Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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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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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′) 21 nt passenger (5′ → 3′)	Seed duplex stability (Tm)	
				Guide	Passenger
nsp2 (NC_045512.2:806-2719)	285–307	TCCCTTAAATTCCATAATCAAGA	UUGAUUAUGGAAUUUAAGGGA CCUUAAAUUCCAUAAUCAAGA	8.7 °C	−4.3 °C
	541–563	CCCCAAAATGCTGTTGTTAAAAT	UUUAACAACAGCAUUUUGGGG CCAAAAUGCUGUUGUUAAAAU	7.2 °C	4.2 °C
	812–834	TTGAAATACTCCAAAAAGAGAAA	UCUCUUUUUGGAGUAUUUCAA GAAAUACUCCAAAAAGAGAAA	10.3 °C	4.6 °C
	1397–1419	GGGAAATTGTTAAATTTATCTCA	AGAUAAAUUUAACAAUUUCCC GAAAUUGUUAAAUUUAUCUCA	1.8 °C	5.3 °C
	1567–1589	TTGAATTTAGGTGAAACATTTGT	AAAUGUUUCACCUAAAUUCAA GAAUUUAGGUGAAACAUUUGU	5.3 °C	−4.3 °C

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 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′) 21 nt passenger (5′ → 3′)	Seed duplex stability (Tm)	
				Guide	Passenger
PLpro/nsp3 (NC_045512.2:2720-8554)	1732–1754	TTGAAAACTGGTGATTTACAACC	UUGUAAAUCACCAGUUUUCAA GAAAACUGGUGAUUUACAACC	6.9 °C	10.3 °C
	88–110	AGGATTGATAAAGTACTTAATGA	AUUAAGUACUUUAUCAAUCCU GAUUGAUAAAGUACUUAAUGA	6.6 °C	8.7 °C
	609–631	GACTATTGAAGTGAATAGTTTTA	AAACUAUUCACUUCAAUAGUC CUAUUGAAGUGAAUAGUUUUA	4.6 °C	8.9 °C
	652–674	GACAATGTATACATTAAAAATGC	AUUUUUAAUGUAUACAUUGUC CAAUGUAUACAUUAAAAAUGC	−9.1 °C	6.7 °C
	727–749	GCCAATGTTTACCTTAAACATGG	AUGUUUAAGGUAAACAUUGGC CAAUGUUUACCUUAAACAUGG	7.2 °C	5.3 °C
	945–967	TGCTTATGAAAATTTTAATCAGC	UGAUUAAAAUUUUCAUAAGCA CUUAUGAAAAUUUUAAUCAGC	2.1 °C	8.9 °C
	1172–1194	AGGAAGTTAAGCCATTTATAACT	UUAUAAAUGGCUUAACUUCCU GAAGUUAAGCCAUUUAUAACU	−8.0 °C	4.9 °C
	1579–1601	GTGCTTAAAAAGTGTAAAAGTGC	ACUUUUACACUUUUUAAGCAC GCUUAAAAAGUGUAAAAGUGC	4.9 °C	−3.8 °C
	1590–1612	GTGTAAAAGTGCCTTTTACATTC	AUGUAAAAGGCACUUUUACAC GUAAAAGUGCCUUUUACAUUC	7.2 °C	4.9 °C
	1743–1765	TTCAACTATACAGCGTAAATATA	UAUUUACGCUGUAUAGUUGAA CAACUAUACAGCGUAAAUAUA	6.9 °C	6.3 °C
	1813–1835	TACTTTTACACCAGTAAAACAAC	UGUUUUACUGGUGUAAAAGUA CUUUUACACCAGUAAAACAAC	8.2 °C	7.2 °C
	1997–2019	CAGCGTATAATGGTTATCTTACT	UAAGAUAACCAUUAUACGCUG GCGUAUAAUGGUUAUCUUACU	6.9 °C	8.5 °C
	2136–2158	AGGTGATAAAAGTGTATATTACA	UAAUAUACACUUUUAUCACCU GUGAUAAAAGUGUAUAUUACA	1.1 °C	8.9 °C
	2138–2160	GTGATAAAAGTGTATATTACACT	UGUAAUAUACACUUUUAUCAC GAUAAAAGUGUAUAUUACACU	1.1 °C	−4.3 °C
	2387–2409	AAGGTAAAACATTTTATGTTTTA	AAACAUAAAAUGUUUUACCUU GGUAAAACAUUUUAUGUUUUA	6.9 °C	8.2 °C
	2490–2512	AGCATTAAATCACACTAAAAAGT	UUUUUAGUGUGAUUUAAUGCU CAUUAAAUCACACUAAAAAGU	4.9 °C	−10.3 °C
	2522–2544	CACAAGTTAATGGTTTAACTTCT	AAGUUAAACCAUUAACUUGUG CAAGUUAAUGGUUUAACUUCU	4.9 °C	4.9 °C
	2531–2553	ATGGTTTAACTTCTATTAAATGG	AUUUAAUAGAAGUUAAACCAU GGUUUAACUUCUAUUAAAUGG	−7.5 °C	8.2 °C
	2868–2890	TTCTTATGAACAATTTAAGAAAG	UUCUUAAAUUGUUCAUAAGAA CUUAUGAACAAUUUAAGAAAG	7.1 °C	8.9 °C
	2913–2935	TGGTAAACAAGCTACAAAATATC	UAUUUUGUAGCUUGUUUACCA GUAAACAAGCUACAAAAUAUC	5.3 °C	7.2 °C
	3047–3069	GTCACTATAAACATATAACTTCT	AAGUUAUAUGUUUAUAGUGAC CACUAUAAACAUAUAACUUCU	6.3 °C	6.3 °C
	3056–3078	AACATATAACTTCTAAAGAAACT	UUUCUUUAGAAGUUAUAUGUU CAUAUAACUUCUAAAGAAACU	7.1 °C	1.1 °C
	3172–3194	ACCATAAAACCAGTTACTTATAA	AUAAGUAACUGGUUUUAUGGU CAUAAAACCAGUUACUUAUAA	6.6 °C	−0.3 °C

(continued on next page)

Table 2 (*continued*)

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)	
				Guide	Passenger
	3224–3246	ACCCTAAGTTGGACAATTATTAT	AAUAAUUGUCCAACUUAGGGU CCUAAGUUGGACAAUUAUUAU	−1.8 °C	9.8 °C
	3524–3546	ATGTTAACAATGCAACTAATAAA	UAUUAGUUGCAUUGUUAACAU GUUAACAAUGCAACUAAUAAA	4.6 °C	7.2 °C
	3782–3804	CAGCAAATAATAGTTTAAAAATT	UUUUUAAACUAUUAUUUGCUG GCAAAUAAUAGUUUAAAAAUU	−9.1 °C	−1.4 °C
	3847–3869	GACAATTCTAGTCTTACTATTAA	AAUAGUAAGACUAGAAUUGUC CAAUUCUAGUCUUACUAUUAA	6.3 °C	6.9 °C
	88–110	GCCTTTTCTTAACAAAGTTGTTA	ACAACUUUGUUAAGAAAAGGC CUUUUCUUAACAAAGUUGUUA	10.3 °C	5.5 °C
	4041–4063	TACTAATTATATGCCTTATTTCT	AAAUAAGGCAUAUAAUUAGUA CUAAUUAUAUGCCUUAUUUCU	10.9 °C	−8.0 °C
	4051–4073	ATGCCTTATTTCTTTACTTTATT	UAAAGUAAAGAAAUAAGGCAU GCCUUAUUUCUUUACUUUAUU	4.9 °C	10.9 °C
	4052–4074	TGCCTTATTTCTTTACTTTATTG	AUAAAGUAAAGAAAUAAGGCA CCUUAUUUCUUUACUUUAUUG	6.6 °C	−4.3 °C
	4053–4075	GCCTTATTTCTTTACTTTATTGC	AAUAAAGUAAAGAAAUAAGGC CUUAUUUCUUUACUUUAUUGC	4.6 °C	2.1 °C
	4073–4095	TGCTACAATTGTGTACTTTTACT	UAAAAGUACACAAUUGUAGCA CUACAAUUGUGUACUUUUACU	4.9 °C	6.9 °C
	4098–4120	AAGTACAAATTCTAGAATTAAAG	UUAAUUCUAGAAUUUGUACUU GUACAAAUUCUAGAAUUAAAG	6.9 °C	6.9 °C
	4220–4242	AACTGATAAATATTATAATTTGG	AAAUUAUAAUAUUUAUCAGUU CUGAUAAAUAUUAUAAUUUGG	−8.0 °C	8.9 °C
	4296–4318	AGGTGTTTTAATGTCTAATTTAG	AAAUUAGACAUUAAAACACCU GUGUUUUAAUGUCUAAUUUAG	6.9 °C	7.2 °C
	4452–4474	TTCTTTAGAAACTATACAAATTA	AUUUGUAUAGUUUCUAAAGAA CUUUAGAAACUAUACAAAUUA	6.9 °C	7.1 °C
	4524–4546	GTGGTTTTTGGCATATATTCTTT	AGAAUAUAUGCCAAAAACCAC GGUUUUUGGCAUAUAUUCUUU	3.5 °C	5.6 °C
	4600–4622	AGCTATTTTGCAGTACATTTTAT	AAAAUGUACUGCAAAAUAGCU CUAUUUUGCAGUACAUUUUAU	6.9 °C	−1.4 °C
	4610–4632	CAGTACATTTTATTAGTAATTCT	AAUUACUAAUAAAAUGUACUG GUACAUUUUAUUAGUAAUUCU	6.3 °C	6.9 °C
	4972–4994	CAGTTTAAAAGACCAATAAATCC	AUUUAUUGGUCUUUUAAACUG GUUUAAAAGACCAAUAAAUCC	−1.4 °C	−9.1 °C
	5100–5122	CTCTCATTTTGTTAACTTAGACA	UCUAAGUUAACAAAAUGAGAG CUCAUUUUGUUAACUUAGACA	9.8 °C	7.4 °C
	5153–5175	TGCCTATTAATGTTATAGTTTTT	AAACUAUAACAUUAAUAGGCA CCUAUUAAUGUUAUAGUUUUU	6.3 °C	−2.3 °C
	5154–5176	GCCTATTAATGTTATAGTTTTTG	AAAACUAUAACAUUAAUAGGC CUAUUAAUGUUAUAGUUUUUG	4.6 °C	−8.0 °C
	5168–5190	TAGTTTTTGATGGTAAATCAAAA	UUGAUUUACCAUCAAAAACUA GUUUUUGAUGGUAAAUCAAAA	8.9 °C	7.7 °C

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 (Rd-Rp), 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). The envelope protein (E), spike glycoprotein (S), nucleocapsid phosphoprotein (N), and membrane glycoprotein (M) are considered to be potential drug/vaccine targets (Naqvi et al., 2020). By comparing the inflammatory pathways and cytokine responses during SARS-CoV, MERS-CoV, and SARS-CoV2 infections, it has been recognized that ORF8 triggers DNA synthesis and ORF3a triggers necrotic cell death. And also nsp2 does proofreading which is necessary for viral replication (Naqvi et al., 2020). The 5′ and 3′-UTR sequences are necessary for RNA replication and transcription (Wu et al., 2005).

The siRNAs can silence the targeted genes and also inhibit the replication of the virus. Similar studies were reported for the previous SARS viruses (Li et al., 2005).

In this study, siRNAs have been designed targeting specifically conserved sequences and also other potential drug targets. These are: nsp5, nsp3, nsp12, nsp7, nsp13, nsp3, envelope protein (E), spike glycoprotein (S), nucleoprotein (N), membrane protein (M), ORF8, ORF3a, nsp2, 5′- and 3′-UTR. These siRNA sequences have the probable capability to inhibit viral replication as well as further viral infection. These sequence designs might support COVID-19 management, if found effective in drug delivery through liposome.

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 Biotechnological 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

Table 3
List of siRNAs with the specifications of nsp5, nsp7 and nsp12 genes.

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)	
				Guide	Passenger
3CLpro/Mpro/nsp5 (NC_045512.2:10055-10972)	151–173	AACCCTAATTATGAAGATTTACT	UAAAUCUUCAUAAUUAGGGUU CCCUAAUUAUGAAGAUUUACU	5.3 °C	10.9 °C
	153–175	CCCTAATTATGAAGATTTACTCA	AGUAAAUCUUCAUAAUUAGGG CUAAUUAUGAAGAUUUACUCA	10.0 °C	−8.0 °C
	444–466	TGGTTTTAACATAGATTATGACT	UCAUAAUCUAUGUUAAAACCA GUUUUAACAUAGAUUAUGACU	8.7 °C	0.0 °C
	526–548	GACTTAGAAGGTAACTTTTATGG	AUAAAAGUUACCUUCUAAGUC CUUAGAAGGUAACUUUUAUGG	4.9 °C	11.7 °C
	538–560	AACTTTTATGGACCTTTTGTTGA	AACAAAAGGUCCAUAAAAGUU CUUUUAUGGACCUUUUGUUGA	10.3 °C	−1.4 °C
	594–616	AACTATTACAGTTAATGTTTTAG	AAAACAUUAACUGUAAUAGUU CUAUUACAGUUAAUGUUUUAG	5.3 °C	8.5 °C
	795–817	TGCTTCATTAAAAGAATTACTGC	AGUAAUUCUUUUAAUGAAGCA CUUCAUUAAAAGAAUUACUGC	10.0 °C	8.9 °C
nsp7 (NC_045512.2:11843-12091)	62–84	GAGTAGAATCATCATCTAAATTG	AUUUAGAUGAUGAUUCUACUC GUAGAAUCAUCAUCUAAAUUG	6.9 °C	16.0 °C
	65–87	TAGAATCATCATCTAAATTGTGG	ACAAUUUAGAUGAUGAUUCUA GAAUCAUCAUCUAAAUUGUGG	−1.4 °C	16.2 °C
RdRp/nsp12 (NC_045512.2:13442-16236)	133–155	TTGCTAAATTCCTAAAAACTAAT	UAGUUUUUAGGAAUUUAGCAA GCUAAAUUCCUAAAAACUAAU	3.2 °C	−4.3 °C
	134–156	TGCTAAATTCCTAAAAACTAATT	UUAGUUUUUAGGAAUUUAGCA CUAAAUUCCUAAAAACUAAUU	4.9 °C	2.1 °C
	297–319	GACTTCTTTAAGTTTAGAATAGA	UAUUCUAAACUUAAAGAAGUC CUUCUUUAAGUUUAGAAUAGA	6.9 °C	7.1 °C
	407–429	AGGTAATTGTGACACATTAAAAG	UUUAAUGUGUCACAAUUACCU GUAAUUGUGACACAUUAAAAG	6.9 °C	6.9 °C
	417–439	GACACATTAAAAGAAATACTTGT	AAGUAUUUCUUUUAAUGUGUC CACAUUAAAAGAAAUACUUGU	4.6 °C	6.9 °C
	457–479	ATGATGATTATTTCAATAAAAAG	UUUUAUUGAAAUAAUCAUCAU GAUGAUUAUUUCAAUAAAAAG	−1.4 °C	8.7 °C
	704–726	TTCTTATTATTCATTGTTAATGC	AUUAACAAUGAAUAAUAAGAA CUUAUUAUUCAUUGUUAAUGC	7.2 °C	−8.0 °C
	792–814	TACATTAAGTGGGATTTGTTAAA	UAACAAAUCCCACUUAAUGUA CAUUAAGUGGGAUUUGUUAAA	5.3 °C	4.6 °C
	1573–1595	ATGCACTTTTCGCATATACAAAA	UUGUAUAUGCGAAAAGUGCAU GCACUUUUCGCAUAUACAAAA	8.2 °C	10.3 °C
	1711–1733	TTCATCAAAAATTATTGAAATCA	AUUUCAAUAAUUUUUGAUGAA CAUCAAAAAUUAUUGAAAUCA	7.4 °C	7.4 °C
	1800–1822	ATGTTAAAAACTGTTTATAGTGA	ACUAUAAACAGUUUUUAACAU GUUAAAAACUGUUUAUAGUGA	−2.3 °C	−9.1 °C
	2066–2088	TGCTAATAGTGTTTTTAACATTT	AUGUUAAAAACACUAUUAGCA CUAAUAGUGUUUUUAACAUUU	7.2 °C	6.3 °C
	2103–2125	GCCAATGTTAATGCACTTTTATC	UAAAAGUGCAUUAACAUUGGC CAAUGUUAAUGCACUUUUAUC	10.3 °C	6.9 °C
	2136–2158	AACAAAATTGCCGATAAGTATGT	AUACUUAUCGGCAAUUUUGUU CAAAAUUGCCGAUAAGUAUGU	6.3 °C	−3.3 °C
	2236–2258	ACGCATATTTGCGTAAACATTTC	AAUGUUUACGCAAAUAUGCGU GCAUAUUUGCGUAAACAUUUC	6.9 °C	−1.8 °C
	2237–2259	CGCATATTTGCGTAAACATTTCT	AAAUGUUUACGCAAAUAUGCG CAUAUUUGCGUAAACAUUUCU	5.3 °C	−1.8 °C
	2340–2362	AACTTTAAGTCAGTTCTTTATTA	AUAAAGAACUGACUUAAAGUU CUUUAAGUCAGUUCUUUAUUA	7.1 °C	4.9 °C
	2362–2384	ATCAAAACAATGTTTTTATGTCT	ACAUAAAAACAUUGUUUUGAU CAAAACAAUGUUUUUAUGUCU	−1.4 °C	5.6 °C

[NC_045512.2:2720-8554], Rd-Rp or non-structural protein 12/nsp12 [NC_045512.2:13442-16236], non-structural protein 7/nsp7 [NC_045512.2:11843-12091], spike glycoprotein (S) [NC_045512.2:21563-25384], envelope protein (E) [NC_045512.2:26245-26472], membrane glycoprotein (M) [NC_045512.2:26523-27191], nucleocapsid phosphoprotein/nucleoprotein (N) [NC_045512.2:28274-29533], Open Reading Frame 8 (ORF8) [NC_045512.2:27894-28259], Open Reading Frame 3a (ORF3a) [NC_045512.2:25393-26220], non-structural protein 2(nsp2) [NC_045512.2:806-2719], Untranslated Region 5′ (5′-UTR) [NC_045512.2:1-265] and Untranslated Region 3′ (3′-UTR) [NC_045512.2:29675-29903].

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′terminus of the siRNA guide strand, (b) G/C in region 19, (c) AU richness (AU ≥4) in regions 1–7, and (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

Table 4
List of siRNAs with the specifications of nsp13, envelope protein (E) and nucleocapsid phosphoprotein (N) genes.

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)	
				Guide	Passenger
ZD, NTPase/HEL/nsp13 (NC_045512.2:16237-18039)	260–282	GACAAGTTTTTGGTTTATATAAA	UAUAUAAACCAAAAACUUGUC CAAGUUUUUGGUUUAUAUAAA	−8.0 °C	3.2 °C
	269–291	TTGGTTTATATAAAAATACATGT	AUGUAUUUUUAUAUAAACCAA GGUUUAUAUAAAAAUACAUGU	6.9 °C	1.4 °C
	568–590	AACAGTAAAGTACAAATAGGAGA	UCCUAUUUGUACUUUACUGUU CAGUAAAGUACAAAUAGGAGA	10.9 °C	9.8 °C
	1410–1432	ATGCTTTAAAATGTTTTATAAGG	UUAUAAAACAUUUUAAAGCAU GCUUUAAAAUGUUUUAUAAGG	−7.5 °C	−3.8 °C
	1516–1538	TGGAGAAAAGCTGTCTTTATTTC	AAUAAAGACAGCUUUUCUCCA GAGAAAAGCUGUCUUUAUUUC	6.9 °C	10.3 °C
	1528–1550	GTCTTTATTTCACCTTATAATTC	AUUAUAAGGUGAAAUAAAGAC CUUUAUUUCACCUUAUAAUUC	−2.3 °C	−9.7 °C
	1665–1687	TTGTAATGTAAACAGATTTAATG	UUAAAUCUGUUUACAUUACAA GUAAUGUAAACAGAUUUAAUG	6.9 °C	8.5 °C
	1700–1722	GAGCAAAAGTAGGCATACTTTGC	AAAGUAUGCCUACUUUUGCUC GCAAAAGUAGGCAUACUUUGC	11.6 °C	10.3 °C
Envelope protein (E) (NC_045512.2:26245-26472)	149–171	GTCTTGTAAAACCTTCTTTTTAC	AAAAAGAAGGUUUUACAAGAC CUUGUAAAACCUUCUUUUUAC	5.5 °C	7.2 °C
	170–192	ACGTTTACTCTCGTGTTAAAAAT	UUUUAACACGAGAGUAAACGU GUUUACUCUCGUGUUAAAAAU	7.2 °C	14.6 °C
Nucleocapsid phosphor protein (N) (NC_045512.2:28274-29533)	1064–1086	AGCATATTGACGCATACAAAACA	UUUUGUAUGCGUCAAUAUGCU CAUAUUGACGCAUACAAAACA	6.9 °C	8.7 °C
	1021–1043	GACAAAGATCCAAATTTCAAAGA	UUUGAAAUUUGGAUCUUUGUC CAAAGAUCCAAAUUUCAAAGA	7.4 °C	14.8 °C

colleagues (Naito et al., 2009). The siRNAs are satisfactory according to the Ui-Tei rule chosen in the default parameter as stated by Ui-Tei, K., and colleagues (Naito and Ui-Tei, 2012).

2.4. Target sequence selection& functional siRNA designing

21 nt targets with 2 nt overhang highly functional sequences were 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.

2.5. Off-target effect-reduced siRNA sequence selection

The siRNAs (both guide and other passenger strands) with low melting temperature (*T*m) were chosen to avoid the seed-dependent off-target silencing. Even though the benchmark was fixed as *T*m of 21.5 °C (Ui-Tei et al., 2008), in this study we tried to select nearly *T*m < 10 °C.

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/nsp5), non-structural protein 7/nsp7, RNA dependent RNA polymerase shortly Rd-Rp or non-structural protein 12/nsp12, 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 necessary for proofreading of viral replication (Naqvi et al., 2020). Both sequences of the 5′ and 3′-UTR are crucial for RNA replication and transcription (Wu et al., 2005).

RNA interference (RNAi) is a widely applied approach by which small interfering RNA (siRNA) also known as silencing RNA, silence a specific target gene with a perfectly complementary sequence, for the purpose of therapeutic usage in human diseases (Ketting, 2011). siRNA is typically 21 nt in length, and is the functional agent in RNAi, and acts as a guide for specific mRNA degradation (Elbashir et al., 2001a; Elbashir et al., 2001b). In the mammal family, siRNA is thought to have potential, not only for the gene silencing necessary for functional genomics, but also for medicinal goals, including antiviral therapy (Gitlin and Andino, 2003; Saadat, 2013).

To design specific and effective siRNA (21 nt), a practical guideline has been proposed by Ui-Tei, K., and colleagues named the “Ui-Tei rule”. The siRNA chosen according to the Ui-Tei rule persuades the subsequent four ambiances concurrently: (a) A/U in region 1 from the 5′terminus of the siRNA guide strand, (b) G/C in region 19, (c) AU richness (AU ≥4) in

Table 5
List of siRNAs with the specifications of spike glycoprotein (S) gene.

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)	
				Guide	Passenger
Spike glycoprotein (S) (NC_045512.2:21563-25384)	167–189	TACCTTTCTTTTCCAATGTTACT	UAACAUUGGAAAAGAAAGGUA CCUUUCUUUUCCAAUGUUACU	12.1 °C	10.3 °C
	222–244	TGGTACTAAGAGGTTTGATAACC	UUAUCAAACCUCUUAGUACCA GUACUAAGAGGUUUGAUAACC	8.9 °C	11.3 °C
	310–332	TGGATTTTTGGTACTACTTTAGA	UAAAGUAGUACCAAAAAUCCA GAUUUUUGGUACUACUUUAGA	9.8 °C	−3.3 °C
	365–387	ACGCTACTAATGTTGTTATTAAA	UAAUAACAACAUUAGUAGCGU GCUACUAAUGUUGUUAUUAAA	6.9 °C	11.3 °C
	366–388	CGCTACTAATGTTGTTATTAAAG	UUAAUAACAACAUUAGUAGCG CUACUAAUGUUGUUAUUAAAG	1.4 °C	6.3 °C
	369–391	TACTAATGTTGTTATTAAAGTCT	ACUUUAAUAACAACAUUAGUA CUAAUGUUGUUAUUAAAGUCU	−4.3 °C	6.9 °C
	390–412	CTGTGAATTTCAATTTTGTAATG	UUACAAAAUUGAAAUUCACAG GUGAAUUUCAAUUUUGUAAUG	7.2 °C	7.4 °C
	413–435	ATCCATTTTTGGGTGTTTATTAC	AAUAAACACCCAAAAAUGGAU CCAUUUUUGGGUGUUUAUUAC	6.9 °C	−3.3 °C
	414–436	TCCATTTTTGGGTGTTTATTACC	UAAUAAACACCCAAAAAUGGA CAUUUUUGGGUGUUUAUUACC	−0.3 °C	−3.3 °C
	486–508	TGCGAATAATTGCACTTTTGAAT	UCAAAAGUGCAAUUAUUCGCA CGAAUAAUUGCACUUUUGAAU	10.3 °C	1.8 °C
	540–562	AGGAAAACAGGGTAATTTCAAAA	UUGAAAUUACCCUGUUUUCCU GAAAACAGGGUAAUUUCAAAA	7.4 °C	10.3 °C
	548–570	AGGGTAATTTCAAAAATCTTAGG	UAAGAUUUUUGAAAUUACCCU GGUAAUUUCAAAAAUCUUAGG	5.3 °C	−0.3 °C
	568–590	AGGGAATTTGTGTTTAAGAATAT	AUUCUUAAACACAAAUUCCCU GGAAUUUGUGUUUAAGAAUAU	7.1 °C	7.4 °C
	569–591	GGGAATTTGTGTTTAAGAATATT	UAUUCUUAAACACAAAUUCCC GAAUUUGUGUUUAAGAAUAUU	6.9 °C	5.3 °C
	583–605	AAGAATATTGATGGTTATTTTAA	AAAAUAACCAUCAAUAUUCUU GAAUAUUGAUGGUUAUUUUAA	−0.3 °C	−1.8 °C
	726–748	TGCTTTACATAGAAGTTATTTGA	AAAUAACUUCUAUGUAAAGCA CUUUACAUAGAAGUUAUUUGA	4.6 °C	6.9 °C
	824–846	TTCTATTAAAATATAATGAAAAT	UUUCAUUAUAUUUUAAUAGAA CUAUUAAAAUAUAAUGAAAAU	8.9 °C	−7.5 °C
	934–956	ATCTATCAAACTTCTAACTTTAG	AAAGUUAGAAGUUUGAUAGAU CUAUCAAACUUCUAACUUUAG	9.8 °C	8.9 °C
	977–999	TTGTTAGATTTCCTAATATTACA	UAAUAUUAGGAAAUCUAACAA GUUAGAUUUCCUAAUAUUACA	−8.0 °C	6.9 °C
	986–1008	TTCCTAATATTACAAACTTGTGC	ACAAGUUUGUAAUAUUAGGAA CCUAAUAUUACAAACUUGUGC	10.3 °C	−2.7 °C
	1245–1267	TGGAAAGATTGCTGATTATAATT	UUAUAAUCAGCAAUCUUUCCA GAAAGAUUGCUGAUUAUAAUU	3.5 °C	5.3 °C
	1254–1276	TGCTGATTATAATTATAAATTAC	AAUUUAUAAUUAUAAUCAGCA CUGAUUAUAAUUAUAAAUUAC	−8.0 °C	8.7 °C
	1577–1599	GACCTAAAAAGTCTACTAATTTG	AAUUAGUAGACUUUUUAGGUC CCUAAAAAGUCUACUAAUUUG	6.3 °C	−3.8 °C
	1578–1600	ACCTAAAAAGTCTACTAATTTGG	AAAUUAGUAGACUUUUUAGGU CUAAAAAGUCUACUAAUUUGG	4.6 °C	−3.8 °C
	1587–1609	GTCTACTAATTTGGTTAAAAACA	UUUUUAACCAAAUUAGUAGAC CUACUAAUUUGGUUAAAAACA	0.0 °C	6.3 °C
	2143–2165	CCCACAAATTTTACTATTAGTGT	ACUAAUAGUAAAAUUUGUGGG CACAAAUUUUACUAUUAGUGU	2.8 °C	5.3 °C
	2271–2293	CAGTTTTTGTACACAATTAAACC	UUUAAUUGUGUACAAAAACUG GUUUUUGUACACAAUUAAACC	−1.4 °C	5.6 °C
	2902–2924	TCCAATTTTGGTGCAATTTCAAG	UGAAAUUGCACCAAAAUUGGA CAAUUUUGGUGCAAUUUCAAG	7.4 °C	−3.3 °C

regions 1–7, and (d) the absence of long GC stretches ≥10 (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 appropriate mode of administration. Further experimental validation and related trials are needed to confirm these findings.

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 21 nt target + 2 nt overhang	RNA oligo sequences 21 nt guide (5′ → 3′) 21 nt passenger (5′ → 3′)	Seed duplex stability (Tm)	
				Guide	Passenger
Membrane glycoprotein (M) (NC_045512.2:26523-27191)	136–158	TTGTATATAATTAAGTTAATTTT	AAUUAACUUAAUUAUAUACAA GUAUAUAAUUAAGUUAAUUUU	4.6 °C	−5.9 °C
	203–225	CTGCTGTTTACAGAATAAATTGG	AAUUUAUUCUGUAAACAGCAG GCUGUUUACAGAAUAAAUUGG	−10.3 °C	11.8 °C
	206–228	CTGTTTACAGAATAAATTGGATC	UCCAAUUUAUUCUGUAAACAG GUUUACAGAAUAAAUUGGAUC	11.3 °C	11.8 °C
ORF3a (NC_045512.2:25393-26220)	402–424	TTCCAAAAACCCATTACTTTATG	UAAAGUAAUGGGUUUUUGGAA CCAAAAACCCAUUACUUUAUG	4.9 °C	5.6 °C
	403–425	TCCAAAAACCCATTACTTTATGA	AUAAAGUAAUGGGUUUUUGGA CAAAAACCCAUUACUUUAUGA	6.6 °C	12.6 °C
ORF8 (NC_045512.2:27894-28259)	1–23	ATGAAATTTCTTGTTTTCTTAGG	UAAGAAAACAAGAAAUUUCAU GAAAUUUCUUGUUUUCUUAGG	5.5 °C	0.4 °C
	243–265	TTCCTGTTTACCTTTTACAATTA	AUUGUAAAAGGUAAACAGGAA CCUGUUUACCUUUUACAAUUA	7.2 °C	11.8 °C
	244–266	TCCTGTTTACCTTTTACAATTAA	AAUUGUAAAAGGUAAACAGGA CUGUUUACCUUUUACAAUUAA	6.9 °C	14.7 °C
	307–329	TCGTTCTATGAAGACTTTTTAGA	UAAAAAGUCUUCAUAGAACGA GUUCUAUGAAGACUUUUUAGA	3.2 °C	13.4 °C
3′-UTR (NC_045512.2:29675-29903)	126–148	GCCCTAATGTGTAAAATTAATTT	AUUAAUUUUACACAUUAGGGC CCUAAUGUGUAAAAUUAAUUU	−9.7 °C	11.6 °C
	127–149	CCCTAATGTGTAAAATTAATTTT	AAUUAAUUUUACACAUUAGGG CUAAUGUGUAAAAUUAAUUUU	−10.3 °C	13.5 °C
	132–154	ATGTGTAAAATTAATTTTAGTAG	ACUAAAAUUAAUUUUACACAU GUGUAAAAUUAAUUUUAGUAG	−4.3 °C	7.2 °C
	192–214	ATGACAAAAAAAAAAAAAAAAAA	UUUUUUUUUUUUUUUUUGUCAU GACAAAAAAAAAAAAAAAAAA	−11.3 °C	5.6 °C
	194–216	GACAAAAAAAAAAAAAAAAAAAA	UUUUUUUUUUUUUUUUUUGUC CAAAAAAAAAAAAAAAAAAAA	−11.3 °C	−11.3 °C
5′-UTR (NC_045512.2:1-265)	123–145	CGCAGTATAATTAATAACTAATT	UUAGUUAUUAAUUAUACUGCG CAGUAUAAUUAAUAACUAAUU	6.3 °C	6.3 °C
	125–147	CAGTATAATTAATAACTAATTAC	AAUUAGUUAUUAAUUAUACUG GUAUAAUUAAUAACUAAUUAC	4.6 °C	−8.0 °C

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Declaration of competing interest

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