | RNA oligo sequences 21 nt guide (5′ → 3′) | Seed duplex stability (Tm) | Guide Passenger |
|------------------|----------------|---------------------------------------------|-------------------------------------------|-----------------------------|----------------|
| PLpro/nsp3 (NC_045512.2:2720-8554) | 1732-1754     | TTGAAAGTTTCGTAGTTTACACC                   | UUGAUAAGUCAUUUUGUGGAA                   | 6.9 °C                     | 10.3 °C        |
|                  | 88-110        | AGAATGTTGATAAGGACTTTAAATGA               | GAAACUCUGUUUUGUAAACCC                   | 6.6 °C                     | 8.7 °C         |
|                  | 609-631       | GACTATTGACTGTAAGTATTTTTA                  | AAAACUUAUUCAUAAACUGU                     | 4.6 °C                     | 8.9 °C         |
|                  | 652-674       | GAAATGTTACATATTTAAAAATGG                 | AUIUUAACAUAAUACAUAAUGUGG                 | −9.1 °C                    | 6.7 °C         |
|                  | 727-749       | GCCTATGTTTTACCTTTAAAAATGG               | AUIUUAACAUAAUACAUAAUGUGG                 | 7.2 °C                     | 5.3 °C         |
|                  | 945-967       | TGCTATGAAATTTTAAATACG                    | UGUAUAACUAUUUAUAAACG                     | 2.1 °C                     | 8.9 °C         |
|                  | 1172-1194     | AGGAATTTAGGCTTTTTAAACTA                  | UUUAACACUAAUUGUAAACCC                   | −8.0 °C                    | 4.9 °C         |
|                  | 1579-1601     | GTGCTTTAAAAGTTGAGAAAAAGTG                | ACUUAACUCUAAUUUUGAC                     | 4.9 °C                     | −3.8 °C        |
|                  | 1590-1612     | GTGAAAATGCTGCTTACATTTC                  | AUIUUAACACUAAUACAUAAUGUGG                 | 7.2 °C                     | 4.9 °C         |
|                  | 1747-1765     | TTCAAATCATACGTTAACAT                   | UUUAACACUAAUACAUAAUGUGG                 | 6.9 °C                     | 6.3 °C         |
|                  | 1813-1835     | TACTTTTACACAGTAAACAC                     | UGUAUAACUGUGAAGUAAACG                     | 8.2 °C                     | 7.2 °C         |
|                  | 1997-2019     | CAGGTATAATGTGTATCTACT                    | UUUAACACUAAUUGUAAACCC                   | 6.9 °C                     | 8.5 °C         |
|                  | 2136-2158     | AGGTATATAAAGAATGTATATTACA                | UUUAACACUAAUUGUAAACG                     | 1.1 °C                     | 8.9 °C         |
|                  | 2138-2160     | GTGATAAAGTGTATATTACT                    | UUUAACACUAAUUGUAAACG                     | 1.1 °C                     | −4.3 °C        |
|                  | 2387-2409     | AAGTAAACATTTTTATGTATTTTA                | AUCUUAACACUAAUUGUAAACG                     | 6.9 °C                     | 8.2 °C         |
|                  | 2490-2512     | AGCATTTAATCACTAAAGATG                   | UUUAACUGUGAAGUAAACG                     | 4.9 °C                     | −10.3 °C       |
|                  | 2522-2544     | CACAAGTTAATGTTTACCTCT                   | AUCUUAACACUAAUUGUAAACG                     | 4.9 °C                     | 4.9 °C         |
|                  | 2531-2553     | ATGGTTACTTTCTTTAAAGT                    | UUUAACACUAAUUGUAAACG                     | −7.5 °C                    | 8.2 °C         |
|                  | 2868-2890     | TTCTTATGAACATTTAAGAG                   | UUUAACACUAAUUGUAAACG                     | 7.1 °C                     | 8.9 °C         |
|                  | 2913-2935     | TGTTAACAGTCTAAATATAC                    | UUUAACACUAAUUGUAAACG                     | 5.3 °C                     | 7.2 °C         |
|                  | 3047-3069     | GTCAACTAAACATATAACCTCT                  | AAGUUAACACUAAUUGUAAACG                     | 6.3 °C                     | 6.3 °C         |
|                  | 3056-3078     | AACATATACTTTAAGAGAC                     | AAGUUAACACUAAUUGUAAACG                     | 7.1 °C                     | 1.1 °C         |
|                  | 3172-3194     | ACCATAAAACCGATTACCTTATA                 | AUAAGUUAACUGUGAAGUAAACG                     | 6.6 °C                     | −0.3 °C        |

(continued on next page)
infected with SARS-CoV2.

Genome SARS-CoV2 contains 14 Open Reading Frames (ORFs), and 27 proteins (A. Wu et al., 2020). ORF1a, as well as ORF1b, is translated as a single large poly-protein. The ORF1a contains two viral proteases; papain-like proteases or PLpro (non-structural protein 3 or nsp3), and main proteases or Mpro also designated as 3-chymotrypsin-like proteases or 3CLpro (non-structural protein 5 or nsp5). Recent clinical trials of multiple antiviral agents have targeted the proteases (Ghosh et al., 2020). The ORF1b contains viral RNA-dependent RNA polymerase (RdRp), which is non-structural protein 12 or nsp12. The site identified, downstream to the Rd-Rp is coding for the viral helicase (non-structural protein 13 or nsp13) (Ghosh et al., 2020). Both ORF1a and ORF1b include highly preserved sequences among the annotated genomes of SARS-CoV2 and earlier beta coronaviruses like SARS and MERS (F. Wu et al., 2020).

2. Materials and methods

2.1. Sequence retrieval & manual extraction

The reference genome of the SARS-CoV2 [NC_045512.2] was achieved from the database available at the National Center for Biotechnology Information (NCBI) (NCBI (accessed 18 February 2021)) and we manually extracted the sequences for 3CLpro/Mpro or non-structural protein 5/nsp5 [NC_045512.2:10055-10972], PLpro or non-structural protein 3/nsp3.

| Target gene | Target position | Target sequence 21 nt target + 2 nt overhang | RNA oligo sequences 21 nt guide (5’ → 3’) | 21 nt passenger (5’ → 3’) | Seed duplex stability (Tm) |
|-------------|----------------|---------------------------------------------|-------------------------------------------|---------------------------|--------------------------|
|             |                |                                             |                                           |                           |                          |
| 3224-3246   | ACCTAAGTTGACAAATTATAT | AAUAAUGGUCCAAUAGGU                   | -1.8 °C                                   | 9.8 °C                    |
|             |                 | CCAAGUUGGCAACUAUUAA |                                     |                           |                          |
| 3524-3546   | AGTGAACATGCAAATCAATATAAA | UAUAGUGUUGCAAUUGUCAUU |                                           |                           |                          |
|             |                 | GUAACAAAGGCAACUAUAAA |                                     |                           |                          |
| 3782-3804   | CAGGAAAATATAGTTAAAATT | UUUUUAACAUUAUAIUGUG |                                           |                           |                          |
|             |                 | GAAAUAAGUGUUAACGAAA |                                     |                           |                          |
| 3847-3869   | GACATAATCTAGTCTATTATTAA | AAUAGUAGACIAUAUUGAU |                                           |                           |                          |
|             |                 | CUAUAUAAUGGCUAUAUUCU |                                     |                           |                          |
| 88-110      | GCCATTTCATCAAAGTGTTGA | ACAUAUUGGUAACUGUGUAU |                                           |                           |                          |
| 4041-4063   | ACTACTAAATATAGCTTATTTTC | AAAUAAGGCAUAUAUAGUA |                                           |                           |                          |
|             |                 | CUAUAUAAUGGCUAUAUUCU |                                     |                           |                          |
| 4051-4073   | AGCTGATTATTCCTATTATTTT | AAUAAUGGUCCAAUAGGU                   | -1.8 °C                                   | 9.8 °C                    |
|             |                 | CCAAGUUGGCAACUAUUAA |                                     |                           |                          |
| 4052-4074   | TGCTATTTCATTTATTGTT | AAUUGUAAAGGAAGAACUGCA |                                           |                           |                          |
|             |                 | GCCUAUUAAGUUAACUUA |                                     |                           |                          |
| 4053-4075   | GCCATTTCATTTCTATTATGC | AAUAAUGUAGAGAAAGAAGC |                                           |                           |                          |
|             |                 | CUAUAUAAUGGCUAUAUUCU |                                     |                           |                          |
| 4073-4093   | TGCTAAAATGTTATCTTACTT | AAUAAGGCAUAUAUAGUA |                                           |                           |                          |
|             |                 | CUAUAUAAUGGCUAUAUUCU |                                     |                           |                          |
| 4098-4120   | AAGTACAAATTTGAAATTTTAGAAG | AAUAAUGGUCCAAUAGGU                   | -1.8 °C                                   | 9.8 °C                    |
|             |                 | CUAUAUAAUGGCUAUAUUCU |                                     |                           |                          |
| 4220-4242   | AACTGATAAATATTATAATTTG | AAUAAUGGUCCAAUAGGU                   | -1.8 °C                                   | 9.8 °C                    |
|             |                 | CUAUAUAAUGGCUAUAUUCU |                                     |                           |                          |
| 4296-4318   | AGGTTGTTTAAATGGTAAATTAG | AAAUAAGGCAUAUAUAGUA |                                           |                           |                          |
|             |                 | CUAUAUAAUGGCUAUAUUCU |                                     |                           |                          |
| 4452-4474   | TCTTATGAAATCATACAATATTA | AAUAAUGGUCCAAUAGGU                   | -1.8 °C                                   | 9.8 °C                    |
|             |                 | CUAUAUAAUGGCUAUAUUCU |                                     |                           |                          |
| 4452-4474   | TGTGTTGTTGCAATATCTTCTT | AAAUAAGGCAUAUAUAGUA |                                           |                           |                          |
|             |                 | CUAUAUAAUGGCUAUAUUCU |                                     |                           |                          |
| 4600-4622   | AGCTATTTTGCAGATCATATTAT | AAAUAAGGCAUAUAUAGUA |                                           |                           |                          |
|             |                 | CUAUAUAAUGGCUAUAUUCU |                                     |                           |                          |
| 4610-4632   | CAGTACATTATTTAAGTATCTT | AAAUAAGGCAUAUAUAGUA |                                           |                           |                          |
|             |                 | CUAUAUAAUGGCUAUAUUCU |                                     |                           |                          |
| 4972-4994   | CAGTTTAAAGGACCATAAATTC | AAAUAAGGCAUAUAUAGUA |                                           |                           |                          |
|             |                 | CUAUAUAAUGGCUAUAUUCU |                                     |                           |                          |
| 5000-5022   | CCTCATTGGTATTACACCTAGACA | AAAUAAGGCAUAUAUAGUA |                                           |                           |                          |
|             |                 | CUAUAUAAUGGCUAUAUUCU |                                     |                           |                          |
| 5100-5122   | TCTCATTGTTGATCTTACGACA | AAAUAAGGCAUAUAUAGUA |                                           |                           |                          |
|             |                 | CUAUAUAAUGGCUAUAUUCU |                                     |                           |                          |
| 5153-5175   | TGCTTATATATGTATGTATT | AAAUAAGGCAUAUAUAGUA |                                           |                           |                          |
|             |                 | CUAUAUAAUGGCUAUAUUCU |                                     |                           |                          |
| 5154-5176   | GCTCTAAATGTTATGATTGGTTTG | AAAUAAGGCAUAUAUAGUA |                                           |                           |                          |
|             |                 | CUAUAUAAUGGCUAUAUUCU |                                     |                           |                          |
| 5168-5190   | TAGTGTGTTGTATGGTTAACAAA | AAAUAAGGCAUAUAUAGUA |                                           |                           |                          |
|             |                 | CUAUAUAAUGGCUAUAUUCU |                                     |                           |                          |
List of siRNAs with the specifications of nsp5, nsp7 and nsp12 genes.

| Target gene | Target position | Target sequence | RNA oligo sequences | Seed duplex stability (Tm) |
|-------------|-----------------|-----------------|--------------------|--------------------------|
| 3CLpr/Mpro/nsp5 (NC_045512.2:10055-10972) | 151–173 | AACCTAATTATGAGATTATCTTACT | UAAACCUUCUAAUUAAGGGGUU | 5.3 °C 10.9 °C |
| | 153–175 | CCGTATTTGAGCTGATTTACTCA | AGUAAAUUCGUUCAAAUGGGGU | 10.0 °C −5.0 °C |
| | 444–466 | TGTGGTTTAACTAGATTATGACT | UCUAAUCAUGUAAAAGAAAACCA | 8.0 °C 0.0 °C |
| | 526–548 | GACTTAGAAGGTAACTTTTAGG | UUAAGAAGCUUUCAAAUGGUUC | 4.9 °C 11.7 °C |
| | 538–560 | AACTTTTGGACCTTTTGGTA | AAACAGGGUCAAUAAUG | 10.3 °C −1.4 °C |
| | 594–616 | AACTTTTGGACCTTTTGGTA | AAACAGGGUCAAUAAUG | 5.3 °C 8.5 °C |
| | 795–817 | TGGTTTCATTTAAGATTACTG | UCUAUCUAAAGUAAUGAAGGC | 10.0 °C 8.9 °C |
| nsp7 (NC_045512.2:11843-12091) | 62–84 | GAGTAGAATCTCATCATTAATTG | AUUUAAGAGUAIUUCUAAUC | 6.9 °C 16.0 °C |
| | 65–87 | TAGAATCATCATCTAAATTG | GUAAGAUCACUAAUUAAGGG | −1.4 °C 16.2 °C |
| RdRp/nsp12 (NC_045512.2:13442-16236) | 133–155 | TTGCTAATTCCTAAAAAATCT | UAGUUAUAGGUAUAAUGGAA | 3.2 °C −4.3 °C |
| | 134–156 | TTGCAATATCTAAAAAAACTT | UGAUUAUAGGUAUAAUGGAA | 4.9 °C 2.1 °C |
| | 297–319 | GACTCTTTTATGTTAGATAAG | UUAUCAUAAGAUAAGAAAGG | 6.9 °C 7.1 °C |
| | 407–429 | AGGTAAATTCGACATACATTAAG | UUAUUAUGUCACAUAUACU | 6.9 °C 6.9 °C |
| | 417–439 | GACACCTAAAAGGAATACTTGT | AUAUAUAUAAUGGUAUGG | 4.6 °C 6.9 °C |
| | 457–479 | ATGATGATTATTTCAAAAAGU | UUUUAUUGAAAUAAUCAUC | −1.4 °C 8.7 °C |
| | 704–726 | TTCTTATATTCTATCTAGTG | AUUAACUAAGUAAUAAGAA | 7.2 °C −8.0 °C |
| | 792–814 | TACATTAGTGGATTGTGTAAC | CIUAUCUAAAAUAUUAUGG | 5.3 °C 4.6 °C |
| | 1573–1595 | ATGACCTTTTGCAGATACAAAAAAGC | UGGAUUGICACAAUCUAAGG | 8.2 °C 10.3 °C |
| | 1711–1733 | TTCACTAAAAAATATTCTAAAACTA | CAUAACUAUAAUAUAUAGG | 7.4 °C 7.4 °C |
| | 1800–1822 | AGTTAAAAAGTTTTATAGTAG | UAAACUAUCAUGUAAUAUGG | −2.3 °C −9.1 °C |
| | 2606–2088 | TGCACTATTGTTCGTATGTTT | AAGUUAUAAACAAUCUAAGG | 7.2 °C 6.3 °C |
| | 2103–2125 | GCCAATGTattaGTGACTTTTATTC | CIUAACUAUAAUAUAUAUGG | 10.3 °C 6.9 °C |
| | 2126–2158 | AACAATAGTTTGGGATAGATGATTG | AAACUAUAGGCAUAAUGG | 6.3 °C −3.3 °C |
| | 2236–2258 | AGCAATATTGGTGAATACATTC | AAACUAUAGGCAUAAUGG | 6.9 °C −1.8 °C |
| | 2237–2259 | GCCGATATTGCCATAGACCTT | AAACUAUAGGCAUAAUGG | 5.3 °C −1.8 °C |
| | 2340–2362 | ACCATTTAATCAGTCTTCTTTATTA | AAACUAACUAAGGCAUAAUGG | 7.1 °C 4.9 °C |
| | 2362–2384 | ATCCAAACACATGTGTATTTATACTC | AAACUAACUAAGGCAUAAUGG | −1.4 °C 5.6 °C |

2.2. siRNA design principles

Ui-Tei, K., and colleagues prescribed the characteristics of the hugely functional siRNAs, named “Ui-Tei rule”. The siRNA chosen according to the Ui-Tei rule persuades the subsequent four ambiances concurrently: (a) A/U at region 1 from the 5′-UTR, (b) G/U at region 2, (c) G/U at region 3, (d) the absence of long GC stretches ≥10 (Ui-Tei et al., 2004).

2.3. siRNA design web-based tool

The web-based siRNA design siDirect2.0 Tool (siDirect version 2.0 (accessed 18 February 2021)) has been used. It is used to design functional and target-specific siRNAs, which was proposed by Naito, Y., and
colleagues (Naito et al., 2009). The siRNAs are satisfactory according to Table 4.

2.5. Off-target effect-reduced siRNA sequence selection

Selected and sequence-specific siRNAs were designed with the web-based siRNA design siDirect2.0 Tool (siDirect version 2.0 (accessed 18 February 2021)) according to the Ui-Tei rule.

2.6. Near-perfect matched off-target gene elimination

In order to exclude the near-perfect matched non-target genes, the siDirect 2.0 homology search option was used, as its accuracy level has been found to the best of all available homology search engines. Both (guide and other passenger) strands of candidate functional siRNAs that have at minimum two inconsistencies to any other non-targeted transcripts were chosen (Naito and Ui-Tei, 2012).

3. Results

In this study siRNA-based specific sequences were designed for therapeutic purposes of SARS-CoV2 with siDirect2.0 (siDirect version 2.0 (accessed 18 February 2021)) by following all the above-mentioned procedures. They are listed in Tables 1–6 with the title of specific genes and also their location in the genome is mentioned in brackets.

4. Discussion

Due to the advancement of modern technologies, it could be possible to produce a vaccine in a shorter time but the acquisition of knowledge related to its effect on the human body may take a much longer time; maybe years or decades.

The question still remains unanswered- how can we combat the waves of COVID-19 disease during the vaccine trial period, as an effective drug does not exist?

Thus, antiviral drugs specific to SARS-CoV2 can be designed and developed by targeting conserved enzymes such as: 3C-like protease or main protease (3CLpro/Mpro or non-structural protein 5/5p5), non-structural protein 7/np7, RNA dependent RNA polymerase shortly Rd-Rp or non-structural protein 12/np12, papain-like protease (PLpro or non-structural protein 3/nsp3), and non-structural protein 13/nsp13 (also known as ZD, NTPase/HEL) (Zumla et al., 2016; Naqvi et al., 2020). These drug targets were confirmed by executing sequence analysis of potential drug target proteins in SARS-CoV2 beside viruses called SARS-CoV and MERS. Also, it was observed that the envelope protein (E), spike glycoprotein (S), nucleocapsid phosphoprotein (N), and membrane glycoprotein (M) are considered as potential drug/vaccine targets (Naqvi et al., 2020). By comparing the pathways in inflammation, and cytokine responses during SARS-CoV, MERS-CoV, and SARS-CoV2 infections, it was revealed that DNA synthesis is triggered by ORF8, while necrotic cell death is triggered by ORF3a. And also nsp2 is related to its effect on the human body may take a much longer time; maybe years or decades.

RNA interference (RNAi) is a widely applied approach by which small interfering RNA (siRNA) also known as silencing RNA, silence a gene.
temperatures ≥10°C (Ui-Tei et al., 2004). To avoid the seed-dependent off-target effects, choosing siRNAs with a low melting temperature (Tm) of the seed-target duplex can minimize the seed-dependent off-target silencing. The melting temperature (Tm) of 21.5°C may serve as the benchmark (Naito and Ui-Tei, 2012) but the seed duplex selected here was nearly Tm < 10°C. The siRNAs that have near-perfect matches to any other non-targeted transcripts were excluded by comparing both their strands, having at minimum two mismatches to any other non-targeted transcripts (Naito and Ui-Tei, 2012). siDirect2.0 (siDirect version 2.0 (accessed 18 February 2021)) provides a functional, target-specific siRNA design web-based tool according to the procedures mentioned above (Naito et al., 2009). siDirect 2.0 would be a more suitable and sensitive homology search engine for short sequences, in comparison to other search engines (Naito and Ui-Tei, 2012).

5. Conclusion

In conclusion, it can be said that our designed RNAi sequences specific for SARS-CoV2 would be a potential weapon against COVID-19 disease all over the world. Nebulization or suspension in the systemic circulation by using a liposome-based delivery system might be an alternative treatment for COVID-19 disease all over the world. Further experimental validation and related trials are needed to confirm these findings.

Table 5

| Target Gene                  | Target position | Target sequence 21 nt target + 2 nt overhang | RNA oligo sequences 21 nt guide (5’ → 3’) | Seed duplex stability (Tm) |
|-----------------------------|-----------------|---------------------------------------------|-------------------------------------------|---------------------------|
| Spike glycoprotein (S) (NC_045512.2:21563-25384) | 167-189 | TACCTTCTTTTCTTTCAATGGTTCTTAC | UACAUUGAAGGAAGAAUGUA | 12.1°C, 10.3°C |
| 222-244 | TGGTACTAAGAGTTTGAATACC | UAAUAACCAAUGUAGGUCG | 8.9°C, 11.3°C |
| 310-332 | TGATTTTTTGGTACTAATTGAAG | UAAAAGCAGGACTAAACCA | 9.8°C, −3.3°C |
| 365-387 | AGCTCTAATTTCTTTATTTAA | UAAUAACAACUAUGAGCU | 6.9°C, 11.3°C |
| 366-388 | CGCTCTAATTTCTTTAATAAG | UUAAUAACCAAUAGUACGA | 1.4°C, 6.3°C |
| 369-391 | TACTAATGTGGTATATTTAG | ACUUAUAACAAACUAUGUA | −4.3°C, 6.9°C |
| 390-412 | CTGTAATTTTCTTTTATTTAAG | UUACAAUAAGCAUUACAGG | 7.2°C, 7.4°C |
| 413-435 | ATCTAATTGGGTTGATTAC | AAUAUAACCCAAAAGUAACGA | 6.9°C, −3.3°C |
| 414-436 | TCTATTTTGTTGTTATTACTAC | CAAUAUAACCAAACAAUCGG | −0.3°C, −3.3°C |
| 486-508 | TGGAAATTTTCTTTTATTTAAG | UUACAAUAAGCAUUACAGG | 10.3°C, 1.8°C |
| 540-562 | AGGAAAAAACGGAATTTTCAAAAA | UUGGAAACUAACGUGUUCUC | 7.4°C, 10.3°C |
| 548-570 | AGGTTAATTTTCAAAAATTTTAG | GAAACAAAAAGGAAGAAUCC | 5.3°C, −0.3°C |
| 568-590 | AGGAAAAATTTTATTTTAGAAAT | UUACCAUAACAAACUUCC | 7.1°C, 7.4°C |
| 569-591 | GGAAATTTTGTGGTATTAAG | CAUAUAACCAAACAAUCGG | 6.9°C, 5.3°C |
| 583-605 | AGGAATTTATGTGGTTATTTAAG | GAUAUAACCAAACAAUCGG | −0.3°C, −1.8°C |
| 726-748 | TGCTTTACATAGAAGTTTTG | AAAUAACCUAAUACAGCA | 4.6°C, 6.9°C |
| 824-846 | TTCTATTTAAATATAATGAAAT | CUUACACAGGAAGAAGUUG | 8.9°C, 7.5°C |
| 934-956 | ATCTATCAACTCTACTTTTAC | GAUAUAACCAAGUAGGAAG | 9.8°C, 8.9°C |
| 977-999 | TGTTATAGTTTCTTATTTAC | UUACAAUAAGCAUUACAAC | −8.0°C, 6.9°C |
| 986-1008 | TTCTATATTAACAAAATTTG | CAUCACACACAAUGUGG | 10.3°C, −2.7°C |
| 1245-1267 | TGGAAAGTATTGTTATATTAC | UUUAUAACCAAACUGCUUCA | 3.5°C, 5.3°C |
| 1254-1276 | MGCTATTATATTATTAATTAC | AAUUAUAACAUAAUCAGC | −8.0°C, 8.7°C |
| 1577-1599 | GACCTAAAAAGCTCATATTTG | AAUAUAACAGAUUUAAACGC | 6.3°C, −3.8°C |
| 1578-1600 | ACCAAAAAGCTAATTTTTTC | CUAUAACAGAUCUUAUUAGG | 4.6°C, −3.8°C |
| 1587-1609 | GTCTAATTTTCTTTAATAAG | UUUAUAACCAAACAGUACG | 0.0°C, 6.3°C |
| 2143-2165 | CCAACAAATTCTTTATTG | CAAUAUAACCAAACAAUUG | 2.8°C, 5.3°C |
| 2271-2293 | CAGTTTTTGTTGCAAAATTTAAC | CAAUAUAACCAAACAAUUG | −1.4°C, 5.6°C |
| 2902-2924 | TCCATTFTTGGCAATTTCAAG | UUUAUAACCAAACAAUUG | 7.4°C, −3.3°C |
Table 6
List of siRNAs with the specifications of membrane glycoprotein (M), ORF3a, ORF8, 3'-UTR and 5'-UTR genes.

| Target gene                  | Target position | Target sequence | RNA oligos sequences | Seed duplex stability (Tm) |
|------------------------------|-----------------|-----------------|----------------------|---------------------------|
| Membrane glycoprotein (M)    | 136-158         | TGTATATAATTAGTTAATTTT   | AAUAAUCAUAUAUAUAUACAA  | 4.6 °C – 5.9 °C          |
|                              | 203-225         | CTGCTTGTACAGAGGTTTAT   | GUAUAUAAUAUAUAUAUU     | –10.3 °C – 11.8 °C       |
|                              | 206-228         | CTGTTTACAGAATATTTAGGATC | CUCUUAAUAUAUAUAACAGG   | 11.3 °C – 11.8 °C        |
| ORF3a (NC_045512.2:25933-26220) | 402-424         | TCCAAAAACCCATTTTCTTGAATG | UAAUGAUUGGUUUGUUUGAAGGA | 4.9 °C – 5.6 °C          |
|                              | 403-425         | TCCAAAAACCCATTTTCTTGAATG | CAAAACACCAUAUAUAUGA     | 6.6 °C – 12.6 °C         |
| ORF8 (NC_045512.2:27894-28259) | 1-23            | ATGAAATTTCTGGTTTTTAGGATC | GAAAUAAUAUAUAUAUAUGAGGA  | 5.5 °C – 0.4 °C          |
|                              | 243-265         | TTCTTGTTACCTTTCTTATTTTTAATG | AUUGUAAAUAUAUAUAUGGAGGA | 7.2 °C – 11.8 °C         |
|                              | 246-266         | TCTCCTTTACTTCTTTCTAATTTTTAATG | CAUUGUAAAUAUAUAUAAGGA  | 6.9 °C – 14.7 °C         |
|                              | 307-329         | TGCTTCTTACAGAAGCTTGTAGGATC | UAAAAGUCUICUAUAAGAAGGA | 3.2 °C – 13.4 °C         |
| 3’-UTR (NC_045512.2:29675-29903) | 126-148         | GCCCTAATGTGTTAAATATTGTTTTTATG | AAUUGUAUGUAUAUAUAUAUUGAAA | –10.3 °C – 13.5 °C       |
|                              | 127-149         | CCAATATTGTTAAATATTGTTTTTATG | AAUUGUAUGUAUAUAUAUAUUGAAA | –4.3 °C – 7.2 °C         |
|                              | 132-154         | ATGTTGTTAATATTTTATTTTATG | ACUAAUAUAUAUAUAUAACAU     | –11.3 °C – 5.6 °C        |
|                              | 192-214         | ATGGACAAAAAAAAAAAAAAA | GUGGUAAUAUAUAUAUAUAUGAGG   | –11.3 °C – 11.3 °C       |
|                              | 194-216         | GCACAAAAAAAACCAUUUUUAAA | UUUUUUUUUUUUUUUUACAU   | –11.3 °C – 11.3 °C       |
| 5’-UTR (NC_045512.2:1-265)   | 123-145         | GCAGATATATATATTACATTAATT | UUGAUAUAUAUAUAUAUAACUGG  | 6.3 °C – 6.3 °C         |
|                              | 125-147         | GCAGATATATATATTACATTAATT | UUGAUAUAUAUAUAUAUAACUGG  | 4.6 °C – 8.0 °C         |

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