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.

Genomics 113 (2021) 1221–1232

Contents lists available at ScienceDirect

Genomics

journal homepage: www.elsevier.com/locate/ygeno

Review

Genetics and genomics of SARS-CoV-2: A review of the literature with the special focus on genetic diversity and SARS-CoV-2 genome detection

Azadeh Rahimi[a], Azin Mirzazadeh[b,c], Soheil Tavakolpour[d,*]

[a] Department of Genetics and Molecular Biology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
[b] Department of Medical Genetics, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[c] Joint Bioinformatics Graduate Program, University of Arkansas Little Rock and University of Arkansas for Medical Sciences, Little Rock, AR, United States
[d] Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02215, United States

ARTICLE INFO

Keywords:
Coronavirus
SARS-CoV-2
COVID-19
Genetics
Vaccine
Treatment
Molecular diagnosis

ABSTRACT

The outbreak of 2019-novel coronavirus disease (COVID-19), caused by SARS-CoV-2, started in late 2019; in a short time, it has spread rapidly all over the world. Although some possible antiviral and anti-inflammatory medications are available, thousands of people are dying daily. Well-understanding of the SARS-CoV-2 genome is not only essential for the development of new treatments/vaccines, but it also can be used for improving the sensitivity and specificity of current approaches for virus detection. Accordingly, we reviewed the most critical findings related to the genetics of the SARS-CoV-2, with a specific focus on genetic diversity and reported mutations, molecular-based diagnosis assays, using interfering RNA technology for the treatment of patients, and genetic-related vaccination strategies. Additionally, considering the unanswered questions or uncertainties in these regards, different topics were discussed.

1. An overview of the SARS-CoV-2

In the late December 2019, a novel virus called Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), also known as 2019 novel coronavirus (2019-nCoV), was reported with an unidentified source [1]. The genomic sequence of this newly emerged virus is highly similar to that of severe acute respiratory syndrome coronavirus (SARS-CoV) with a 79.6% sequence identity [2]. The SARS-CoV-2 causes coronavirus disease 2019 (COVID-19), a respiratory tract infection, with a clinical spectrum ranging from asymptomatic to acute respiratory distress syndrome. On March 11, 2020, COVID-19 was declared as a global pandemic and the sixth public health emergency worldwide by the World Health Organization (WHO). During the recent months, some antivirals (e.g., remdesivir) and anti-inflammatory medications (e.g., tocilizumab) showed acceptable outcomes to viral clearance and amelioration of patients' symptoms [3,4,184]. Additionally, following the initial release of some researches for coronavirus vaccine, the spectrum of hope moves from cure to hope for prevention. As of September 19, 2020, 27 vaccines are in Phase 1, 15 in Phase 2, nine in Phase 3, and five have been approved for limited use [5,6]. However, no approval has been granted by the United States Food and Drug Administration (FDA) so far. Understanding the genetics of SARS-CoV-2 may open some new avenues for the development of more effective and safer targeted therapies and even new generations of vaccines for this highly mutable coronavirus. In this review, we tried to cover the most critical and up-to-date findings related to the genetics of SARS-CoV-2 to be used for developing the current strategies of diagnosis, and treatment platforms.

2. The origin and genomic structure of SARS-CoV-2

Firstly, the genome sequence of SARS-CoV-2 was released in GenBank on January 11, 2020 (accession no. MN908947.3) [7]. Based on the sequence alignment and evolutionary tree analysis, SARS-CoV-2 is now considered as the newest member of the lineage B of genus *Betacoronavirus* (β-CoV) in the family of Coronaviridae of the order *Nidovirales* [1]. The initial comparative genomic analysis illustrated that SARS-CoV-2 has almost 79% and 50% sequence identity with SARS-CoV and the Middle East respiratory syndrome (MERS), respectively [2,8]. According to phylogenetic analysis, SARS-CoV-2 is more similar to SARS-CoV than MERS-CoV. It is worthy to note that based on homology modeling studies, it has been shown that SARS-CoV-2 shares 96.2% homology with the BatCoV RaTG13, a bat coronavirus from *Rhinolophus affinis* [2].

SARS-CoV-2 virus belongs to the B lineage of the β-coronaviruses (β-CoVs); this family comprises an enveloped, non-segmented, positive-

* Corresponding author.
E-mail addresses: Soheil_tavakolpour@dfci.harvard.edu, soheil.tavakolpour@gmail.com (S. Tavakolpour).

https://doi.org/10.1016/j.ygeno.2020.09.059
Received 30 June 2020; Received in revised form 26 September 2020; Accepted 28 September 2020
Available online 30 September 2020

sense single-stranded RNA virus genome, with a 5′ cap structure and 3′ poly-A tail, allowing to perform as an mRNA for translation of the replicase polyproteins [9]. Based on the data of whole-genome analyses, the genome of β-CoVs encodes several non-structural and four structural proteins, including spike (S), envelope (E), membrane (M), and nucleocapsid (N) [10,11]. Among the known RNA viruses, coronaviruses, which are single-stranded and positive-sense RNA viruses have the largest genome between other RNA viruses, with the GC content ranging from 32% to 43% [12,13]. The SARS-CoV-2 genomic sequences exhibit a diverse length from 29.8 kb to 29.9 kb with 12 open reading frames (ORFs) encoding 27 proteins [14,15]. The genomic organization includes 5′- leader sequence- ORF1/ab- S- ORF3a- E- M- ORF6a- ORF7a- ORF7b- ORF8- N- ORF10-3′ from left to right and lacks the hemagglutinin-esterase gene which is detected in some β-CoVs [16]. A series of transcription regulatory sequences (TRS) is situated at the junction between each of these ORFs as well as at the 5′ end of the genomic RNA downstream of the leader sequence of β-CoVs [17]. About two-thirds of SARS-CoV-2's RNA comprises ORF1a/b region, which with 16 non-structural proteins (nsp1-16) is considered as the largest ORF. The remaining one-third of the genome near the 3′-terminus contains ORFs encode structural and accessory proteins [10].

Fig. 1 shows a schema of the SARS-CoV-2 genome along with the most critical reported diversities in its different parts, as well as the general structure of the SARS-CoV-2.

3. Genetic diversity of SARS-CoV-2

Similar to the other RNA viruses, genetic diversity in SARS-CoV-2 is critical for its fitness, survival, and probably its pathogenesis. In a study performed on the origin of SARS-CoV-2, it was shown that random mutations and recombination are two main sources for genetic diversity in this virus [18,19]. In this regard, nine putative recombinant patterns were identified in the SARS-CoV-2 genome, including six critical recombination regions in the *S* gene, and one in each of *RdRp*, *nsp13*, and *ORF3a* [19]. Additionally, the results of the genomic analyses suggest that the receptor binding motif in SARS-CoV-2 could possibly arise from the occurrence of a recombination between the strains of coronavirus found in the pangolin and RaTG13 [20,21]. According to data extracted from the public database of the Global Initiative on Sharing All Influenza Data (GISAID) as of September 2020, SARS-CoV-2 virus has a mutation rate of about 8×10^{-4} nucleotides/genome per year [22]. Although this rate is too much for an RNA virus [23–25]. Analyzing 220 genomic sequences from the GISAID database revealed that the number and the occurrence of mutations are significantly higher in Europe, and North America as compared to Asia; this suggests probably different mutation patterns [26]. The distribution of SARS-CoV-2 mutation patterns shows a difference in time, geography, and age, but the similarity in gender [27]. However, according to a recent study on SARS-CoV-2 mutations in the United States, genome samples isolated from women patients have shown a higher mutation rate compared to those isolated from men. Likewise, a female-dominated pattern was seen in mutation 27964C > T (S24L) on the ORF8 protein [28].

Considering the role of mutation as one of the most important mechanism of evolution in RNA viruses [29], several studies have been conducted so far to detect genomic variations of SARS-CoV-2, which resulted in finding rich genetic variations of different types, including missense, synonymous, insertion, deletion, and non-coding mutations [14,27,30–34]. Reportedly, missense and synonymous mutations were the most frequent type of mutations along the SARS-CoV-2 genome [27,35]. Of more than thousands of known variants with different frequencies, 17 high-frequency mutations have been reported in the literature, summarized in Table 1 and located on the SARS-CoV-2 genome in Fig. 1.

The genomic analysis of the SARS-CoV-2 in several studies has reported mutations in various genes, including *ORF1ab*, *ORF3a*, *ORF6*, *ORF7*, *ORF8*, *ORF10*, *S*, *M*, *E*, and *N* [32,35–37]. Among them, *nsp1*, *nsp2* *nsp3*, *nsp12*, and *nsp15* of ORF1ab, *S*, as well as *ORF8* genes were reported to have remarkably more mutations than other genes [14,32,35,38]. Furthermore, two insertions with unknown effects were detected on *ORF1ab* [8]. Based on the critical role of hydrophobicity and charge of amino acid residues in the understanding of the protein functionalities, in a study on detecting homoplasic sites in the SARS-CoV-2, hydrophobic changes (hydrophilic → hydrophobic mutations) on *ORF1ab* and *N* genes were significantly found more than hydrophilic mutations. Also, neutral hydrophobic changes occurred more on the S protein [39]. Reportedly, the results of mutational profiles analysis have demonstrated a strong mutation bias towards U in the SARS-CoV-2 genome. Improved immunogenicity, selection for higher expression, and increased mRNA stability could be considered possible mechanisms for this bias [40].

Among the known mutations, 241C > T on 5′ untranslated region (UTR), 3037C > T on nsp3, 14408C > Ton nsp12, and 23403A > G on S are considered to be the most common mutations [33,41]. Furthermore, non-coding mutations are placed on 5′ UTR, 3′ UTR, or intergenic regions and may impact SARS-CoV-2 packaging and titers [8,32,42]. According to the results of several studies, different deletions (in-frame or frameshift) were found in various regions of SARS-CoV-2 genomes, but no mutations have been reported on the *M* gene so far. These nucleotide deletions may play some roles in virulence and pathogenesis by affecting the third structure and the function of viral proteins, as well as an altered innate immune response [8,30,32,43–48].

According to Shannon entropy, Ceraolo and Giorgi have reported two hypervariable hotspots at positions 8789 and 28151. Both positions are characterized by the presence of either base C or U. Position 8789 is located in the area of the polyprotein gene and leads to a synonymous variation with no phenotypic differences between different strains. On the other hand, position 28151 on *ORF8* generates a Ser/Leu replacement in amino acid 84, which seems to be non-conserved in even other coronaviridae [49]. Surprisingly, in a recent study of Feng Wen et al., no genomic hotspot in the SARS-CoV-2 population was reported. They integrated all entirely identical sequences into one because the separate calculation of each mutation in the identical sequences causes a sharp increase in Shannon entropy, and also to omit biases in collection time and location in Shannon entropy analysis [38]. In a recent study, ten hotspot mutations including D614G (23403A > G) on *S*, L84S (28144T > C) on *ORF8*, S5932F on *nsp14*, M5865V on *nsp13*, L37F (10818G > C) on *nsp6*, T85I (1059C > T) of *nsp2*, Q57H (25563G > T) on *ORF3a*, G251V (26144G > T) on *ORF3a*, R203K (28881G > A) on *N*, as well as G204R (28883G > C) on *N* with a frequency of over 0.10 were reported in SARS-CoV-2 genomes [35].

Due to the role of more mutable regions in viral replication, transmission, and the induced immune responses accordingly, further surveys required to determine the effects of these mutations in the outbreak of SARS-CoV-2 [30,38,43]. Compared to the genome sequence of SARS-CoV-2 isolated from Asia, coinciding mutation 14408C > T (P323L) on *nsp12* with more spot mutations was shown in European viral genomes [26]. Reportedly, co-mutations 241C > T (in 5′ UTR) with 3037C > T (F105F), 23403A > G (D614G), and 28144T > C (L84S), as well as 8782C > T (S75S) with 28144T > C (L84S) and 18060C > T > C (L6L), were found. Also, the leader sequence mutation 241C > T tends to coincide with three mutations including 3037C > T (F105F), 14408C > T(P323L), and 23403A > G (D614G) in the virus isolates extracted from European population with a high COVID-19 infection rate reports, thus, these four co-mutations may have a pivotal role in raising virus transmission [31]. In a recent study, the co-occurrence of 8782C > T, 29095C > T, and 28144T > C variants were observed in various samples. Moreover, both single-base substitutions (8782C > T and 29095C > T) are synonymous variants; in contrast, 28144T > C caused a nonsynonymous substitution, where lysine was replaced with serine (L84S) on *ORF8* [14].

Since publishing complete SARS-CoV-2 sequences, various studies have been conducted to categorize globally circulating SARS-CoV-2

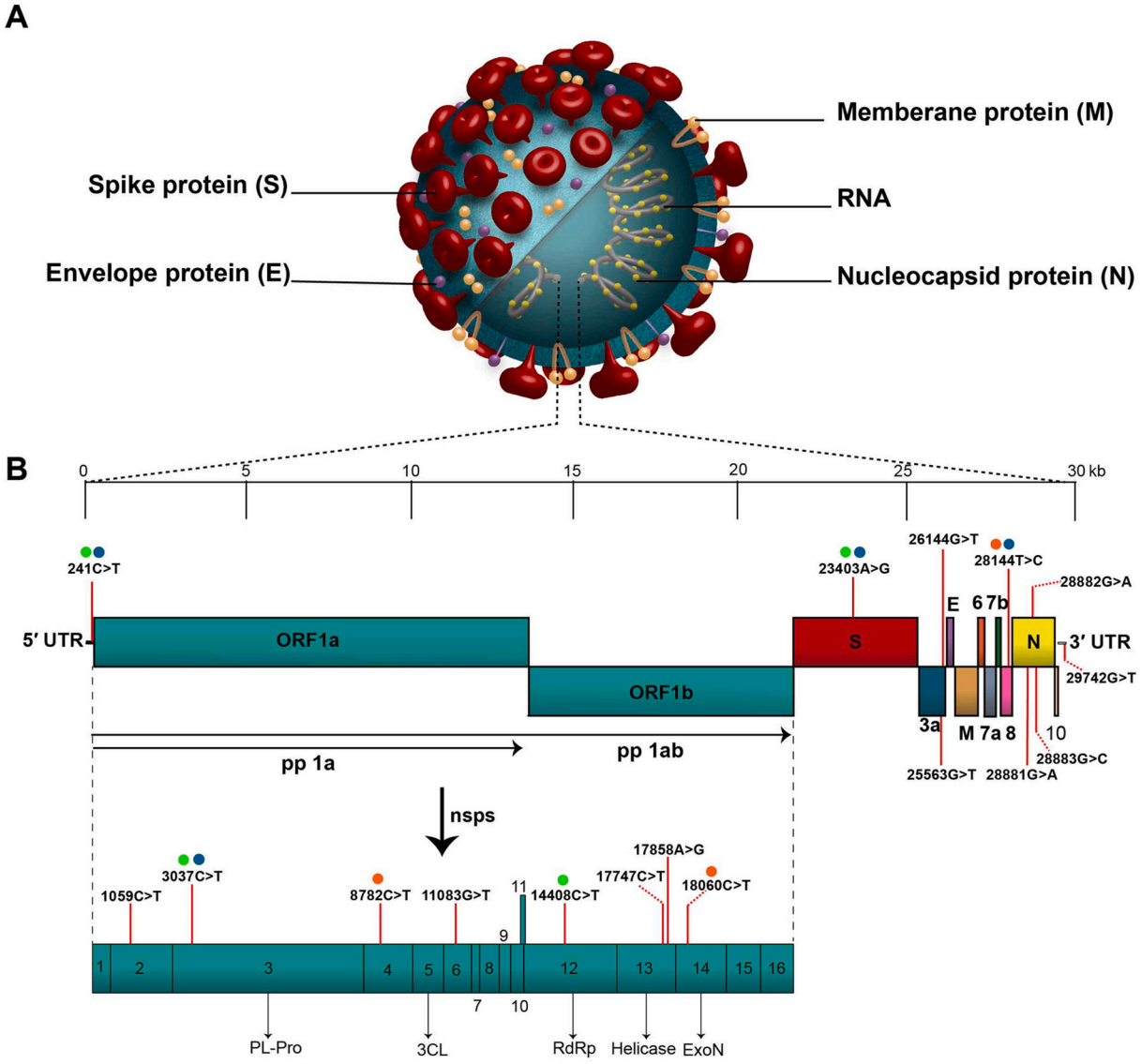

Fig. 1. Schematic presentation of structure and genome organization of SARS-CoV-2 based on reference sequence (EPI_ISL_412026). (A) The virion is covered by the spike (S) proteins as well as the membrane (M) and envelope (E) proteins are placed among the S proteins in the virus envelope. The genomic RNA is surrounded by phosphorylated nucleocapsid (N) proteins inside phospholipid bilayers. (B) The SARS-CoV-2 genome (29903 nucleotides) comprises of the 5′ UTR, ORF1a/b encoding 16 nsps for replication, four genes that encode structural proteins including S, E, M, and N proteins, six accessory genes that encode six accessory proteins such as ORF3a, ORF6, ORF7a, ORF7b, ORF8, and ORF10, as well as the 3′ UTR. The location of the seventeen high-frequency mutations and co-mutations reported in the literature are shown on the genome by vertical red lines and circles with similar color, respectively. *Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; 5′ UTR, 5′ untranslated region; OFR, open reading frame; nsp, non-structural protein.* (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

population, based on mutation profiles [41,50–52]. For instance, a recent study on the genomic distribution of SARS-CoV-2 mutations has grouped detected mutations into five distinct clades, G (241C > T, 3037C > T, 14408C > T, and 23403A > G), GH(241C > T, 3037C > T, 14408C > T, 23403A > G, and 25563G > T), GR (241C > T, 3037C > T, 14408C > T, 23403A > G, and 28881GGG > AAC), S (8782C > T and 28144T > C), and V (11083G > T and 26144G > T), each clade being identified by a specific set of mutations. Considerably, clades allow tracking of the SARS-CoV-2 genetic diversity and prevalence over time [33].

There is presently little evidence on the clinical and molecular effects of mutations detected in the SARS-CoV-2 genome. The results of several preliminary studies suggested a strong link between raising the case fatality rate with the proportion of viruses bearing mutation 23403A > G (D614G), a mutation outside the receptor-binding domain (RBD), over a time period in different regions, as well as the association of this variant with viral infectivity and greater transmissibility [53–58]. Using *in silico* methods, the effect of mapped mutations R408I, L455Y, F486L, Q493N, Q498Y, N501T on RBD, and A930V, D936Y on heptad repeat 1 (HR1) domain identified deleterious and cause instability of spike glycoprotein [59]. Furthermore, the substitution of proline to leucine (P323L) caused by the 14408C > T mutation on *RdRp* appears to increase mutation rates. The results of studies have suggested that this mutation leads to rigidify the RdRp protein structure and subsequently change interactions of RdRp with other components of the replication or transcription machinery [26,60–62]. In addition to P323L, A97V and A185V mutations on *RdRp* have also been reported to alter the secondary structure of the protein [62]. According to a cohort study, COVID-19 patients with a 382-nucleotide deletion on *ORF8* have reportedly illustrated a milder infection compared with patients infected with wild-type virus only [63].

Table 1
High-frequency mutations reported in the literature.

Mutation type	Nucleotide change	Amino acid change	Gene/location	References
Non-coding	241C > T	NA	5′ UTR	[14,27,31,34,42,179,180]
Synonymous mutation	3037C > T	F105F	ORF1ab (*nsp3*)	[26,27,31,34,42,179,180]
Missense	1059C > T	T85I	ORF1ab (*nsp2*)	[28,34,41,50]
Synonymous mutation	8782C > T	S75S	ORF1ab (*nsp4*)	[14,26,27,30–32,42]
Missense	11083G > T	L37F	ORF1ab (*nsp6*)	[8,27,28,31,32,42]
Missense	14408C > T	P323L	ORF1ab (*nsp12*)	[26–28,31,34,42,179]
Missense	17747C > T	P504L	ORF1ab (*nsp13*)	[26,27,31,42]
Missense	17858A > G	Y541C	ORF1ab (*nsp13*)	[26,27,31,42]
Synonymous mutation	18060C > T	L6L	ORF1ab (*nsp14*)	[8,26,27,30–32,42]
Missense	23403A > G	D614G	*S*	[8,26–28,31,34,42,179]
Missense	25563G > T	Q57H	*ORF3a*	[28,34,36]
Missense	26144G > T	G251V	*ORF3a*	[14,27,30–32,42]
Missense	28144T > C	L84S	*ORF8*	[8,27,28,30–32,42]
Missense	28881G > A	R203K	*N*	[26,27,31,34,42,179]
Synonymous mutation	28882G > A	R203R	*N*	[27,31,34,42,179]
Missense	28883G > C	G204R	*N*	[27,34,42,179]
Non-coding	29742G > T	NA	3′ UTR	[41,181]

NA: not applicable

4. Molecular diagnostic techniques for the SASR-CoV-2

Since the first release of genetic information of SARS-CoV-2, various molecular methods including reverse transcription-polymerase chain reaction (RT-PCR), reverse transcription loop-mediated isothermal amplification (RT-LAMP), real-time RT-LAMP, recombinase polymerase amplification (RPA), high-throughput sequencing of the whole genome, and clustered regularly interspersed short palindromic repeats/CRISPR associated (CRISPR/Cas)-based approach have been employed for genetically detection of SARS-CoV-2 [64].

4.1. Real-time RT-PCR

Real-time RT-PCR, which has been recommended by WHO could be applied as the effective, straightforward, and gold standard method for the detection of SARS-CoV-2 genome in respiratory secretions, serum, stool, sputum, or ocular secretions, [65–67] although a study indicated that sampling by nasopharyngeal swab is associated with more accurate results than other types of samples [68]. Generally, RT-PCR has been performed as a one-step or two-step format. In the one-step procedure, which is also preferred to identify SARS-CoV-2, a single tube is used for the whole RT-PCR reaction. This method has been considered in SARS-CoV-2 detection due to the possibility of quick start-up as well as reducing the possibility of contamination and pipetting technique errors [69,70].

However, the possibility of false-negative results is considered to be one of the major challenges in real-time RT-PCR test, [71] which has been reported to range between 21% and 67% [72–76]. The most important suggested underlying causes include thermal inactivation, storage time and temperature of specimen preservation, inadequate viral material, laboratory error, and test sample transfer limitation [77,78]. It was also suggested that inappropriate timing of sample collection and deficiency in sampling technique, especially of nasopharyngeal swabs are other reasons for false-negative results of real-time RT-PCR [79].

Furthermore, mutations in the primer- and probe-target regions caused by the rapid evolution of the SARS-CoV-2 genome, infection routes, issues related to sampling (timing and method), and co-infection with other viruses would probably have specific effects on RT-PCR test accuracy [71,72,80]. According to the need to test asymptomatic patients or test before quarantine release as well as the high false-negative rate of RT-PCR for detection of SARS-CoV-2, digital, and digital-droplet PCR methods have received attention due to their high sensitivity. In a study, digital PCR has demonstrated 96.3% accuracy for SARS-CoV-2 detection from pharyngeal swab samples and identified four patients' samples, which were considered negative based on the RT-PCR test [81]. It was shown that digital-droplet PCR capability for SARS-CoV-2 detection with lower minimum detection range is 500 times more sensitive than RT-PCR [82]. The use of the latest laboratory standards, quality sampling at different times from multiple sample types, and selection of RT-PCR kits with the lowest rate of reported false-negative results can effectively prevent the occurrence of false-negative results. Moreover, considering real-time RT-PCR results along with clinical features, especially computed tomography imaging, can be impactful in the timely diagnosis of SARS-CoV-2 infection and COVID-19 management [83,84]. According to the association between the quality of specimens and satisfactory RT-PCR results, evaluating cellular content and considering an internal reference such as *RPP30* has been suggested. Indeed, the reliability of the SARS-CoV-2 RNA test and detection of false-negative results could be assessed using the cycle threshold cutoff values of *RPP30* RT-PCR [85].

Until now, myriads of real-time RT-PCR kits with different qualities and methods of optimization have been introduced for SARS-CoV-2 detection by clinical laboratories and companies around the world [84,86,87]. Also, many SARS-CoV-2 primers and probes were designed and published by WHO as well as many laboratories worldwide. Employed primers and probes address different SARS-CoV-2 genomic regions target, including *ORF1ab* or *ORF8* regions as well as *E*, *N*, *S,* and *RdRP* genes [88–92]. Additionally, a two-target system with one universally primer for detection of diverse coronaviruses and a second primer set for the only identification of SARS-CoV-2 can be employed in RT-PCR assay [1,91,93].

4.2. Loop-mediated isothermal amplification (RT-LAMP)

As another diagnostic technique, RT-LAMP is considered as a rapid, effective, and one-step nucleic acid amplification method used to identify different viruses including influenza virus, MERS-CoV, Ebola virus, Zika virus, yellow fever virus, and West Nile virus [94–97]. This method is performed at a constant temperature using DNA polymerase and four to six specific primers to detect six to eight sequences of the target gene in the only 60 min [98]. Recently, RT-LAMP has been applied as an alternative test for SARS-CoV-2 detection [99–102]. Compared with the RT-PCR method, the RT-LAMP assay is faster, easier, more cost-effective as well as less time-consuming for diagnosing COVID-19 [103]. Besides, the sensitivity of this assay has been reported to be similar to the RT-PCR assay [93]. Importantly, the RT-LAMP method can be used as a point-of-care diagnostic test [101]. Dao Thi *et al.* recently tested a two-color RT-LAMP assay by several hundred clinical SARS-CoV-2 samples. Their results showed the colorimetric RT-LAMP could detect patients with a high or moderate SARS-CoV-2 viral

load. Additionally, they developed direct swab–to–RT-LAMP assay, a simplified version of RT-LAMP without the need for prior RNA isolation [104]. In different studies, in order to use RT-LAMP assay to diagnose SARS-CoV-2 in patients, several primer sets have been designed that target the *ORF1ab*, *nsp3*, *S,* and *N* genes/regions for optimization of the assay, which are summarized in supplementary Table S1.

4.3. Recombinase polymerase amplification

Recombinase Polymerase Amplification (RPA) is another sensitive isothermal alternative to the PCR, which has been considered due to its high amplification speed and performance at a low constant temperature [105]. El-Tholoth et al. using a 2-stage dubbed rapid amplification, a combination of LAMP and RPA techniques, provided a method 100-fold more sensitive than LAMP and RT-PCR in mimic patient samples for SARS-CoV-2 detection [99].

4.4. Metagenomic next-generation sequencing

As another alternative approach, metagenomic next-generation sequencing (mNGS) allows unbiased detection of pathogenic genomes for epidemiological aims, as well as identification of co-infections that may lead to increase morbidity and mortality in emerging infectious diseases [106]. Additionally, this offers an opportunity to identify unknown pathogens with sudden onset in a particular area, similar to the Ebola virus in West Africa [107]. In recent studies, mNGS has been reported as a feasible and efficient approach for detecting a wide range of pathogens, especially the SARS-CoV-2 in clinical samples [108,109]. Moreover, the amplicon-based metagenomic sequencing approach which is a combination of amplicon-based sequencing and metagenomic sequencing has been utilized to recognize the genome sequence of SARS-CoV-2 and the other microbiome from nasopharyngeal swabs of patients with COVID-19 [108].

4.5. CRISPR/Cas systems

CRISPR/Cas systems are recently characterized as rapid and versatile platforms for nucleic acid detection [109]. Further, the analysis of the results of CRISPR-based methods can be done using fluorescent, lateral flow strips, or agarose gels in less than an hour [110,111]. Compared to the PCR strategies, CRISPR-Cas diagnostic tools are significantly more sensitive and less costly than PCR-based approaches [112]. Additionally, in a study on the performance of the three technology platforms including mNGS, RT-PCR, and CRISPR in identifying the SARS-CoV-2, results indicated a great sensitivity and specificity of CRISPR-based assay for SARS-CoV-2 detection in clinical specimens with a shorter turn-around time than other methods [113]. Since the beginning of the COVID-19 outbreak, several CRISPR-Cas-based platforms have been developed to detect the SARS-CoV-2, which are compared in Table 2. For instance, using a combination of CRISPR–Cas12-based assay and LAMP, Broughton et al. were able to reduce the SARS-CoV-2 detection time to around 30 min, while maintaining a relatively low sensitivity (10 copies of RNA per μL) [114]. In contrast, All-In-One Dual CRISPR-Cas12a (AIOD-CRISPR) platform was able to detect 4.6 copies of SARS-CoV-2 RNA per μL of input in about 1 h [115].

4.6. Multiplex diagnostic approaches

In contrast to the singleplex reaction, in the multiplex diagnostic methods, more than one viral gene can be detected in a short time, which makes these methods acceptable for routine diagnostic tests. Furthermore, the multiplex approach has some advantages including increased sample throughput, as well as a reduced amount of sample required and turnaround time than a singleplex diagnostic approach [116]. Until now, several multiplex assays including FTD21 kit [117],

Table 2
SARS-CoV-2 detection methods based on CRISPR/Cas.

Platform	Sensitivity	Time	Equipment required	Cas protein	Gene or reign detected	Pre-amplification technique	Positive control	References
DETECTR	10–100 copies of RNA per μL	30–40 min	Eppendorf tubes with reagents, heat blocks or water bath (37°C and 62°C), nuclease-free water, pipettes, and tips as well as lateral flow strips.	Cas12a	*E* gene and *N* gene (N2 region)	RT-LAMP	human *RNase P* gene	[114]
SHERLOCK	10–100 copies of RNA per μL	about 60 min	37°C and 42°C water bath and Microcentrifuge for spinning down 1.5 mL test tubes	Cas13	*S* gene and *ORF1ab* gene	qRT-PCR	Synthetic virus fragment	[111]
AIOD-CRISPR	4.6 copies of SARS-CoV-2 RNA per μL of input	40 min	37°C heat block, LED blue light illuminator or UV light illuminator	Cas12a	*N* gene	RT-RPA	human *RPP30* gene.	[115]
CREST	10 copies of RNA per μL	Not mentioned, likely about 1–2 h	Mini PCR machines by DIY-Bio, P51 cardboard fluorescence visualizer with 9V battery	Cas13	N1, N2, and N3 regions in *N* gene	qRT-PCR	Synthetic, T7 RNA polymerase gene	[182]
FELUDA	110 femtomolar	about 1 h	PCR machines/Heat blocks, Lateral flow strips, Agarose gel equipment, Fluorescent emission reader	FnCas9	*nsp8* and *N* gene	RT-PCR/RPA	human *ACTB*	[110]
CASdetec	5 copies of RNA per μL	40–60 min	42°C heat, fluorescence microplate reader, ssDNA-fluorescently quenched (FQ) reporter	Cas12b	*RdRp*	RT-RAA[a]	NA	[183]

[a] Recombinase-aided amplification (RAA).

GeXP assay [118], Qiagen ResPlex II V2.0 kit [119], and FilmArray multiplex PCR system [120] have been developed for the detection of respiratory viruses as well as some common human coronaviruses (HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1) in particular. According to the operation assessment results of AusDiagnostics respiratory multiplex tandem PCR assay including SARS-CoV-2, this assay has shown high specificity (98.4%) and considerable concordance with the reference laboratory for the identification of SARS-CoV-2 by the *ORF1ab* gene [121]. The QIAstat-Dx Respiratory SARS-CoV-2 panel (QIAstat-SARS) has also been indicated the ability to detect 1000 copies per milliliter with 100% sensitivity and 93% specification by targeting the *E* and *ORF1ab* genes from SARS-CoV-2. Moreover, the latest multiplex platform for pathogen detection was developed, called Combinatorial Arrayed Reactions for Multiplexed Evaluation of Nucleic acids (CARMEN). This method, in combination with Cas13 created CARMEN-Cas13 technology, which can be used for highly efficient virus detection. Through using CARMEN-Cas13 assay clinicians are able to track and diagnose all 169 human-associated viruses, as well as SARS-CoV-2 [122].

4.7. Point-of-care tests

There are some approaches with the aim of moving nucleic acid testing from a laboratory-based setup to the point of care testing in the clinic [88]. For example, a point-of-care system using a smartphone-based LAMP and panel of equine respiratory infectious diseases, as a model for human pathogens like SARS-CoV-2 has recently developed for finding live virus from the nasal swab [123]. Likewise, hybridization chain reactions (HCRs) were suggested as screening strategies for the prevention of widespread infection, including SARS-CoV-2. Based on the HCR designer, SARS-CoV-2 nucleocapsid phosphoprotein gene and human RNase P have been recently targeted for performing HCR reactions on complementary DNA of SARS–CoV-2, which has been shown to be useful for COVID-19 screening [124]. In addition, HCR technology features including the lack of thermal cycler or enzymes need for amplification, as well as the possibility of testing at room temperature, have made HCR technology as a suitable method for quick detection of some viruses such as the hepatitis B and influenza A [125].

5. Using RNA interference as a weapon for combating SARS-CoV-2: possible challenges and opportunities

RNA interference (RNAi) is a molecular process in which small noncoding RNAs (of endogenous or exogenous origin) match with the target mRNA in a sequence-specific manner and silence its expression [126]. During the past decade, based on the therapeutic potential of RNAi and their abilities to limit gene expression, many of them have been investigated and employed for medical application. RNAi-based technology has a simple and unique design and can be simultaneously used against multiple strains of a virus, making it a tractable and viable option to battle and treat severe respiratory viral infections [127]. Several features, containing thermodynamic characteristics, small interfering RNA (siRNA) structure, percentage of GC content, approachability of the target site, nucleotides at siRNA termini, and selection of highly conservative sequences in the targeted virus genome affect the design of siRNAs with high antiviral activity [128–130]. It has been found that different positive- and negative-strand of RNA viruses induce mammalian antiviral RNAi activity. On the other hand, as a countermeasure, virus-encoded suppressors of RNAi (VSRs) are produced in diverse families of plant, insect, and mammalian viruses to oppose the RNAi pathway at various steps [131,132].

From the chronological perspective, targeting viral RNAs have been used for the suppression of the replication in numerous viruses, including human immunodeficiency virus (HIV), human papilloma virus (HPV), Hepatitis A virus, Hepatitis B virus, Influenza A and B viruses, respiratory syncytial virus (RSV), SARS-CoV, and Adenoviruses [133–140]. Some siRNA-based antivirals have been examined in clinical trials such as ALN-RSV01 to target respiratory syncytial virus nucleocapsid gene (phase II) [141], NucB1000 against four separate targets (Pre-C, Pre-S1, Pre-S2, and X) of chronic hepatitis B virus (phase I) [142], SPC3649 for blocking miR-122 and its interaction with hepatitis C virus RNA (phase II) [143], pHIV7-shI-TAR-CCR5RZ for targeting several human immunodeficiency virus genes (Tat, Tar, and CCR5) (phase I) [144], and TKM-Ebola against some transcripts (L, VP24, and VP35) of Ebola virus (phase I) [145].

In CAS content collection, more than seventy patents reveal the use of RNAi, siRNA molecules, antisense oligonucleotides, RNA aptamers, ribozymes, and microRNA inhibitors in treating SARS-CoV, a close relative of the SARS-CoV-2 [146]. These patents have presented medicines for treatment and inhibition of the SARS, as well as supplied various information about siRNAs against SARS-CoV and techniques for their delivery [147]. Moreover, several studies supported that siRNAs are able to prevent gene expression and the replication of SARS-CoV by targeting some genes or the leader sequence of this virus in cultured cells [148–151]. For example, in 2005, Shi et al. exhibited short interfering RNAs as molecules targeting the open reading frames of the *E*, *M*, and *N* genes of SARS-CoV, which blocked the replication of this human Coronavirus in Vero-E6 cells [152]. In another study, two siRNAs targeted the SARS-CoV genome at S and nsp12 regions, which led to the depletion of fever caused by the virus as well as SARS-CoV viral loads. Likewise, it reduced acute diffuse alveoli damage in Rhesus macaques [140]. Furthermore, the previous study reported the N protein of SARS-CoV as a VSR in mammalian cells by a cellular reversal-of-silencing assay regarding viral infection [153].

5.1. The use of siRNA-based therapeutics for COVID-19

In order to target the RNA genome of SARS-CoV-2 and as the first step in the production of antiviral siRNAs, Chen et al. [154] suggested nine potential siRNA targets with 21–25 nucleotides in length within ORF1ab, S, ORF3a, M, and N regions of the SARS-CoV-2 genome and detected those regions as conserved areas. In another study, considering siRNA as a potential therapy for COVID-19, nsp5, and nsp12 were introduced to be targeted using siRNA based therapeutics [147]. Besides, in a study related to the interaction between antiviral RNAi immunity and SARS-CoV-2 has been reported structural protein N of the SARS-CoV-2 as an indicator of viral suppressor of RNAi (VSR) activity, which is a challenge in developing siRNA therapeutic and a factor which opposes the antiviral activity of RNAi in different steps [155].

A recent study found that host cell entry of SARS-CoV-2 is contingent on ACE2, as well as the transmembrane protease, serine 2 (TMPRSS2) produced by the host cell is needed to cleave and activate spike protein in the invasive process of the SARS-CoV-2 [156]. Therefore, siRNA targeting TMPRSS2 and ACE2 can be considered as therapeutic options for blocking SARS-CoV-2 infections, as shown in Fig. 2. Regarding the strategy of inhibiting viral entry for antiviral treatment, Lu et al [157]. reported a reduction in the replication of SARS-CoV in the ACE2-silenced cells using siRNA technology. However, there is a concern that the selective elimination of ACE2 in vulnerable organs to SARS-CoV-2 may have unanticipated outcomes because ACE2 has critical roles in a variety of pathological and physiological processes [158]. Moreover, siRNA targeting of TMPRSS2 has formerly indicated a significant decrease in SARS-CoV entry into Calu-3 cells [159]. Fig. 2 briefly shows the use of RNAi as a preventive or therapeutic agent against SARS-CoV-2.

5.2. miRNAs as the potential therapeutic option for SARS-CoV-2

In viral infections, many known human miRNAs appear to play an important role in regulating immune responses and interfering with functions of viral genes including reproduction, translation, and expression by targeting viral genes. Consequently, miRNA targeting can

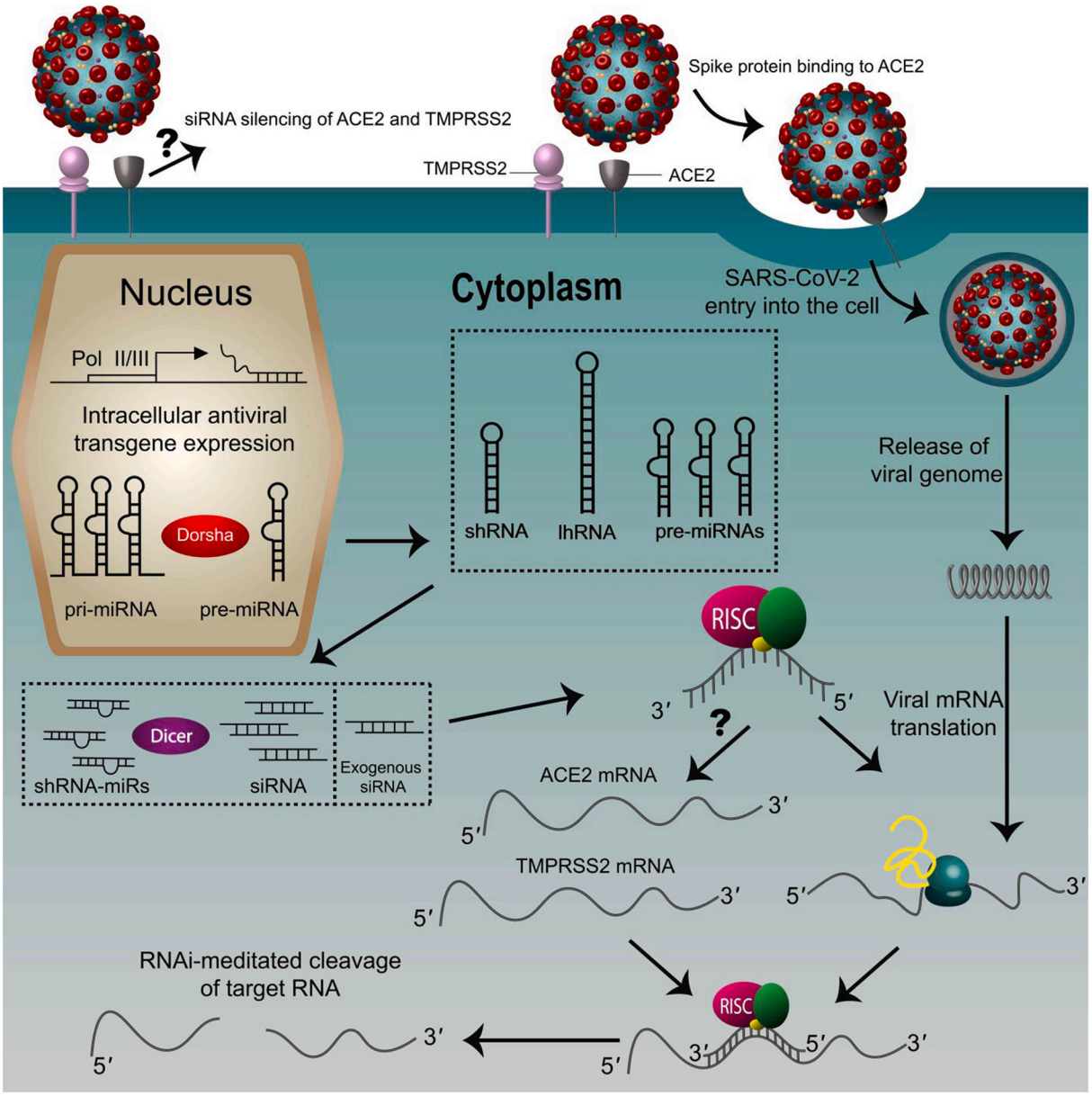

Fig. 2. Antiviral applications of RNAi against SARS-CoV-2. Generally, RNAi can either be induced by transfection of chemically synthesized siRNAs or the intracellular expression of double-stranded siRNA precursors stably or temporary. After transcription of pri-miRNA from the corresponding miRNA gene by RNA Pol II/III, and then processed by Drosha to construct pre-miRNA in the nucleus, double-stranded siRNA precursors (shRNA, lhRNA or pre-miRNA) are exported to the cytoplasm and processed into mature siRNAs using Dicer. Subsequently, the antisense strand of the siRNAs/miRNAs load into the RISC. The complex can finally cleave the target mRNA. The siRNA-based strategies against the SARS-CoV-2 can either be directed against the SARS-CoV-2 itself or against the ACE2 receptor or TMPRSS2, whose silencing will inhibit virus entry into the cell. Following the interaction of the SARS-CoV-2 spike protein with the human ACE2 receptor after spike protein activation by TMPRSS2, the virus is endocytosed. Then, the virion releases its RNA for translation into proteins by the cell's machinery. *Abbreviations: RNAi, RNA interference; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; siRNA, small interfering RNA; pri-miRNA, primary miRNA; RNA Pol II/III, RNA polymerase II/III; pre-miRNA, precursor-miRNA; shRNA, short hairpin RNA; lhRNA, long hairpin RNA; RISC, RNA-induced silencing complex; ACE2, angiotensin-converting enzyme-2; TMPRSS2, transmembrane protease serine-2.*

be considered as an antiviral therapy approach [160]. In a recent computational study, a list of predicted possible host genes targeted by viral miRNAs and viral genes targeted by cellular miRNAs was presented by computational analysis to understand the basic mechanisms of SARS-CoV-2 infection [161]. Additionally, in a recent study, six antiviral host- miRNAs comprising hsa-let-7a, hsa-miR101, hsa-miR126, hsa-miR23b, hsa-miR378, and hsa-miR98 were predicted to act on nsps, S, and N genes of SARS-CoV2 by cleaving their target sites or translation inhibition. The association between these miRNAs and several viruses, including Hepatitis C virus, Herpes simplex virus 1, and Enterovirus 71 has already been identified [44]. Moreover, in a current study on the prediction of miRNAs in SARS-CoV-2 genomes, seven miRNAs, including miR-8066, miR-5197-3p, miR-3611, miR-3934-3p, miR-1307-3p, miR-3691-3p, and miR-1468-5p were introduced that were related to viral pathogenesis and host response using KEGG pathway analysis [162]. In another *in silico* prediction-based study, the SARS-CoV-2 genome was searched for finding virus-encoded miRNA seed sponges that could bind to human miRNA seed sites and subsequently disrupt the interaction of host miRNA with their native targets. Nearly 80 human miRNAs that can interact with the SARS-CoV-2 genes have been identified. According to the literature, these miRNAs are related to pulmonary and cardiac disorders [163].

5.3. Circular RNAs

Circular RNAs (circRNAs) is a novel class of non-coding RNAs with pivotal regulatory roles in human cells. Zhang et al. articulated that MERS-CoV infection leads to considerable changes in the expression of many host cell circRNAs, miRNAs, and mRNAs. Moreover, they reported a significant decrease in viral load by knocking down specific DE circRNAs in MERS-CoV infection. Thus, findings proposed that circRNAs may act as potential antiviral targets [164].

Regarding viral derived miRNA, according to a machine learning based miRNA prediction analysis, PANTHER gene function analysis results represented the ability of viral derived miRNA candidates for targeting various human genes involved in crucial cellular processes including transcription, metabolism, defense system and several signaling pathways such as Wnt and EGFR signalings. Furtheremore, many known human miRNAs appeared to be able to target viral genes involved in viral life cycle such as S, M, N, E proteins and ORF1ab, ORF3a, ORF8, ORF7a and ORF10. Regarding the importance of miRNA-based therapies, comprehending mode of actions of miRNAs and their possible roles during SARS-CoV-2 infections could create new opportunities for the development and improvement of new therapeutics [165].

6. Nucleotide-based technology for vaccine development against SARS-CoV-2

Among the vaccinations under evaluation, there are three major groups of DNA vaccines, RNA vaccines, and Epitope-based vaccines, which could be categorized into the nucleotide-based vaccines.

6.1. DNA vaccine

The past few years have witnessed a surge in the development of highly efficacious delivery materials for nucleic acids with some remarkable results. Following viral sequencing technology, nucleic acid therapeutics have emerged as promising alternatives to conventional vaccine approaches. Regarding previous strains of coronavirus, some studies had been performed to develop DNA-vaccines [166,167]. One of the most successful DNA-based vaccines for MERS is INO-4700, which led to durable neutralizing antibodies and T cell immune responses through targeting MERS-CoV S protein [167]. As another effort with a similar strategy, Smith et al. [168] generated a DNA vaccine, INO-4800, for targeting SARS-CoV-2 S protein. The American company INOVIO started a phase I/IIa trial NCT04336410, NCT0444778, in April, and July 2020 articulating that INO-4800 could recruit humoral and T cell responses in mice. They assessed the biodistribution of SARS-CoV-2 targeting antibodies to the lungs to validate INO-4800 as a capable potential SARS-CoV-2 vaccine candidate in mice and pigs, which resulted in the detection of anti-SARS-CoV-2 specific antibody in the lungs following immunization with INO-4800. In another achievement related to DNA vaccines for SARS-CoV-2, Jingyou et al. [169] developed a series of DNA vaccines, which recruited humoral and cellular immune responses in Rhesus Macaques. The titer of developed neutralizing antibodies was as high as the naturally antibody developed following with SARS-CoV-2. In addition to the INO-4800, Indian vaccine-maker Zydus Cadila began testing a DNA-based vaccine in July, becoming the second company in India to enter the Covid-19 vaccine race after Bharat Biotech, and recently they launched a Phase 2 trial on Aug. 6. Similarly, The Korean company Genexine, as well as the Japanese biotechnology company AnGes started testing the safety of a DNA-based vaccine in June. Furthermore, fifteen other DNA vaccines are in pre-clinical and clinical trial evaluations. The list of such COVID-19 candidate vaccines in both clinical and pre-clinical phases brought in a new report published by WHO in June 2020 [170,171].

6.2. RNA vaccine

RNA-based therapies and vaccines have remarkable potential for the treatment and prevention of a wide range of diseases [172]. RNA vaccines offer a promising alternative to conventional vaccine approaches because of their high potency, capacity for rapid development, safe administration, and low-cost production. However, their application has been restricted until recently by the instability and inefficient in vivo delivery of mRNA [173]. Gradually, some techniques served to reduce toxicity and improve the translation of the mRNA. Various modifications including the incorporation of modified nucleosides (particularly modified uridine), optimization of coding sequences, and stringent purification of in vitro transcribed mRNA by high-performance liquid chromatography have been applied to remove double-stranded RNA contaminants [174]. Given that the 5' and 3' UTRs of mRNA can significantly influence the rate of translation and half-life of the transcript, optimization of the UTRs is of paramount value in the design of mRNA vaccines [173].

As of September 2020, the phase III, (NCT04470427) clinical trial of a novel lipid nanoparticle-encapsulated mRNA-based vaccine, mRNA-1273, encoding the S protein of SARS-CoV-2, began in the United States by the Moderna Inc. [175] The German company BioNTech has recently entered into collaborations with Pfizer, based in New York, and the Chinese drug maker Fosun Pharma to develop their mRNA vaccine. They found that one version, called BNT162b2, produced significantly fewer side effects, such as fevers and fatigue, and so they chose it to move into Phase 2/3 trials. On September 12, the companies announced the launch of a Phase 2/3 trial with 43,000 volunteers in the United States and other countries including Argentina, Brazil, and Germany [171].

In addition to these two RNA-based vaccines, which are entered into the clinical trials, there are at least sixteen other ongoing studies in pre-clinical evaluation (based on WHO's report on September, 2020). In addition to the aforementioned active clinical trials, there is a competition between those in pre-clinical for entering the clinic. Imperial College London, which has developed a ”self-amplifying” RNA vaccine with the aim of boosting the production of a viral protein to stimulate the immune system. They begun Phase I/II trials on June 15, and have partnered with Morningside Ventures to manufacture and distribute the vaccine through a new company called VacEquity Global Health. Also, CureVac is working on a similar vaccine and launched a Phase II trial of its mRNA vaccine in August [171].

6.3. Epitope-based vaccine

In addition to the DNA and RNA-based vaccines, epitope-based vaccines are other approaches, which somehow rely on the genetic aspect of SARS-CoV-2. In the new era of medicine, the immunoinformatics approaches have been desperately used to provide putative epitopes using a genome database. Recently, a group of computational scientists obtained immunogenic epitopes for all critical proteins of the virus. This list is composed of top-ranked cytotoxic T cell helper and epitopes common across MHC alleles, covering all predominant human leukocyte antigen (HLA) supertypes in population [176]. By immunogenicity predictive models, researchers provide the immunogenicity of detected peptides and their binding potential to HLA alleles [177].

Currently, a total of 63 peptides with a high immunogenicity potential have been identified for SARS-CoV-2. Identification of a ranked list of immunogenic peptides shows that they can be used as potential targets for SARS-CoV-2 vaccine development, and it accelerates the development pipeline. A detailed screen of candidate peptides based on comparison with immunogenic peptides was used using deposited data in the immune epitope database, which resulted in a de novo prediction from SARS-CoV-2-associated 9-mer peptides [177]. It has been shown that these peptides bind various HLA alleles (both class I and class II),

although with a higher tendency towards HLA-A:02:01, and can cause activation of effector T cells. In another study to provide a fast-immunogenic profile of these epitopes, linear B-cell epitopes, along with their sequence, position, and length, were introduced for SARS-CoV-2. In a recent study, 13 Major Histocompatibility Complex-(MHC) I and 3 MHC-II epitopes were recognized to have antigenic properties [178]. These epitopes molecularly dock on toll-like receptor-5 to get binding affinity and are usually linked to specific linkers to build vaccine components. Reportedly, a list of 22 SARS-CoV-2 peptides has been revealed, which have a higher predicted immunogenicity score than their target peptides. Further predictive models and algorithms for the characterization of immunogenic peptides, as well as several in vitro and in vivo validations, are required for the most efficient vaccine development [177].

7. Concluding remarks

Currently, the genome of SARS-CoV-2 has been comprehensively studied. Without a doubt, our understanding of this regard caused molecular-based diagnosis, some targeted therapies, and vaccine developments. Focusing on targeting the SARS-CoV-2 genome via RNAi technology could lead to emerging effective and safe treatments, which probably could prevent disease severity and decrease COVID-19 associated mortality. Although many efforts have been made to reveal the behavior of SARS-CoV-2, based on its genetics, there are still some concerns regarding emerging variation/mutations. These could significantly affect targeted therapies and, more importantly, vaccine development. Moving forward, we must recognize that we have had a new major coronavirus epidemic every decade in the twenty-first century SARS in the 2000s, MERS in the 2010s, and now COVID-19. It is, therefore, a global security priority to advance coronavirus vaccines and to identify international funding support resources, as well as other data regarding their development, manufacture, and storage. This recent outbreak should be considered as a sine qua non to alert the international research community to both react and prepare for the next coronavirus transmission to mammals. We urgently need to manufacture a pan-coronavirus vaccine, and it is something that appears feasible if sufficient resources are provided in an appropriate time. Besides, further molecular research of SARS-CoV-2 is required to develop more sensitive genetic-based detection methods to facilitate the diagnosis of COVID-19. In addition, the development of broad-spectrum antiviral drugs and vaccines based on the genetic profile of both virus and host cells are highly recommended.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygeno.2020.09.059.

Declaration of competing interest

None.

References

[1] N. Zhu, D. Zhang, W. Wang, X. Li, B. Yang, J. Song, X. Zhao, B. Huang, W. Shi, R. Lu, P. Niu, F. Zhan, X. Ma, D. Wang, W. Xu, G. Wu, G.F. Gao, W. Tan, A novel coronavirus from patients with pneumonia in China, 2019, N. Engl. J. Med. 382 (2020) 727–733.

[2] P. Zhou, X.L. Yang, X.G. Wang, B. Hu, L. Zhang, W. Zhang, H.R. Si, Y. Zhu, B. Li, C.L. Huang, H.D. Chen, J. Chen, Y. Luo, H. Guo, R.D. Jiang, M.Q. Liu, Y. Chen, X.R. Shen, X. Wang, X.S. Zheng, K. Zhao, Q.J. Chen, F. Deng, L.L. Liu, B. Yan, F.X. Zhan, Y.Y. Wang, G.F. Xiao, Z.L. Shi, A pneumonia outbreak associated with a new coronavirus of probable bat origin, Nature 579 (2020) 270–273.

[3] X. Xu, M. Han, T. Li, W. Sun, D. Wang, B. Fu, Y. Zhou, X. Zheng, Y. Yang, X. Li, X. Zhang, A. Pan, H. Wei, Effective treatment of severe COVID-19 patients with tocilizumab, Proc. Natl. Acad. Sci. U. S. A. 117 (2020) 10970–10975.

[4] J. Grein, N. Ohmagari, D. Shin, G. Diaz, E. Asperges, A. Castagna, T. Feldt, G. Green, M.L. Green, F.X. Lescure, E. Nicastri, R. Oda, K. Yo, E. Quiros-Roldan, A. Studemeister, J. Redinski, S. Ahmed, J. Bernett, D. Chelliah, D. Chen, S. Chihara, S.H. Cohen, J. Cunningham, A. D'Arminio Monforte, S. Ismail, H. Kato, G. Lapadula, E. L'Her, T. Maeno, S. Majumder, M. Massari, M. Mora-Rillo, Y. Mutoh, D. Nguyen, E. Verweij, A. Zoufaly, A.O. Osinusi, A. DeZure, Y. Zhao, L. Zhong, A. Chokkalingam, E. Elboudwarej, L. Telep, L. Timbs, I. Henne, S. Sellers, H. Cao, S.K. Tan, L. Winterbourne, P. Desai, R. Mera, A. Gaggar, R.P. Myers, D.M. Brainard, R. Childs, T. Flanigan, Compassionate use of remdesivir for patients with severe Covid-19, N. Engl. J. Med. 382 (2020) 2327–2336.

[5] S.P. Kaur, V. Gupta, COVID-19 Vaccine: a comprehensive status report, Virus Res. 288 (2020) 198114.

[6] CBCnews, How Close are We to a Vaccine for COVID-19? [online] Available at https://newsinteractives.cbc.ca/coronavirusvaccinetracker/, (2020) [Accessed 15 September 2020].

[7] F. Wu, S. Zhao, B. Yu, Y.-M. Chen, W. Wang, Z.-G. Song, Y. Hu, Z.-W. Tao, J.-H. Tian, Y.-Y. Pei, M.-L. Yuan, Y.-L. Zhang, F.-H. Dai, Y. Liu, Q.-M. Wang, J.-J. Zheng, L. Xu, E.C. Holmes, Y.-Z. Zhang, A new coronavirus associated with human respiratory disease in China, Nature 579 (2020) 265–269.

[8] R. Lu, X. Zhao, J. Li, P. Niu, B. Yang, H. Wu, W. Wang, H. Song, B. Huang, N. Zhu, Y. Bi, X. Ma, F. Zhan, L. Wang, T. Hu, H. Zhou, Z. Hu, W. Zhou, L. Zhao, J. Chen, Y. Meng, J. Wang, Y. Lin, J. Yuan, Z. Xie, J. Ma, W.J. Liu, D. Wang, W. Xu, E.C. Holmes, G.F. Gao, G. Wu, W. Chen, W. Shi, W. Tan, Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding, Lancet 395 (2020) 565–574.

[9] A.R. Fehr, S. Perlman, Coronaviruses: an overview of their replication and pathogenesis, Methods Mol. Biol. 1282 (2015) 1–23.

[10] J. Cui, F. Li, Z.L. Shi, Origin and evolution of pathogenic coronaviruses, Nat. Rev. Microbiol. 17 (2019) 181–192.

[11] Y.J. Ruan, C.L. Wei, A.L. Ee, V.B. Vega, H. Thoreau, S.T. Su, J.M. Chia, P. Ng, K.P. Chiu, L. Lim, T. Zhang, C.K. Peng, E.O. Lin, N.M. Lee, S.L. Yee, L.F. Ng, R.E. Chee, L.W. Stanton, P.M. Long, E.T. Liu, Comparative full-length genome sequence analysis of 14 SARS coronavirus isolates and common mutations associated with putative origins of infection, Lancet 361 (2003) 1779–1785.

[12] P.S. Masters, The molecular biology of coronaviruses, Adv. Virus Res. 66 (2006) 193–292.

[13] P.C.Y. Woo, Y. Huang, S.K.P. Lau, K.-Y. Yuen, Coronavirus genomics and bioinformatics analysis, Viruses 2 (2010) 1804–1820.

[14] J.F. Chan, K.H. Kok, Z. Zhu, H. Chu, K.K. To, S. Yuan, K.Y. Yuen, Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan, Emerg. Microbes Infect. 9 (2020) 221–236.

[15] A. Wu, Y. Peng, B. Huang, X. Ding, X. Wang, P. Niu, J. Meng, Z. Zhu, Z. Zhang, J. Wang, J. Sheng, L. Quan, Z. Xia, W. Tan, G. Cheng, T. Jiang, Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China, Cell Host Microbe 27 (2020) 325–328.

[16] F. Wu, S. Zhao, B. Yu, Y.-M. Chen, W. Wang, Y. Hu, Z.-G. Song, Z.-W. Tao, J.-H. Tian, Y.-Y. Pei, M.-L. Yuan, Y.-L. Zhang, F.-H. Dai, Y. Liu, Q.-M. Wang, J.-J. Zheng, L. Xu, E.C. Holmes, Y.-Z. Zhang, Complete genome characterisation of a novel coronavirus associated with severe human respiratory disease in Wuhan, China, bioRxiv : the preprint server for biology (2020) 2020.2001.2024.919183.

[17] D. Yang, J.L. Leibowitz, The structure and functions of coronavirus genomic 3' and 5' ends, Virus Res. 206 (2015) 120–133.

[18] E. Domingo, Mechanisms of viral emergence, Vet. Res. 41 (2010) 38.

[19] S.U. Rehman, L. Shafique, A. Ihsan, Q. Liu, Evolutionary trajectory for the emergence of novel coronavirus SARS-CoV-2, Pathogens 9 (2020) 240.

[20] M.C. Wong, S.J. Javornik Cregeen, N.J. Ajami, J.F. Petrosino, Evidence of recombination in coronaviruses implicating pangolin origins of nCoV-2019, bioRxiv : the preprint server for biology (2020) 2020.2002.2007.939207.

[21] A. Flores-Alanis, L. Sandner-Miranda, G. Delgado, A. Cravioto, R. Morales-Espinosa, The receptor binding domain of SARS-CoV-2 spike protein is the result of an ancestral recombination between the bat-CoV RaTG13 and the pangolin-CoV MP789, BMC Res. Notes 13 (2020) 398.

[22] Y. Shu, J. McCauley, GISAID: Global initiative on sharing all influenza data – from vision to reality, Eurosurveillance 22 (2017) 30494.

[23] Y. Jia, G. Shen, Y. Zhang, K.-S. Huang, H.-Y. Ho, W.-S. Hor, C.-H. Yang, C. Li, W.-L. Wang, Analysis of the mutation dynamics of SARS-CoV-2 reveals the spread history and emergence of RBD mutant with lower ACE2 binding affinity, bioRxiv : the preprint server for biology (2020) 2020.2004.2009.034942.

[24] E.C. Holmes, G. Dudas, A. Rambaut, K.G. Andersen, The evolution of Ebola virus: Insights from the 2013-2016 epidemic, Nature 538 (2016) 193–200.

[25] P. Domingo-Calap, B. Schubert, M. Joly, M. Solis, M. Untrau, R. Carapito, P. Georgel, S. Caillard, S. Fafi-Kremer, N. Paul, O. Kohlbacher, F. González-Candelas, S. Bahram, An unusually high substitution rate in transplant-associated BK polyomavirus in vivo is further concentrated in HLA-C-bound viral peptides, PLoS Pathog. 14 (2018) e1007368.

[26] M. Pachetti, B. Marini, F. Benedetti, F. Giudici, E. Mauro, P. Storici, C. Masciovecchio, S. Angeletti, M. Ciccozzi, R.C. Gallo, D. Zella, R. Ippodrino, Emerging SARS-CoV-2 mutation hot spots include a novel RNA-dependent-RNA polymerase variant, J. Transl. Med. 18 (2020) 179.

[27] Z.-w. Chen, Z. Li, H. Li, H. Ren, P. Hu, Global genetic diversity patterns and transmissions of SARS-CoV-2, medRxiv (2020) 2020.2005.2005.20091413.

[28] R. Wang, J. Chen, K. Gao, Y. Hozumi, C. Yin, G. Wei, Characterizing SARS-CoV-2 mutations in the United States, Res Sq (2020) rs.3.rs-49671.

[29] Z. Zhao, H. Li, X. Wu, Y. Zhong, K. Zhang, Y.P. Zhang, E. Boerwinkle, Y.X. Fu, Moderate mutation rate in the SARS coronavirus genome and its implications, BMC Evol. Biol. 4 (2004) 21.

[30] C. Wang, Z. Liu, Z. Chen, X. Huang, M. Xu, T. He, Z. Zhang, The establishment of reference sequence for SARS-CoV-2 and variation analysis, J. Med. Virol. 92 (2020) 667–674.

[31] C. Yin, Genotyping coronavirus SARS-CoV-2: methods and implications, Genomics 112 (2020) 3588–3596.

[32] D.P.L.P.T. Koyama, Variant Analysis of COVID-19 Genomes, [Preprint] Bull World Health Organ, 2020.

[33] D. Mercatelli, F.M. Giorgi, Geographic and genomic distribution of SARS-CoV-2 mutations, Front. Microbiol. 11 (2020) 1800.

[34] T.A.T. Soratto, H. Darban, A. Bjerkner, M. Coorens, J. Albert, T. Allander, B. Andersson, Four SARS-CoV-2 genome sequences from late April in Stockholm, Sweden, reveal a rare mutation in the spike protein, Microbiol. Resour. Announc. 9 (2020).

[35] M. Laamarti, T. Alouane, S. Kartti, M.W. Chemao-Elfihri, M. Hakmi, A. Essabbar, M. Laamart, H. Hlali, L. Allam, N.E.L. Hafidi, R.E.L. Jaoudi, I. Allali, N. Marchoudi, J. Fekkak, H. Benrahma, C. Nejjari, S. Amzazi, L. Belyamani, A. Ibrahimi, Large scale genomic analysis of 3067 SARS-CoV-2 genomes reveals a clonal geo-distribution and a rich genetic variations of hotspots mutations, bioRxiv (2020) 2020.2005.2003.074567.

[36] S.S. Hassan, P.P. Choudhury, B. Roy, S.S. Jana, Missense mutations in SARS-CoV2 genomes from Indian patients, Genomics 112 (2020) 4622–4627.

[37] S. Laha, J. Chakraborty, S. Das, S.K. Manna, S. Biswas, R. Chatterjee, Characterizations of SARS-CoV-2 mutational profile, spike protein stability and viral transmission, Infection, Genet. Evol. 85 (2020) 104445.

[38] F. Wen, H. Yu, J. Guo, Y. Li, K. Luo, S. Huang, Identification of the hyper-variable genomic hotspot for the novel coronavirus SARS-CoV-2, J. Infect. 80 (2020) 671–693.

[39] L. van Dorp, M. Acman, D. Richard, L.P. Shaw, C.E. Ford, L. Ormond, C.J. Owen, J. Pang, C.C.S. Tan, F.A.T. Boshier, A.T. Ortiz, F. Balloux, Emergence of genomic diversity and recurrent mutations in SARS-CoV-2, Infection, Genet. Evol. 83 (2020) 104351.

[40] A.M. Rice, A.C. Morales, A.T. Ho, C. Mordstein, S. Mühlhausen, S. Watson, L. Cano, B. Young, G. Kudla, L.D. Hurst, Evidence for strong mutation bias towards, and selection against, U content in SARS-CoV-2: implications for vaccine design, Mol. Biol. Evol. (2020) 2020.2005.2011.088112.

[41] T. Koyama, D. Platt, L. Parida, Variant analysis of SARS-CoV-2 genomes, Bull. World Health Organ. 98 (2020) 495–504.

[42] A. Mishra, A.K. Pandey, P. Gupta, P. Pradhan, S. Dhamija, J. Gomes, B. Kundu, P. Vivekanandan, M.B. Menon, Mutation landscape of SARS-CoV-2 reveals three mutually exclusive clusters of leading and trailing single nucleotide substitutions, bioRxiv (2020) 2020.2005.2007.082768.

[43] T. Phan, Genetic diversity and evolution of SARS-CoV-2, Infect. Genet. Evol. 81 (2020) 104260.

[44] R. Sardar, D. Satish, S. Birla, D. Gupta, Integrative analyses of SARS-CoV-2 genomes from different geographical locations reveal unique features potentially consequential to host-virus interaction, pathogenesis and clues for novel therapies, Heliyon 6 (2020) e04658.

[45] R. Islam, N. Hoque, S. Rahman, A. Puspo, M. Akhter, S. Akter, A.S.M. Rubayet-Ul-Alam, M. Sultana, K. Crandall, A. Hossain, Genome Wide Analysis of Severe Acute Respiratory Syndrome Coronavirus-2 Implicates World-Wide Circulatory Virus Strains Heterogeneity, Preprints.org, 2020.

[46] L.A. Holland, E.A. Kaelin, R. Maqsood, B. Estifanos, L.I. Wu, A. Varsani, R.U. Halden, B.G. Hogue, M. Scotch, E.S. Lim, An 81 base-pair deletion in SARS-CoV-2 ORF7a identified from sentinel surveillance in Arizona (Jan-Mar 2020), J Virol 94 (2020) e00711-00720.

[47] G. Taiaroa, D. Rawlinson, L. Featherstone, M. Pitt, L. Caly, J. Druce, D. Purcell, L. Harty, T. Tran, J. Roberts, M. Catton, D. Williamson, L. Coin, S. Duchene, Direct RNA sequencing and early evolution of SARS-CoV-2, bioRxiv (2020) 2020.2003.2005.976167.

[48] A. Addetia, H. Xie, P. Roychoudhury, L. Shrestha, M. Loprieno, M.-L. Huang, K.R. Jerome, A.L. Greninger, Identification of multiple large deletions in ORF7a resulting in in-frame gene fusions in clinical SARS-CoV-2 isolates, J. Clin. Virol. 129 (2020) 104523.

[49] C. Ceraolo, F.M. Giorgi, Genomic variance of the 2019-nCoV coronavirus, J. Med. Virol. 92 (2020) 522–528.

[50] E. Alm, E.K. Broberg, T. Connor, E.B. Hodcroft, A.B. Komissarov, S. Maurer-Stroh, A. Melidou, R.A. Neher, Á. O'Toole, D. Pereyaslov, W.H.O.E.R.s. laboratories, G.E. group, W.H.O.E.R.s. laboratories, G.E. group, Geographical and temporal distribution of SARS-CoV-2 clades in the WHO European Region, January to June 2020, Euro Surveill. 25 (2020) 2001410.

[51] P. Forster, L. Forster, C. Renfrew, M. Forster, Phylogenetic network analysis of SARS-CoV-2 genomes, Proc. Natl. Acad. Sci. U. S. A. 117 (2020) 9241–9243.

[52] Q. Guan, M. Sadykov, S. Mfarrej, S. Hala, R. Naeem, R. Nugmanova, A. Al-Omari, S. Salih, A.A. Mutair, M.J. Carr, W.W. Hall, S.T. Arold, A. Pain, A genetic barcode of SARS-CoV-2 for monitoring global distribution of different clades during the COVID-19 pandemic, Int. J. Infect. Dis. S1201-9712 (1220) (2020) 30680–30681.

[53] M. Becerra-Flores, T. Cardozo, SARS-CoV-2 viral spike G614 mutation exhibits higher case fatality rate, Int. J. Clin. Pract. 74 (2020) e13525.

[54] B. Korber, W. Fischer, S. Gnanakaran, H. Yoon, J. Theiler, W. Abfalterer, B. Foley, E. Giorgi, T. Bhattacharya, M. Parker, D. Partridge, C. Evans, T. Freeman, T. de Silva, C. LaBranche, D. Montefiori, Spike mutation pipeline reveals the emergence of a more transmissible form of SARS-CoV-2, bioRxiv (2020) 2020.2004.2029.069054.

[55] Q. Li, J. Wu, J. Nie, L. Zhang, H. Hao, S. Liu, C. Zhao, Q. Zhang, H. Liu, L. Nie, H. Qin, M. Wang, Q. Lu, X. Li, Q. Sun, J. Liu, L. Zhang, X. Li, W. Huang, Y. Wang, The impact of mutations in SARS-CoV-2 spike on viral infectivity and antigenicity, Cell 182 (2020) 1284-1294.e1289.

[56] Z. Daniloski, X. Guo, N.E. Sanjana, The D614G mutation in SARS-CoV-2 Spike increases transduction of multiple human cell types, bioRxiv (2020) 2020.2006.2014.151357.

[57] Y. Toyoshima, K. Nemoto, S. Matsumoto, Y. Nakamura, K. Kiyotani, SARS-CoV-2 genomic variations associated with mortality rate of COVID-19, J. Hum. Genet. (2020) 1–8.

[58] SARS-CoV-2 Genomes from Nigeria Reveal Community Transmission, Multiple Virus Lineages and Spike Protein Mutation Associated with Higher Transmission and Pathogenicity, [online] Available at: https://virological.org/t/sars-cov-2-genomes-from-nigeria-reveal-community-transmission-multiple-virus-lineages-and-spike-protein-mutation-associated-with-higher-transmission-and-pathogenicity/494 [Accessed 15 September 2020].

[59] S. Ahamad, H. Kanipakam, D. Gupta, Insights into the structural and dynamical changes of spike glycoprotein mutations associated with SARS-CoV-2 host receptor binding, J. Biomol. Struct. Dyn. (2020) 1–13.

[60] F. Begum, D. Mukherjee, S. Das, D. Thagriki, P.P. Tripathi, A.K. Banerjee, U. Ray, Specific mutations in SARS-CoV2 RNA dependent RNA polymerase and helicase alter protein structure, dynamics and thus function: effect on viral RNA replication, bioRxiv (2020) 2020.2004.2026.063024.

[61] D. Eskier, G. Karakülah, A. Suner, Y. Oktay, RdRp mutations are associated with SARS-CoV-2 genome evolution, PeerJ 8 (2020) e9587.

[62] G.B. Chand, A. Banerjee, G.K. Azad, Identification of novel mutations in RNA-dependent RNA polymerases of SARS-CoV-2 and their implications on its protein structure, PeerJ 8 (2020) e9492.

[63] B.E. Young, S.-W. Fong, Y.-H. Chan, T.-M. Mak, L.W. Ang, D.E. Anderson, C.Y.-P. Lee, S.N. Amrun, B. Lee, Y.S. Goh, Y.C.F. Su, W.E. Wei, S. Kalimuddin, L.Y.A. Chai, S. Pada, S.Y. Tan, L. Sun, P. Parthasarathy, Y.Y.C. Chen, T. Barkham, R.T.P. Lin, S. Maurer-Stroh, Y.-S. Leo, L.-F. Wang, L. Renia, V.J. Lee, G.J.D. Smith, D.C. Lye, L.F.P. Ng, Effects of a major deletion in the SARS-CoV-2 genome on the severity of infection and the inflammatory response: an observational cohort study, Lancet 396 (2020) 603–611.

[64] M. Forouzesh, A. Rahimi, R. Valizadeh, N. Dadashzadeh, A. Mirzazadeh, Clinical display, diagnostics and genetic implication of novel Coronavirus (COVID-19) epidemic, Eur. Rev. Med. Pharmacol. Sci. 24 (2020) 4607–4615.

[65] Clinical and virologic characteristics of the first 12 patients with coronavirus disease 2019 (COVID-19) in the United States, Nat. Med. 26 (2020) 861–868.

[66] G.G. Chen Chen, Yanli Xu, Lin Pu, Qi Wang, Liming Wang, Wenling Wang, Yangzi Song, Meiling Chen, Linghang Wang, Fengting Yu, Siyuan Yang, Yunxia Tang, Li Zhao, Huijuan Wang, Yajie Wang, Hui Zeng, Fujie Zhang, SARS-CoV-2–positive sputum and feces after conversion of pharyngeal samples in patients with COVID-19, Ann. Intern. Med. 172 (2020) 832–834.

[67] J. Xia, J. Tong, M. Liu, Y. Shen, D. Guo, Evaluation of coronavirus in tears and conjunctival secretions of patients with SARS-CoV-2 infection, J. Med. Virol. 92 (2020) 589–594.

[68] W. Wang, Y. Xu, R. Gao, R. Lu, K. Han, G. Wu, W. Tan, Detection of SARS-CoV-2 in Different types of clinical specimens, JAMA 323 (2020) 1843–1844.

[69] M.L. Wong, J.F. Medrano, Real-time PCR for mRNA quantitation, BioTechniques 39 (2005) 75–85.

[70] L.J. Carter, L.V. Garner, J.W. Smoot, Y. Li, Q. Zhou, C.J. Saveson, J.M. Sasso, A.C. Gregg, D.J. Soares, T.R. Beskid, S.R. Jervey, C. Liu, Assay techniques and test development for COVID-19 diagnosis, ACS Cent. Sci. 6 (2020) 591–605.

[71] A. Tahamtan, A. Ardebili, Real-time RT-PCR in COVID-19 detection: issues affecting the results, Expert. Rev. Mol. Diagn. 20 (2020) 453–454.

[72] D. Li, D. Wang, J. Dong, N. Wang, H. Huang, H. Xu, C. Xia, False-negative results of real-time reverse-transcriptase polymerase chain reaction for severe acute respiratory syndrome coronavirus 2: role of deep-learning-based CT diagnosis and insights from two cases, Korean J. Radiol. 21 (2020) 505–508.

[73] L.M. Kucirka, S.A. Lauer, O. Laeyendecker, D. Boon, J. Lessler, Variation in false-negative rate of reverse transcriptase Polymerase chain reaction-based SARS-CoV-2 tests by time since exposure, Ann. Intern. Med. (2020) M20–1495.

[74] X. Xie, Z. Zhong, W. Zhao, C. Zheng, F. Wang, J. Liu, Chest CT for typical 2019-nCoV pneumonia: relationship to negative RT-PCR testing, Radiology 200343 (2020).

[75] L. Song, G. Xiao, X. Zhang, Z. Gao, S. Sun, L. Zhang, Y. Feng, G. Luan, S. Lin, M. He, X. Jia, A case of SARS-CoV-2 carrier for 32 days with several times false negative nucleic acid tests, medRxiv (2020) 2020.2003.2031.20045401.

[76] C. Long, H. Xu, Q. Shen, X. Zhang, B. Fan, C. Wang, B. Zeng, Z. Li, X. Li, H. Li, Diagnosis of the coronavirus disease (COVID-19): rRT-PCR or CT? Eur. J. Radiol. 126 (2020) 108961.

[77] Y. Li, L. Yao, J. Li, L. Chen, Y. Song, Z. Cai, C. Yang, Stability issues of RT-PCR testing of SARS-CoV-2 for hospitalized patients clinically diagnosed with COVID-19, J. Med. Virol. 92 (2020) 903–908.

[78] Y. Pan, L. Long, D. Zhang, T. Yuan, S. Cui, P. Yang, Q. Wang, S. Ren, Potential false-negative nucleic acid testing results for severe acute respiratory syndrome coronavirus 2 from thermal inactivation of samples with low viral loads, Clin. Chem. 66 (2020) 794–801.

[79] N. Sethuraman, S.S. Jeremiah, A. Ryo, Interpreting diagnostic tests for SARS-CoV-2, JAMA 323 (2020) 2249–2251.

[80] K.A. Khan, P. Cheung, Presence of mismatches between diagnostic PCR assays and coronavirus SARS-CoV-2 genome, R. Soc. Open Sci. 7 (2020) 200636.

[81] R. Lu, J. Wang, M. Li, Y. Wang, J. Dong, W. Cai, SARS-CoV-2 detection using digital PCR for COVID-19 diagnosis, treatment monitoring and criteria for discharge, medRxiv (2020) 2020.2003.2024.20042689.

[82] T. Suo, X. Liu, J. Feng, M. Guo, W. Hu, D. Guo, H. Ullah, Y. Yang, Q. Zhang, X. Wang, M. Sajid, Z. Huang, L. Deng, T. Chen, F. Liu, K. Xu, Y. Liu, Q. Zhang, Y. Liu, Y. Xiong, G. Chen, K. Lan, Y. Chen, ddPCR: a more sensitive and accurate tool for SARS-CoV-2 detection in low viral load specimens, Emerging microbes & infections 9 (2020) 1259–1268 2020.2002.2029.20029439.

[83] G. Jiang, X. Ren, Y. Liu, H. Chen, W. Liu, Z. Guo, Y. Zhang, C. Chen, J. Zhou, Q. Xiao, H. Shan, Application and optimization of RT-PCR in diagnosis of SARS-CoV-2 infection, medRxiv (2020) 2020.2002.2025.20027755.

[84] L. Shen, F. Huang, X. Chen, Z. Xiong, X. Yang, H. Li, F. Cheng, J. Guo, G. Gong, Diagnostic efficacy of three test kits for SARS-CoV-2 nucleic acid detection, Zhejiang da xue xue bao. Yi xue ban =, J. Zhejiang Univ. (Med. Sci.) 49 (2020) 185–190.

[85] Y. Zhang, C. Wang, M. Han, J. Ye, Y. Gao, Z. Liu, T. He, T. Li, M. Xu, L. Zhou, G. Zou, M. Lu, Z. Zhang, Discrimination of false negative results in RT-PCR detection of SARS-CoV-2 RNAs in clinical specimens by using an internal reference, Virol. Sin. (2020) 1–10.

[86] K. Okamaoto, K. Shirato, N. Nao, S. Saito, T. Kageyama, H. Hasegawa, T. Suzuki, S. Matsuyama, M. Takeda, An assessment of real-time RT-PCR kits for SARS-CoV-2 detection, Jpn. J. Infect. Dis. 73 (2020) 366–368.

[87] P.B. van Kasteren, B. van der Veer, S. van den Brink, L. Wijsman, J. de Jonge, A. van den Brandt, R. Molenkamp, C. Reusken, A. Meijer, Comparison of seven commercial RT-PCR diagnostic kits for COVID-19, J. Clin. Virol. 128 (2020) 104412.

[88] B. Udugama, P. Kadhiresan, H.N. Kozlowski, A. Malekjahani, M. Osborne, V.Y.C. Li, H. Chen, S. Mubareka, J.B. Gubbay, W.C.W. Chan, Diagnosing COVID-19: the disease and tools for detection, ACS Nano 14 (2020) 3822–3835.

[89] L. Bordi, A. Piralla, E. Lalle, F. Giardina, F. Colavita, M. Tallarita, G. Sberna, F. Novazzi, S. Meschi, C. Castilletti, A. Brisci, G. Minnucci, V. Tettamanzi, F. Baldanti, M.R. Capobianchi, Rapid and sensitive detection of SARS-CoV-2 RNA using the Simplexa™ COVID-19 direct assay, J. Clin. Virol. 128 (2020) 104416.

[90] J. Pang, M.X. Wang, I.Y.H. Ang, S.H.X. Tan, R.F. Lewis, J.I. Chen, R.A. Gutierrez, S.X.W. Gwee, P.E.Y. Chua, Q. Yang, X.Y. Ng, R.K. Yap, H.Y. Tan, Y.Y. Teo, C.C. Tan, A.R. Cook, J.C. Yap, L.Y. Hsu, Potential rapid diagnostics, vaccine and therapeutics for 2019 Novel coronavirus (2019-nCoV): a systematic review, J. Clin. Med. 9 (2020).

[91] V.M. Corman, O. Landt, M. Kaiser, R. Molenkamp, A. Meijer, D.K. Chu, T. Bleicker, S. Brünink, J. Schneider, M.L. Schmidt, D.G. Mulders, B.L. Haagmans, B. van der Veer, S. van den Brink, L. Wijsman, G. Goderski, J.-L. Romette, J. Ellis, M. Zambon, M. Peiris, H. Goossens, C. Reusken, M.P. Koopmans, C. Drosten, Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR, Euro Surveill. 25 (2020) 2000045.

[92] A. Coronavirus Test Tracker, Commercially Available COVID-19 Diagnostic Tests, 360Dx, 2020. www.360dx.com/coronavirus-test-tracker-launched-covid-19-tests.

[93] D.K.W. Chu, Y. Pan, S.M.S. Cheng, K.P.Y. Hui, P. Krishnan, Y. Liu, D.Y.M. Ng, C.K.C. Wan, P. Yang, Q. Wang, M. Peiris, L.L.M. Poon, Molecular diagnosis of a novel coronavirus (2019-nCoV) causing an outbreak of pneumonia, Clin. Chem. 66 (2020) 549–555.

[94] A. Kwallah, S. Inoue, A.W. Muigai, T. Kubo, R. Sang, K. Morita, M. Mwau, A real-time reverse transcription loop-mediated isothermal amplification assay for the rapid detection of yellow fever virus, J. Virol. Methods 193 (2013) 23–27.

[95] H. Li, X. Wang, W. Liu, X. Wei, W. Lin, E. Li, P. Li, D. Dong, L. Cui, X. Hu, B. Li, Y. Ma, X. Zhao, C. Liu, J. Yuan, Survey and visual detection of zaire ebolavirus in clinical samples targeting the nucleoprotein gene in sierra leone, Front. Microbiol. 6 (2015) 1332.

[96] N. Chotiwan, C.D. Brewster, T. Magalhaes, J. Weger-Lucarelli, N.K. Duggal, C. Rückert, C. Nguyen, S.M. Garcia Luna, J.R. Fauver, B. Andre, M. Gray, W.C.t. Black, R.C. Kading, G.D. Ebel, G. Kuan, A. Balmaseda, T. Jaenisch, E.T.A. Marques, A.C. Brault, E. Harris, B.D. Foy, S.L. Quackenbush, R. Perera, J. Rovnak, Rapid and specific detection of Asian- and African-lineage Zika viruses, Sci. Transl. Med. 9 (2017).

[97] Y. Ge, B. Wu, X. Qi, K. Zhao, X. Guo, Y. Zhu, Y. Qi, Z. Shi, M. Zhou, H. Wang, L. Cui, Rapid and sensitive detection of novel avian-origin influenza A (H7N9) virus by reverse transcription loop-mediated isothermal amplification combined with a lateral-flow device, PLoS One 8 (2013) e69941.

[98] T. Notomi, H. Okayama, H. Masubuchi, T. Yonekawa, K. Watanabe, N. Amino, T. Hase, Loop-mediated isothermal amplification of DNA, Nucleic Acids Res. 28 (2000) e63.

[99] E.-T. Mohamed, B. Haim, H.S. Jinzhao, A single and two-stage, closed-tube, molecular test for the 2019 novel coronavirus (COVID-19) at home, Clin. Points Entry (2020).

[100] Y. Zhang, N. Odiwuor, J. Xiong, L. Sun, R.O. Nyaruaba, H. Wei, N.A. Tanner, Rapid molecular detection of SARS-CoV-2 (COVID-19) virus RNA using colorimetric LAMP, medRxiv (2020) 2020.2002.2026.20028373.

[101] C. Yan, J. Cui, L. Huang, B. Du, L. Chen, G. Xue, S. Li, W. Zhang, L. Zhao, Y. Sun, H. Yao, N. Li, H. Zhao, Y. Feng, S. Liu, Q. Zhang, D. Liu, J. Yuan, Rapid and visual detection of 2019 novel coronavirus (SARS-CoV-2) by a reverse transcription loop-mediated isothermal amplification assay, Clin. Microbiol. Infect. 26 (2020) 773–779.

[102] M.A. Lalli, X. Chen, S.J. Langmade, C.C. Fronick, C.S. Sawyer, L.C. Burcea, R.S. Fulton, M. Heinz, W.J. Buchser, R.D. Head, R.D. Mitra, J. Milbrandt, *Rapid and extraction-free detection of SARS-CoV-2 from saliva with colorimetric LAMP*, medRxiv (2020) the preprint server for health sciences. 2020.2005.2007.20093542.

[103] J. Kashir, A. Yaqinuddin, Loop mediated isothermal amplification (LAMP) assays as a rapid diagnostic for COVID-19, Med. Hypotheses 141 (2020) 109786.

[104] V.L. Dao Thi, K. Herbst, K. Boerner, M. Meurer, L.P. Kremer, D. Kirrmaier, A. Freistaedter, D. Papagiannidis, C. Galmozzi, M.L. Stanifer, S. Boulant, S. Klein, P. Chlanda, D. Khalid, I. Barreto Miranda, P. Schnitzler, H.-G. Kräusslich, M. Knop, S. Anders, A colorimetric RT-LAMP assay and LAMP-sequencing for detecting SARS-CoV-2 RNA in clinical samples, Sci. Transl. Med. 12 (2020) eabc7075.

[105] I.M. Lobato, C.K. O'Sullivan, Recombinase polymerase amplification: Basics, applications and recent advances, TrAC Trends Anal. Chem. 98 (2018) 19–35.

[106] Y. Li, X. Deng, F. Hu, J. Wang, Y. Liu, H. Huang, J. Ma, J. Zhang, F. Zhang, C. Zhang, Metagenomic analysis identified co-infection with human rhinovirus C and bocavirus 1 in an adult suffering from severe pneumonia, J. Infect. 76 (2018) 311–313.

[107] N.R. Pollock, B. Wonderly, Evaluating novel diagnostics in an outbreak setting: lessons learned from ebola, J. Clin. Microbiol. 55 (2017) 1255–1261.

[108] S.C. Moore, R. Penrice-Randal, M. Alruwaili, X. Dong, S.T. Pullan, D. Carter, K. Bewley, Q. Zhao, Y. Sun, C. Hartley, E.-m. Zhou, T. Solomon, M.B.J. Beadsworth, J. Cruise, D. Bogaert, D.W.T. Crook, D.A. Matthews, A.D. Davidson, Z. Mahmood, W. Aljabr, J. Druce, R.T. Vipond, L. Ng, L. Renia, P. Openshaw, J.K. Baillie, M.W. Carroll, C. Semple, L. Turtle, J.A. Hiscox, Amplicon based MinION sequencing of SARS-CoV-2 and metagenomic characterisation of nasopharyngeal swabs from patients with COVID-19, medRxiv (2020) 2020.2003.2005.20032011.

[109] Y. Li, S. Li, J. Wang, G. Liu, CRISPR/Cas systems towards next-generation biosensing, Trends Biotechnol. 37 (2019) 730–743.

[110] M. Azhar, R. Phutela, A.H. Ansari, D. Sinha, N. Sharma, M. Kumar, M. Aich, S. Sharma, K. Singhal, H. Lad, P.K. Patra, G. Makharia, G.R. Chandak, D. Chakraborty, S. Maiti, Rapid, field-deployable nucleobase detection and identification using FnCas9, bioRxiv (2020) 2020.2004.2007.028167.

[111] M.J. Kellner, J.G. Koob, J.S. Gootenberg, O.O. Abudayyeh, F. Zhang, SHERLOCK: nucleic acid detection with CRISPR nucleases, Nat. Protoc. 14 (2019) 2986–3012.

[112] J.S. Gootenberg, O.O. Abudayyeh, J.W. Lee, P. Essletzbichler, A.J. Dy, J. Joung, V. Verdine, N. Donghia, N.M. Daringer, C.A. Freije, C. Myhrvold, R.P. Bhattacharyya, J. Livny, A. Regev, E.V. Koonin, D.T. Hung, P.C. Sabeti, J.J. Collins, F. Zhang, Nucleic acid detection with CRISPR-Cas13a/C2c2, Science (New York, N.Y.) 356 (2017) 438–442.

[113] T. Hou, W. Zeng, M. Yang, W. Chen, L. Ren, J. Ai, J. Wu, Y. Liao, X. Gou, Y. Li, X. Wang, H. Su, B. Gu, J. Wang, T. Xu, Development and evaluation of a rapid CRISPR-based diagnostic for COVID-19, PLoS Pathog 16 (2020) e1008705-e1008705.

[114] J.P. Broughton, X. Deng, G. Yu, C.L. Fasching, V. Servellita, J. Singh, X. Miao, J.A. Streithorst, A. Granados, A. Sotomayor-Gonzalez, K. Zorn, A. Gopez, E. Hsu, W. Gu, S. Miller, C.Y. Pan, H. Guevara, D.A. Wadford, J.S. Chen, C.Y. Chiu, CRISPR-Cas12-based detection of SARS-CoV-2, Nature biotechnology 38 (2020) 870–874.

[115] X. Ding, K. Yin, Z. Li, C. Liu, All-in-one dual CRISPR-Cas12a (AIOD-CRISPR) assay: a case for rapid, ultrasensitive and visual detection of novel coronavirus SARS-CoV-2 and HIV virus, bioRxiv (2020) 2020.2003.2019.998724.

[116] J.H.K. Chen, H.Y. Lam, C.C.Y. Yip, S.C.Y. Wong, J.F.W. Chan, E.S.K. Ma, V.C.C. Cheng, B.S.F. Tang, K.Y. Yuen, Clinical evaluation of the new high-throughput luminex NxTAG respiratory pathogen panel assay for multiplex respiratory pathogen detection, J. Clin. Microbiol. 54 (2016) 1820–1825.

[117] D. Huzly, K. Korn, S. Bierbaum, B. Eberle, V. Falcone, A. Knöll, P. Steininger, M. Panning, Influenza A virus drift variants reduced the detection sensitivity of a commercial multiplex nucleic acid amplification assay in the season 2014/15, Arch. Virol. 161 (2016) 2417–2423.

[118] J. Li, N.Y. Mao, C. Zhang, M.J. Yang, M. Wang, W.B. Xu, X.J. Ma, The development of a GeXP-based multiplex reverse transcription-PCR assay for simultaneous detection of sixteen human respiratory virus types/subtypes, BMC Infect. Dis. 12 (2012) 189.

[119] J. Deng, Z. Ma, W. Huang, C. Li, H. Wang, Y. Zheng, R. Zhou, Y.W. Tang, Respiratory virus multiplex RT-PCR assay sensitivities and influence factors in hospitalized children with lower respiratory tract infections, Virol. Sin. 28 (2013) 97–102.

[120] M.J. Loeffelholz, D.L. Pong, R.B. Pyles, Y. Xiong, A.L. Miller, K.K. Bufton, T. Chonmaitree, Comparison of the FilmArray Respiratory Panel and Prodesse real-time PCR assays for detection of respiratory pathogens, J. Clin. Microbiol. 49 (2011) 4083–4088.

[121] L.O. Attwood, M.J. Francis, J. Hamblin, T.M. Korman, J. Druce, M. Graham, Clinical evaluation of AusDiagnostics SARS-CoV-2 multiplex tandem PCR assay, J. Clin. Virol. 128 (2020) 104448.

[122] C.M. Ackerman, C. Myhrvold, S.G. Thakku, C.A. Freije, H.C. Metsky, D.K. Yang, S.H. Ye, C.K. Boehm, T.F. Kosoko-Thoroddsen, J. Kehe, T.G. Nguyen, A. Carter, A. Kulesa, J.R. Barnes, V.G. Dugan, D.T. Hung, P.C. Blainey, P.C. Sabeti, Massively multiplexed nucleic acid detection with Cas13, Nature 582 (2020) 277–282.

[123] F. Sun, A. Ganguli, J. Nguyen, R. Brisbin, K. Shanmugam, D.L. Hirschberg, M.B. Wheeler, R. Bashir, D.M. Nash, B.T. Cunningham, Smartphone-based multiplex 30-minute nucleic acid test of live virus from nasal swab extract, Lab Chip 20 (2020) 1621–1627.

[124] T.H. Wu, C.C. Chang, C.H. Yang, W.Y. Lin, T.J. Ee, C.W. Lin, Hybridization chain reactions targeting the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), Int. J. Mol. Sci. 21 (2020).

[125] G. Xu, H. Zhao, J. Reboud, J.M. Cooper, Cycling of rational hybridization chain reaction to enable enzyme-free dna-based clinical diagnosis, ACS Nano 12 (2018) 7213–7219.

[126] V.N. Kim, J. Han, M.C. Siomi, Biogenesis of small RNAs in animals, Nat. Rev. Mol. Cell Biol. 10 (2009) 126–139.

[127] A. Qureshi, V.G. Tantray, A.R. Kirmani, A.G. Ahangar, A review on current status of antiviral siRNA, Rev. Med. Virol. 28 (2018) e1976.

[128] H.S. Lee, J. Ahn, Y. Jee, I.S. Seo, E.J. Jeon, E.S. Jeon, C.H. Joo, Y.K. Kim, H. Lee, Universal and mutation-resistant anti-enteroviral activity: potency of small interfering RNA complementary to the conserved cis-acting replication element within the enterovirus coding region, J. Gen. Virol. 88 (2007) 2003–2012.

[129] X. Wang, X. Wang, R.K. Varma, L. Beauchamp, S. Magdaleno, T.J. Sendera, Selection of hyperfunctional siRNAs with improved potency and specificity, Nucleic Acids Res. 37 (2009) e152.

[130] J. Kurreck, siRNA efficiency: structure or sequence-that is the question, J. Biomed. Biotechnol. 2006 (2006) 83757.

[131] S.W. Ding, Q. Han, J. Wang, W.X. Li, Antiviral RNA interference in mammals, Curr. Opin. Immunol. 54 (2018) 109–114.

[132] P.V. Maillard, A.G. van der Veen, E.Z. Poirier, C. Reis e Sousa, Slicing and dicing viruses: antiviral RNA interference in mammals, EMBO J. 38 (2019).

[133] B. Berkhout, RNA interference as an antiviral approach: targeting HIV-1, Curr. Opin. Mol. Ther. 6 (2004) 141–145.

[134] M. Jiang, J. Milner, Selective silencing of viral gene expression in HPV-positive human cervical carcinoma cells treated with siRNA, a primer of RNA interference, Oncogene 21 (2002) 6041–6048.

[135] Y. Kusov, T. Kanda, A. Palmenberg, J.-Y. Sgro, V. Gauss-Müller, Silencing of hepatitis A virus infection by small interfering RNAs, J. Virol. 80 (2006) 5599–5610.

[136] F. Jia, Y.Z. Zhang, C.M. Liu, A retrovirus-based system to stably silence hepatitis B virus genes by RNA interference, Biotechnol. Lett. 28 (2006) 1679–1685.

[137] Y.C. Li, L.H. Kong, B.Z. Cheng, K.S. Li, Construction of influenza virus siRNA expression vectors and their inhibitory effects on multiplication of influenza virus, Avian Dis. 49 (2005) 562–573.

[138] K. Asha, P. Kumar, M. Sanicas, C.A. Meseko, M. Khanna, B. Kumar, Advancements in nucleic acid based therapeutics against respiratory viral infections, J. Clin. Med. 8 (2018) 6.

[139] A. Eckstein, T. Grössl, A. Geisler, X. Wang, S. Pinkert, T. Pozzuto, C. Schwer, J. Kurreck, S. Weger, R. Vetter, W. Poller, H. Fechner, Inhibition of adenovirus infections by siRNA-mediated silencing of early and late adenoviral gene functions, Antivir. Res. 88 (2010) 86–94.

[140] B.J. Li, Q. Tang, D. Cheng, C. Qin, F.Y. Xie, Q. Wei, J. Xu, Y. Liu, B.J. Zheng, M.C. Woodle, N. Zhong, P.Y. Lu, Using siRNA in prophylactic and therapeutic regimens against SARS coronavirus in Rhesus macaque, Nat. Med. 11 (2005) 944–951.

[141] J. DeVincenzo, R. Lambkin-Williams, T. Wilkinson, J. Cehelsky, S. Nochur, E. Walsh, R. Meyers, J. Gollob, A. Vaishnaw, A randomized, double-blind, placebo-controlled study of an RNAi-based therapy directed against respiratory syncytial virus, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 8800–8805.

[142] R.G. Gish, C. Satishchandran, M. Young, C. Pachuk, RNA interference and its potential applications to chronic HBV treatment: results of a Phase I safety and tolerability study, Antivir. Ther. 16 (2011) 547–554.

[143] L.F. Gebert, M.A. Rebhan, S.E. Crivelli, R. Denzler, M. Stoffel, J. Hall, Miravirsen (SPC3649) can inhibit the biogenesis of miR-122, Nucleic Acids Res. 42 (2014) 609–621.

[144] B.L. Davidson, P.B. McCray Jr., Current prospects for RNA interference-based therapies, Nat. Rev. Genet. 12 (2011) 329–340.

[145] J.H. Choi, M.A. Croyle, Emerging targets and novel approaches to Ebola virus prophylaxis and treatment, BioDrugs 27 (2013) 565–583.

[146] C. Liu, Q. Zhou, Y. Li, L.V. Garner, S.P. Watkins, L.J. Carter, J. Smoot, A.C. Gregg, A.D. Daniels, S. Jervey, D. Albaiu, Research and development on therapeutic agents and vaccines for COVID-19 and related human coronavirus diseases, ACS Cent Sci 6 (2020) 315–331.

[147] S. Ghosh, S.M. Firdous, A. Nath, siRNA could be a potential therapy for COVID-19, EXCLI J. 19 (2020) 528–531.

[148] M.L. He, B. Zheng, Y. Peng, J.S. Peiris, L.L. Poon, K.Y. Yuen, M.C. Lin, H.F. Kung, Y. Guan, Inhibition of SARS-associated coronavirus infection and replication by RNA interference, JAMA 290 (2003) 2665–2666.

[149] A. Lu, H. Zhang, X. Zhang, H. Wang, Q. Hu, L. Shen, B.S. Schaffhausen, W. Hou, L. Li, Attenuation of SARS coronavirus by a short hairpin RNA expression plasmid targeting RNA-dependent RNA polymerase, Virology 324 (2004) 84–89.

[150] Y. Zhang, T. Li, L. Fu, C. Yu, Y. Li, X. Xu, Y. Wang, H. Ning, S. Zhang, W. Chen, L.A. Babiuk, Z. Chang, Silencing SARS-CoV Spike protein expression in cultured cells by RNA interference, FEBS Lett. 560 (2004) 141–146.

[151] T. Li, Y. Zhang, L. Fu, C. Yu, X. Li, Y. Li, X. Zhang, Z. Rong, Y. Wang, H. Ning, R. Liang, W. Chen, L.A. Babiuk, Z. Chang, siRNA targeting the leader sequence of SARS-CoV inhibits virus replication, Gene Ther. 12 (2005) 751–761.

[152] Y. Shi, D.H. Yang, J. Xiong, J. Jia, B. Huang, Y.X. Jin, Inhibition of genes expression of SARS coronavirus by synthetic small interfering RNAs, Cell Res. 15 (2005) 193–200.

[153] L. Cui, H. Wang, Y. Ji, J. Yang, S. Xu, X. Huang, Z. Wang, L. Qin, P. Tien, X. Zhou, D. Guo, Y. Chen, The nucleocapsid protein of coronaviruses Acts as a viral suppressor of RNA silencing in mammalian cells, J. Virol. 89 (2015) 9029–9043.

[154] W. Chen, P. Feng, K. Liu, M. Wu, H. Lin, Computational identification of small interfering RNA targets in SARS-CoV-2, Virol. Sin. (2020) 1–3.

[155] J. Mu, J. Xu, L. Zhang, T. Shu, D. Wu, M. Huang, Y. Ren, X. Li, Q. Geng, Y. Xu, Y. Qiu, X. Zhou, SARS-CoV-2-encoded nucleocapsid protein acts as a viral suppressor of RNA interference in cells, Sci. China Life Sci. (2020) 1–4.

[156] M. Hoffmann, H. Kleine-Weber, S. Schroeder, N. Krüger, T. Herrler, S. Erichsen, T.S. Schiergens, G. Herrler, N.H. Wu, A. Nitsche, M.A. Müller, C. Drosten, S. Pöhlmann, SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor, Cell 181 (2020) 271-280.e278.

[157] C.Y. Lu, H.Y. Huang, T.H. Yang, L.Y. Chang, C.Y. Lee, L.M. Huang, siRNA silencing of angiotensin-converting enzyme 2 reduced severe acute respiratory syndrome-associated coronavirus replications in Vero E6 cells, Eur. J. Clin. Microbiol. Infect. Dis. 27 (2008) 709–715.

[158] I. Hamming, M.E. Cooper, B.L. Haagmans, N.M. Hooper, R. Korstanje, A.D. Osterhaus, W. Timens, A.J. Turner, G. Navis, H. van Goor, The emerging role of ACE2 in physiology and disease, J. Pathol. 212 (2007) 1–11.

[159] M. Kawase, K. Shirato, L. van der Hoek, F. Taguchi, S. Matsuyama, Simultaneous treatment of human bronchial epithelial cells with serine and cysteine protease inhibitors prevents severe acute respiratory syndrome coronavirus entry, J. Virol. 86 (2012) 6537–6545.

[160] E. Girardi, P. López, S. Pfeffer, On the importance of host MicroRNAs during viral infection, Front. Genet. 9 (2018) 439.

[161] M.D. Saçar Demirci, A. Adan, Computational analysis of microRNA-mediated interactions in SARS-CoV-2 infection, PeerJ 8 (2020) e9369-e9369.

[162] E.D. Arisan, A. Dart, G.H. Grant, S. Arisan, S. Cuhadaroglu, S. Lange, P. Uysal-Onganer, The prediction of miRNAs in SARS-CoV-2 genomes: hsa-miR databases identify 7 key miRs linked to host responses and virus pathogenicity-related KEGG pathways significant for comorbidities, Viruses 12 (2020).

[163] A. Guterres, C.H. de Azeredo Lima, R.L. Miranda, M.R. Gadelha, What is the potential function of microRNAs as biomarkers and therapeutic targets in COVID-19? Infect. Genet. Evol. 85 (2020) 104417.

[164] X. Zhang, H. Chu, L. Wen, H. Shuai, D. Yang, Y. Wang, Y. Hou, Z. Zhu, S. Yuan, F. Yin, J.F. Chan, K.Y. Yuen, Competing endogenous RNA network profiling reveals novel host dependency factors required for MERS-CoV propagation, Emerg. Microbes Infect. 9 (2020) 733–746.

[165] M.D. Saçar Demirci, A. Adan, Computational analysis of microRNA-mediated interactions in SARS-CoV-2 infection, PeerJ 8 (2020) e9369.

[166] Z.Y. Yang, W.P. Kong, Y. Huang, A. Roberts, B.R. Murphy, K. Subbarao, G.J. Nabel, A DNA vaccine induces SARS coronavirus neutralization and protective immunity in mice, Nature 428 (2004) 561–564.

[167] K. Modjarrad, C.C. Roberts, K.T. Mills, A.R. Castellano, K. Paolino, K. Muthumani, E.L. Reuschel, M.L. Robb, T. Racine, M.D. Oh, C. Lamarre, F.I. Zaidi, J. Boyer, S.B. Kudchodkar, M. Jeong, J.M. Darden, Y.K. Park, P.T. Scott, C. Remigio, A.P. Parikh, M.C. Wise, A. Patel, E.K. Duperret, K.Y. Kim, H. Choi, S. White, M. Bagarazzi, J.M. May, D. Kane, H. Lee, G. Kobinger, N.L. Michael, D.B. Weiner, S.J. Thomas, J.N. Maslow, Safety and immunogenicity of an anti-Middle East respiratory syndrome coronavirus DNA vaccine: a phase 1, open-label, single-arm, dose-escalation trial, Lancet Infect. Dis. 19 (2019) 1013–1022.

[168] T.R.F. Smith, A. Patel, S. Ramos, D. Elwood, X. Zhu, J. Yan, E.N. Gary, S.N. Walker, K. Schultheis, M. Purwar, Z. Xu, J. Walters, P. Bhojnagarwala, M. Yang, N. Chokkalingam, P. Pezzoli, E. Parzych, E.L. Reuschel, A. Doan, N. Tursi, M. Vasquez, J. Choi, E. Tello-Ruiz, I. Maricic, M.A. Bah, Y. Wu, D. Amante, D.H. Park, Y. Dia, A.R. Ali, F.I. Zaidi, A. Generotti, K.Y. Kim, T.A. Herring, S. Reeder, V.M. Andrade, K. Buttigieg, G. Zhao, J.M. Wu, D. Li, L. Bao, J. Liu, W. Deng, C. Qin, A.S. Brown, M. Khoshnejad, N. Wang, J. Chu, D. Wrapp, J.S. McLellan, K. Muthumani, B. Wang, M.W. Carroll, J.J. Kim, J. Boyer, D.W. Kulp, L. Humeau, D.B. Weiner, K.E. Broderick, Immunogenicity of a DNA vaccine candidate for COVID-19, Nat. Commun. 11 (2020) 2601.

[169] J. Yu, L.H. Tostanoski, L. Peter, N.B. Mercado, K. McMahan, S.H. Mahrokhian, J.P. Nkolola, J. Liu, Z. Li, A. Chandrashekar, D.R. Martinez, C. Loos, C. Atyeo, S. Fischinger, J.S. Burke, M.D. Slein, Y. Chen, A. Zuiani, N.L. FJ, M. Travers, S. Habibi, L. Pessaint, A. Van Ry, K. Blade, R. Brown, A. Cook, B. Finneyfrock, A. Dodson, E. Teow, J. Velasco, R. Zahn, F. Wegmann, E.A. Bondzie, G. Dagotto, M.S. Gebre, X. He, C. Jacob-Dolan, M. Kirilova, N. Kordana, Z. Lin, L.F. Maxfield, F. Nampanya, R. Nityanandam, J.D. Ventura, H. Wan, Y. Cai, B. Chen, A.G. Schmidt, D.R. Wesemann, R.S. Baric, G. Alter, H. Andersen, M.G. Lewis, D.H. Barouch, DNA vaccine protection against SARS-CoV-2 in rhesus macaques, Science (New York, N.Y.) 369 (2020) 806–811.

[170] World Health Organization, Draft Landscape of COVID-19 Candidate Vaccines, (9 September 2020) Accessed September 15, 2020.

[171] Nytimes.com, Coronavirus Vaccine Tracker, [online] Available at: https://www.nytimes.com/interactive/2020/science/coronavirus-vaccine-tracker.html>, (2020) [Accessed 09 September 2020].

[172] Y. Eygeris, S. Patel, A. Jozic, G. Sahay, Deconvoluting lipid nanoparticle structure for messenger RNA delivery, Nano Lett. 20 (2020) 4543–4549.

[173] N. Pardi, M.J. Hogan, F.W. Porter, D. Weissman, mRNA vaccines – a new era in vaccinology, Nat. Rev. Drug Discov. 17 (2018) 261–279.

[174] N. Pardi, M.J. Hogan, D. Weissman, Recent advances in mRNA vaccine technology, Curr. Opin. Immunol. 65 (2020) 14–20.

[175] F. Wang, R.M. Kream, G.B. Stefano, An evidence based perspective on mRNA-SARS-CoV-2 vaccine development, Med. Sci. Monit. 26 (2020) e924700.

[176] D.S. Mishra, T cell epitope-based vaccine design for pandemic novel coronavirus 2019-nCoV, ChemRxiv (2020), https://doi.org/10.26434/chemrxiv.12029523.v1.

[177] C.H. Lee, H. Koohy, In silico identification of vaccine targets for 2019-nCoV, F1000Res 9 (2020) 145.

[178] M. Bhattacharya, A.R. Sharma, P. Patra, P. Ghosh, G. Sharma, B.C. Patra, S.-S. Lee, C. Chakraborty, Development of epitope-based peptide vaccine against novel coronavirus 2019 (SARS-COV-2): Immunoinformatics approach, J. Med. Virol. 92 (2020) 618–631.

[179] L. Kozlovskaya, A. Piniaeva, G. Ignatyev, A. Selivanov, A. Shishova, A. Kovpak, I. Gordeychuk, Y. Ivin, A. Berestovskaya, E. Prokhortchouk, D. Protsenko, M. Rychev, A. Ishmukhametov, Isolation and phylogenetic analysis of SARS-CoV-2 variants collected in Russia during the COVID-19 outbreak, Int. J. Infect. Dis. 99 (2020) 40–46.

[180] I. Saha, N. Ghosh, D. Maity, N. Sharma, J.P. Sarkar, K. Mitra, Genome-wide analysis of Indian SARS-CoV-2 genomes for the identification of genetic mutation and SNP, Infect. Genet. Evol. 85 (2020) 104457.

[181] M. Mukherjee, S. Goswami, Global cataloguing of variations in untranslated regions of viral genome and prediction of key host RNA binding protein-microRNA interactions modulating genome stability in SARS-CoV-2, PLoS One 15 (2020) e0237559.

[182] J.N. Rauch, E. Valois, S.C. Solley, F. Braig, R.S. Lach, N.J. Baxter, K.S. Kosik, C. Arias, D. Acosta-Alvear, M.Z. Wilson, A scalable, easy-to-deploy, protocol for Cas13-Based detection of SARS-CoV-2 genetic material, bioRxiv (2020) 2020.2004.2020.052159.

[183] L. Guo, X. Sun, X. Wang, C. Liang, H. Jiang, Q. Gao, M. Dai, B. Qu, S. Fang, Y. Mao, Y. Chen, G. Feng, Q. Gu, L. Wang, R.R. Wang, Q. Zhou, W. Li, SARS-CoV-2 detection with CRISPR diagnostics, Cell Discovery 6 (2020) 34 2020.2004.2010.023358.

[184] A. Nili, A. Farbod, A. Neishabouri, M. Mozafarihashjin, S. Tavakolpour, H.Remdesivir Mahmoudi, A beacon of hope from Ebola virus disease to COVID-19, Rev Med Virol. (2020) e2133, https://doi.org/10.1002/rmv.2133.