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.

Virus Research 294 (2021) 198282

Contents lists available at ScienceDirect

Virus Research

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

Review

CRISPR systems: Novel approaches for detection and combating COVID-19

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ARTICLE INFO

Keywords:
CRISPR-Cas system
Single-stranded RNA viruses
SARS-CoV-2
Diagnosis
SHERLOCK
DETECTR
PAC-MAN
ABACAS

ABSTRACT

Type V and VI CRISPR enzymes are RNA-guided, DNA and RNA-targeting effectors that allow specific gene knockdown. Cas12 and Cas13 are CRISPR proteins that are efficient agents for diagnosis and combating single-stranded RNA (ssRNA) viruses. The programmability of these proteins paves the way for the detection and degradation of RNA viruses by targeting RNAs complementary to its CRISPR RNA (crRNA). Approximately two-thirds of viruses causing diseases contain ssRNA genomes. The Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) has caused the outbreak of the coronavirus disease 2019 (COVID-19), which has infected more than 88 million people worldwide with near 2 million deaths since December 2019. Thus, accurate and rapid diagnostic and therapeutic tools are essential for early detection and treatment of this widespread infectious disease. For us, the CRISPR based platforms seem to be a plausible new approach for an accurate detection and treatment of SARS-CoV-2.

In this review, we talk about Cas12 and Cas13 CRISPR systems and their applications in diagnosis and treatment of RNA virus mediated diseases. In continue, the SARS-CoV-2 pathogenicity, and its conventional diagnostics and antivirals will be discussed. Moreover, we highlight novel CRISPR based diagnostic platforms and therapies for COVID-19. We also discuss the challenges of diagnostic CRISPR based platforms as well as clarifying the proposed solution for high efficient selective in vivo delivery of CRISPR components into SARS-CoV-2-infected cells.

1. Introduction

Clustered regularly interspaced short palindromic repeats (CRISPR) technology are seen as a powerful tool for editing genomes (Safari et al., 2018, 2020b). This allows researchers to alter DNA sequences and modify gene function easily. CRISPR and CRISPR-associated Cas proteins carry many potential applications which include correcting genetic defects, treating and preventing the spread of diseases. Using the immense potential of this system, novel applications have been in development in bench science as well as clinical research. Recent advances in the CRISPR system have enabled researchers to make proper use of this technology in both therapeutic and most recently as a tool for molecular diagnostics of different infectious diseases (Safari et al., 2017). For this purpose, the CRISPR/Cas9-based tools have been recently used as an antiviral agent for the treatment of HIV infection as well as detection of infection with Zika virus and Methicillin-resistant *Staphylococcus aureus* (Strich and Chertow, 2019). On the other hand, the invention of RNA-guided, RNA-targeting CRISPR effectors (Zhang

Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, Coronavirus disease 2019; ssRNA viruses, single-stranded RNA viruses; CRISPR, clustered regularly interspaced short palindromic repeats; CRISPR-Cas, CRISPR and CRISPR-associated proteins; SHERLOCK, specific high sensitive enzymatic reporter unlocking; PAC-MAN, prophylactic antiviral crispr in human cells; crRNAs, CRISPR RNAs; DSB, DNA double-strand break; PAM, protospacer adjacent motif; DETECTR, DNA endonuclease-targeted CRISPR trans reporter; HOLMES, a one-hour low-cost multipurpose highly efficient system; DR, direct repeat; (+) ssRNA virus, positive-sense single-stranded RNA virus; RTC, replication-transcription complex; RT-LAMP, reverse transcription simultaneous with isothermal amplification by using loop-mediated amplification; AIOD-CRISPR, all-in-one dual CRISPR-Cas12a; CRISPR/Cas12a-NER, CRISPR/Cas12a-based-detection with naked eye readout; CONAN, Cas3-operated nucleic acid detection; GFP, green fluorescent protein; NLS, nuclear localization sequence; MFE, minimum free energy.

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https://doi.org/10.1016/j.virusres.2020.198282
Received 11 November 2020; Received in revised form 13 December 2020; Accepted 27 December 2020
Available online 8 January 2021
0168-1702/

et al., 2019b) sets the stage for CRISPR-Cas13 based tool kit in clinical diagnosis as well as defending against RNA viruses (Freije et al., 2019).

The single-stranded RNA viruses (ssRNA), including negative-sense (Ebola, Marburg, and Influenza) (Leung, 2019) and positive-sense (Corona and HIV) (Prajapat et al., 2020) are critical human pathogens worldwide. RNA viruses like Corona, Ebola, Zika, and different strains of influenza A viruses mediate recent virus outbreaks that have been led to a considerable number of human deaths. Respiratory RNA viruses are highly contagious and result in occasional pandemic outbreaks and as well as the worldwide epidemics which happen annually (Badolo et al., 2019).

Currently, the emerging novel (new) coronavirus (SARS-CoV-2) outbreak has caused a rapid increase in morbidity and mortality rate all over the world. SARS-CoV-2 is known as the third highly pathogenic coronavirus in the human population after the severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV) (Kouhpayeh et al., 2020). The SARS-CoV-2 belongs to a family of positive-sense RNA viruses that often infect both upper and lower respiratory tracks (Lu et al., 2020). This ssRNA virus has infected more than 88 million people globally with close to 2 million deaths since December 2019 (https://www.worldometers.info/coronavirus/). The lack of any viable therapy in addition to the urgent need for the invention of rapid diagnostic tests offer the use of novel techniques like CRISPR technology as a plausible candidate for a diagnostic and therapeutic vehicles for diagnosis and treatment of SARS-CoV-2 infection.

Specific High sensitive Enzymatic Reporter Unlocking (SHERLOCK) technology described by Feng Zhang laboratory and DNA endonuclease-targeted CRISPR trans reporter (DETECTR) provide rapid and accurate diagnostic assays based on CRISPR-Cas13 for emerging 2019 Novel coronavirus disease (COVID-19) (Udugama et al., 2020).

Isothermal amplification methods such as Loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA) has been employed to increase the sensitivity of CRISPR based platforms and using them in low resource condition. LAMP is a highly rapid, efficient and specific amplification method operating at 60−65 °C. This method recruits a DNA polymerase and a set of four LAMP specific primers that recognize six distinct sequences on the target DNA. The LAMP cycling reaction results in accumulation of 10^9 copies of target in less than an hour (Wong et al., 2018). RPA amplifies the target sequence with high sensitivity and selectivity under isothermal condition (37–42 °C). This method requires minimal sample preparation and has the ability of amplifying as low as 1–10 target copies in less than 20 min (Lobato and O'Sullivan, 2018).

Prophylactic Antiviral CRISPR in huMAN cells (PAC-MAN) strategy has been introduced for fighting COVID-19 based on Cas13d (Abbott et al., 2020). Hence, CRISPR/Cas based technology has a robust application potential as a fast, sensitive, and specific diagnostic and therapeutic tool kit for emerging infectious diseases. The following review aims to describe the overall aspects of CRISPR systems, their classification and applications in viral diseases detection and treatment.

2. General aspects of CRISPR/Cas systems

CRISPR/Cas systems are defined as the adaptive system of 45 and 87 percent of bacteria and archaeal organisms, respectively (Safari et al., 2019). Via an adaptive activity, the CRISPR/Cas system scans the genome of the invader phage and induces a DNA double-strand break (DSB) in a fragment of the phage genome that flanks in the upstream of an adjacent protospacer motif (PAM). Dissected DNA fragments, also known as spacers, become exposed to the CRISPR array, and afterwards are flanked between the direct repeats (Mojica et al., 2005; Rahmatabadi et al., 2016). These spacer fragments produce the CRISPR RNAs (crRNAs) post-transcriptionally. crRNAs play a guiding role for Cas protein to bind and cleave the specific nucleic acids during the next exposure of phage and bacteria (Garneau et al., 2010). The Cas genes which are often located adjacent to the CRISPR array are seen as crucial components of CRISPR systems for exerting immune function (Maxwell et al., 2016; Wright et al., 2016). Importantly, Cas proteins enable the cleavage of the foreign genomes(Garneau et al., 2010), integrating the phage sequences into the CRISPR array(Makarova et al., 2011), and thereby producing the mature crRNAs (Cong, 2017).

CRISPR-Cas systems are classified into two classes; each contains specific types (Safari et al., 2020c). Class 1 CRISPR-Cas systems include type I, type III, and type IV, which are complex and composed of RNA-guided multiunit-protein complexes (Negahdaripour et al., 2017). Class 2 is a mono protein CRISPR system and subdivided into three types such as type II, V and VI systems (Makarova et al., 2015). Type II is recognized by the presence of Cas9 enzyme (Barrangou and van Pijkeren, 2016); type V systems, identified by the Cas12a, C2c1, or C2c3 nuclease enzymes (Nakade et al., 2017) and type VI systems encode the Cas13 effector enzyme (East-Seletsky et al., 2017). Among these Cas proteins, Cas12 and Cas13 are used to devise platforms for detection and treatment of viral diseases which will be elaborated further.

2.1. Cas12a characterization and diagnostic platforms

Cas12a, also known as Cpf1, which is found in Prevotella and Francisella genus of bacteria are classified as the RNA-guided monomeric endonuclease protein (1200–1500 amino acids in length) that belong to class 2 type V-A CRISPR/Cas system (Li et al., 2018a). The Cas12a CRISPR array is composed of nine spacer sequences isolated by 36 nucleotide-long repeated sequences. Cas12a identifies a 5′-TTTV-3′ PAM within a DNA target, which allows the base pairing of the crRNA spacer segment with the complementary target DNA. Simultaneously, RNase and DNase activity of Cas12a leads to an independent crRNA biogenesis which lacks the need for tracrRNAs. Thereby, Cas12a itself recognizes and cleaves the pre-crRNA to form a pseudoknot structure (Li et al., 2019a).

Cas12a introduces DSBs with the staggered ends containing 5 or 8 nucleotides 5′ overhang depending on crRNA length (Zetsche et al., 2015). Along with the specific DSB, Cas12a also undergoes nonspecific cleaves on other single-stranded DNA molecules. These nonspecific trends are triggered only upon binding of crRNA to its complementary target called collateral activity (Li et al., 2019b).

The type V-B CRISPR-Cas12b (C2c1) system is a dual-RNA-guided DNA endonuclease system. It is reported that Cas12b generates staggered ends distal to the PAM site and possesses a smaller size than the most widely-used SpCas9 and Cas12a. This effector recognizes simpler PAM sequences include 5′-TTN, which can significantly enhance the targeting range of the genome. Most importantly, the minimal off-target effects of Cas12b serves it as a safer choice for clinical applications (Teng et al., 2018).

The promising features of Cas12a represent it as an emerging genome editing tool, which can be used in the context of both diagnostic and therapeutic approaches which include the detection and treatment of infectious diseases. DETECTR is a diagnostic tool based on Cas12a effector protein. This diagnostic platform is based on the Cas12a collateral cleavage of a reporter nucleic acid composed of a single stranded DNA (ssDNA) bearing a fluorophore and a quencher at either end. Since the reporter DNA remains intact, fluorescence is suppressed, and when the cas12a mediates the collateral cleavage and degrades the intervening DNA, the fluorophore is released and leads to the emission of a fluorescent signal. A pre-amplification step using an isothermal enzymatic reaction enhances the sensitivity of DETECTR. An isothermal amplification increases the analytical sensitivity via replication of nucleic acids inside an unknown sample. Notably, this CRISPR based diagnostic agent functions quickly and can be assembled on the dipstick without any prior need for laborious equipment (Li et al., 2019b). HOLMES (a one-hour low-cost multipurpose highly efficient system) is a promiscuous Cas12a based detector platform for specific detection of the target DNA. In the presence of target DNA, Cas12a/crRNA forms a target

DNA-RNA hybridization complex and leads to the trans-cleavage of non-targeted ssDNA. The cleavage of reporter DNA allows the illumination of the fluorophores such as HEX, N12, and BHQ1 (Safari et al., 2019). HOLMES could be used to detect DNA viruses such as pseudorabies virus (PRV), and RNA viruses like Japanese encephalitis virus (JEV). The sensitivity of HOLMES detection for both viruses seems quite promising and could be as low as 1–10 aM (Broughton et al., 2020).

2.2. Cas13 characterization and diagnostic platforms

Type VI CRISPR nuclease has recently been discovered as site-specific RNA-guided, RNA-targeting Cas effector (Abudayyeh et al., 2017). The nuclease activity of these proteins allows gene knockdown without genomic alteration. To detect the target RNA, Cas13 proteins recruit crRNA fragment that consists of a direct repeat (DR) stem-loop and a spacer RNA (gRNA). This RNA complex recognizes target RNA by RNA–RNA hybridization. As well as other types of Cas proteins, Cas13 requires a protospacer flanking sequence (PFS) to specify the target RNA but with minimal limitations. Cas13 proteins are divided into various subtypes including Cas13a, Cas13b (Wang et al., 2019) and Cas13d (Yan et al., 2018).

Cas13a protein (formerly C2c2) was first introduced by the Broad Institute in 2015 (Shmakov et al., 2015). Structurally, two higher eukaryotes and prokaryotes nucleotide (HEPN) binding domains are catalytic domains of Cas13a that play a critical role in RNA cleavage. Similar to the Cas12a, the length of Cas13a crRNA is short (24 bp) which only uses for binding and cleavage of target RNA (Knott et al., 2017). The PFS of Cas13a is located at the 3′ end of the spacer, consisting of single A, U or C nucleotide (East-Seletsky et al., 2016). Cas13a becomes enzymatically “active” following the first single-strand RNA (ssRNA) break and thereby cleaves other RNAs regardless of being complementary to the crRNA or the presence of a PFS (East-Seletsky et al., 2017; Liu et al., 2017). This promiscuous collateral effect was used for rapid DNA or RNA detection with high sensitivity in addition to the single-base mismatch specificity (Gootenberg et al., 2017). Cas13b, (also called C2c6, belongs to the type VI-B CRISPR-Cas systems), carries two HEPN domains as well as Cas13a and also has a collateral RNase activity (Smargon et al., 2017).

Recently, a distinct variant of type VI CRISPR-Cas called subtype VI-D (Cas13d) has been introduced. These variants exert robust target cleavage and collateral RNase activities along with their ability to process pre-crRNA. The notably smaller effector size of Cas13d (967 amino acids), high specificity, and vigorous catalytic activity designated Cas13d rather than other Cas13 proteins for RNA interference (Zhang et al., 2019a). To date, three variants of Cas13 effectors includes PguCas13b, PspCas13b, and RfxCas13d have been introduced which characterized by high RNA on-target activity with minimal unintended (off-target) effects (Freije et al., 2019).

Specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) is the first platform based on CRISPR-Cas13 systems. It is composed of isothermal RPA (recombinase polymerase amplification) or reverses transcription (RT)-RPA and Cas13a (Gootenberg et al., 2017). This Cas13a based complex recognizes explicitly and cleaves target nucleic acid. The collateral effect of Cas13a causes cleavage of non-target RNA coupled to a fluorescent reporter. These cleavages release the quenchers and provide a fluorescent signal for rapid and specific detection of viruses even in quite low concentrations.

SHERLOCK is capable of bio-sensing with a sensitivity for the detection of both DNA and RNA viruses with single-base discrimination. This Cas13a based system has been used for the robust detection of Zika and dengue viruses (Gootenberg et al., 2017). Cas13b was applied in the 2.0 version of SHERLOCK, which can detect Zika and Dengue virus ssRNA as well as mutations in patient liquid biopsy samples via dipstick. These findings have displayed the potential for SHERLOCK to be utilized as a rapid, multiplexable, portable, and quantitative detection platform for emerging viral infections (Gootenberg et al., 2018).

3. Diagnostic and therapeutic applications of CRISPR/Cas systems for viral diseases

CRISPR effectors possess the potential to aid in combating with both DNA and RNA viruses. Recent literature have demonstrated that in addition to double-stranded DNA viruses, CRISPR-Cas9 can impede the replication of ssRNA viruses that produce DNA intermediates in mammalian cells (Liu et al., 2015; Ophinni et al., 2018). Approximately two-thirds of viruses cause human diseases have ssRNA genomes. Among them, only 2.5 % produce the DNA intermediates that could be disrupted by the Cas9. SARS-CoV-2 is an emerging RNA virus with a highly contagious nature that has infected a large number of individuals. To date, the lack of any proper therapies for COVID-19 disease mediated by the ssRNA virus has prompted the scientists to design novel antiviral strategies. In the following lines, we will be describing the genetic features of SARS-CoV-2, the pathophysiology of disease caused by this virus and the CRISPR-based diagnostic and therapeutic platforms that have been developed for detection and treatment of this global pandemic.

4. Coronaviridae SARS-CoV-2

SARS-CoV-2 is an enveloped positive-sense single-stranded RNA virus (or (+) ssRNA virus) that typically infects both upper and lower respiratory tract (Bojkova et al., 2020). It prompts the disease by direct cytotoxic effects and induction of host cytokines. The genomic structure of SARS-CoV-2 contains approximately 30 kb RNA, which is the largest known RNA virus, in terms of the size (Davidson et al., 2020). The life cycle of SARS-CoV-2 is partially close to related coronaviruses, such as the SARS disease mediated virus (Zhang et al., 2020c).

Infection begins with the virus entry via endocytosis or direct fusion of viral envelope with the host membrane, and RNA being released to the cytoplasm following the virus uncoating. The transcription is triggered via the replication-transcription complex (RTC) that settled in double-membrane vesicles. Subgenomic RNAs are the products of RTCs that subsequently work as templates for synthesis of positive-sense viral genomic new copy (Dhama et al., 2020). Notably, the SARS-CoV-2 genome encodes 16 non-structural (nsps) and 4 structural proteins (including envelope, spike, membrane, and nucleocapsid proteins) (Kandeel et al., 2020). The pathophysiology and virulence of SARS-CoV-2, is closely related to the activity of the nsps and structural proteins. For instance, nsps enable to block the host innate immune response. Also, the envelope as a structural protein promotes the virus assembly and release (Gorla and Rao, 2021) (Fig. 1).

The spike glycoproteins (S proteins: containing S1 and S2 subunits) of SARS-CoV-2 play a crucial role in binding to the host receptors. S proteins are the gateways of virus for viral entry to the host cell through binging to angiotensin-converting enzyme 2 (ACE2) receptors. These proteins become heavily N-glycosylated in the endoplasm reticulum (ER) during the entrance to the host cells which allows the virus to circulate undetected by the host immune system (Nunes-Santos et al., 2020).

Glycosylation also facilitates the specific binding of S proteins to their cognate receptors on the membrane of the host cell. The subsequent cleavage of S proteins by proteases provides virus endocytosis via membrane fusion. The S2 subunit of SARS-CoV-2 is highly conserved and contains a fusion peptide, as well as a transmembrane domain, and a cytoplasmic domain. Hence, the S2 subunit may be a promising target for antiviral (anti-S2) therapeutic approaches. Despite the presence of the S2 subunit, the spike receptor-binding domain is not conserved and represents only a 40 % amino acid similarity to other SARS-CoVs. Recent literature insinuates that a spike mutation in the transmembrane helical segments is cardinal to be elaborated upon in the context of potential disease relapses when compared with the SARS-CoV (Walls et al., 2020).

The pathophysiology of SARS-CoV-2 causing pneumonia as well as acute respiratory distress syndrome (ARDS) happens to be particularly complex. Clinical and preclinical research will have to explain many

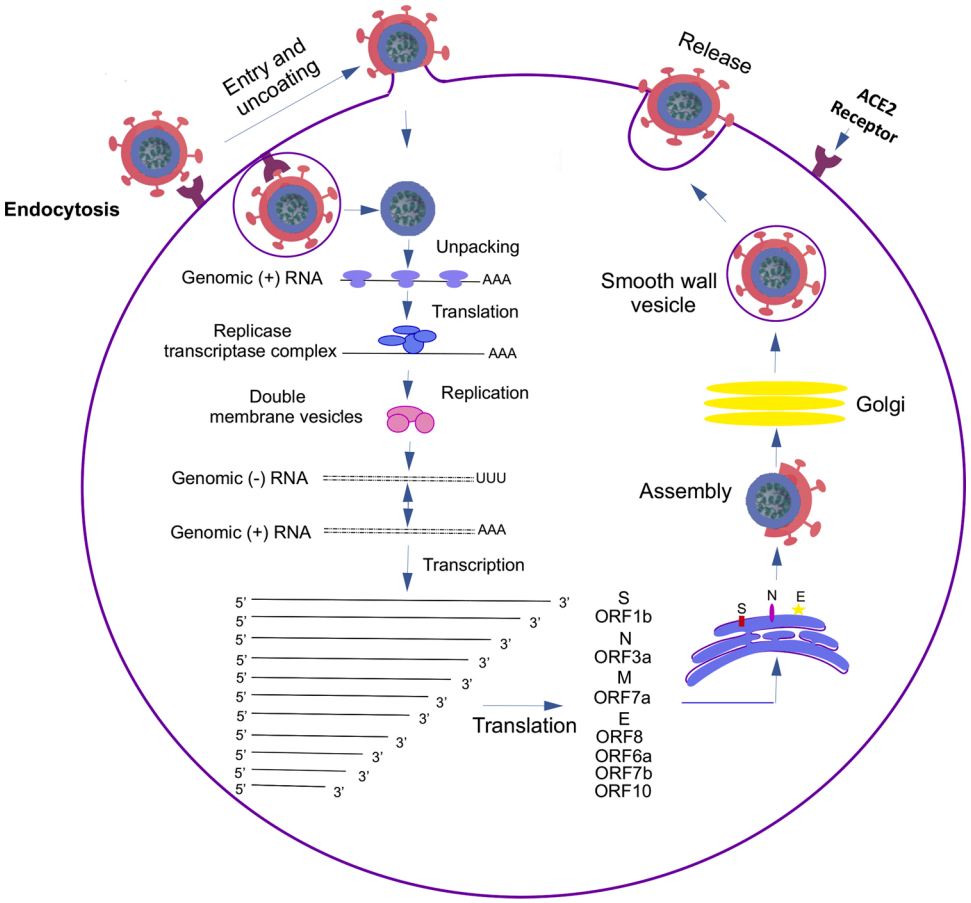

Fig. 1. The SARS-CoV2 life cycle in host cells. SARS-CoV 2 starts its life cycle by biding the S protein to the ACE2 receptor. After receptor binding, the endosomal pathway facilitates the viral envelope fusion with the cell membrane. Subsequently, the SARS-CoV 2 RNA releases into the host cell. The translation of genome RNA resulted in the production of viral replicase polyproteins (pp1a and 1ab). Simultaneously, polymerase produces a series of subgenomic mRNAs by transcription, which are finally translated into the relevant viral proteins. Genomic RNA and viral proteins are subsequently assembled into virions. These virions are budding from Golgi complex and then are transported via vesicles and released out of the cell.

aspects that underlie the primary clinical presentations of the disease. So far, the published data has unveiled that viral infection can produce an excessive host immune reaction. In some patients, this immune reaction results in a cytokine storm that leads to extensive tissue damage. Interleukin 6 (IL-6) produced by activated leukocytes is a significant orchestrator of this storm (Pedersen and Ho, 2020). A large number of cells and tissues are affected by IL-6. For instance, it promotes the differentiation and growth of B lymphocytes and some categories of cells but inhibits the growth of others. IL-6 is also implicated in the pathogenesis of an acute systemic inflammatory syndrome characterized by fever and multiple organ dysfunction called cytokine release syndrome (CRS) (Zhang et al., 2020a).

4.1. Conventional COVID-19 diagnostics

The conventional diagnostic tests for COVID-19 are categorized into two main groups include immunological and molecular methods. Serological tests as an immunological assays can mainly detect viral antigens in respiratory secretions or antibodies in blood. Molecular assays are on the basis of SARS-CoV-2 RNA detection mainly in nasopharyngeal samples. Reverse transcription of SARS-CoV-2 RNA followed by real-time reverse transcription polymerase chain reaction (RT-PCR) is the gold standard for COVID-19 diagnosis. The first protocol suggested by the WHO is based on TaqMan technology using primers and probes to detect the envelope protein (E), RNA-dependent RNA polymerase (RdRp), and nucleocapsid protein (N) genes. Serological test detected by ELISA have more than 95 % specificity for COVID-19 detection (Pizzol et al., 2020). But, the sensitivity and specificity of the RT-PCR test is not absolutely 100 %, and importantly depends on the personnel skill in the relevant techniques, laboratory practice standard and safety procedures (Tahamtan and Ardebili, 2020).

4.2. Conventional COVID-19 therapeutics

Various strategies have been assessed to identify probable treatment choices for COVID-19 with more than 300 distinct therapeutics and over 200 vaccines which are in preclinical stage or in different stages of clinical trials. Remdesivir as a nucleotide analog is one of several known antiviral drugs being assessed in clinical trials for COVID-19 patients. Remdesivir is an inhibitor of RNA-dependent RNA polymerase (RdRP) and has been used in patients with COVID-19 in 75 centers globally. Findings show that administration of remdesivir in hospitalized adult patients infected with COVID-19 resulted in shortening the time to recovery and reduced the respiratory tract infection (Beigel et al., 2020). Dexamethasone is the other drug of choice which has a potential benefit in critically ill COVID-19 patients (Horby et al., 2020). Hydroxychloroquine was suggested as therapeutic in SARS-CoV-2 infected patients, however, recent data showed the inefficiency of this antimalarial drug in treatment of COVID-19 patients (Self et al., 2020).

mRNA-1273 a candidate vaccine that manufactured by ModernaTX, Inc is based on a mRNA that produces the full-length S protein of SARS-CoV-2. This vaccine is coated by lipid nanoparticles consisting four lipid molecules (NCT04470427). Second immunization of older adults with mRNA-1273 demonstrated serum neutralizing activity. In addition, it evoked a strong CD4 cytokine response involving type 1 helper T cells (Anderson et al., 2020). Vaccines based on recombinant S protein include AZD-1222 nCoV-19 (produced in a chimpanzee adenovirus Oxford 1 vector, NCT04324606) from University of Oxford and Ad5-nCoV (produced in an adenovirus type 5 vector, NCT04313127) from CanSino Biologics (Mullard, 2020; Zhu et al., 2020a). A multi-site, AZD1222 has been evaluated in phase 3 clinical trial by enrolling approximately 30,000 adult volunteers at 80 sites in the United States. Phase 2 clinical trial evaluating an investigational Ad5- nCoV vaccine

has begun by assessing 382 adults (age ≥ 18) using a single dose which showed cellular or humoral immune responses in 91–95 % of recipients depending on the dose (Zhu et al., 2020b). DNA vaccines, live-attenuated and inactivated vaccines are the other vaccine candidates that have been evaluated in preclinical or early clinical stages (Jeyanathan et al., 2020).

Neutralizing antibodies (nAb) against SARS-CoV-2 is another active area of COVID-19 treatment investigation. There are two sources of antibodies used for treatment of COVID-19 include polyclonal derived from convalescent plasma of COVID-19 recovered individuals and synthetic monoclonal antibodies. Convalescent plasma is routinely used for patients with COVID-19 but recombinant antibodies need more in vitro and in vivo evaluation to investigate their prophylactic and therapeutic efficacy. Fascinatingly, LY-CoV555, a monoclonal antibody derived from a recovered COVID-19 patient (NCT004497987) (Jones et al., 2020) and REGN-COV-2, a combination of two monoclonal antibodies targeting two regions of S protein (NCT04452318) (Baum et al., 2020) are being progressed to phase 3 clinical trial (Table 1).

5. Novel COVID-19 diagnostics and therapeutics

5.1. CRISPR based COVID-19 diagnostic platforms

Various novel diagnostics have been devised on the basis of CRISPR systems include SHERLOCK, Cas13-based Rugged Equitable Scalable Testing (CREST), DETECTR, All-In-One Dual CRISPR-Cas12a (AIOD-CRISPR), CRISPR/Cas12a-based-detection with naked eye readout (CRISPR/Cas12a-NER) and Cas3-Operated Nucleic Acid detectioN (CONAN) (Table 2).

The potential of CRISPR technology to cleave SARS-CoV-2 genetic material resulted in using Cas13d and crRNAs to devise a therapeutic platform called PAC-MAN. However, PAC-MAN seems to be promising approach for COVID-19 treatment but it's bottlenecks propelled the researchers to propose a novel platform termed AntiBody And CAS fusion (ABACAS).

5.1.1. SHERLOCK

Recently, SHERLOCK has been recruited for the detection of COVID-19 by Feng Zhang et al.(Zhang et al.) Detection can be started with RNA purification from patient samples, and read by using a lateral flow in less than an hour. The sensitivity of COVID-19 target sequences using SHERLOCK method is calculated to be in a range between10 to 100 copies per microliter of input (20 and 200 aM). To detect the COVID-19 RNA in nucleic acid extractions of specimens, two targets from the S gene and Orf1ab gene were gathered. In order to maximize the specificity of the assay, the primers and LwaCas13a CRISPR gRNAs were designed to detect COVID-19 RNA in specimens carrying the minimum off-targets of related human respiratory virus genomes (Zhang et al.) (Fig. 2).

Recently STOP (SHERLOCK Testing in One Pot) has been developed as a simple test chemistry for detection of SARS-CoV-2 in one hour which is suitable for point-of-care applications. The sensitivity of STOPCovid is comparable with RT-qPCR-based SARS-CoV-2 tests and has the LOD of 100 copies of viral genome per microliter. By utilization of dipstick, the result of test returns in 70 min, and by using fluorescence readout, the result of the test returns in 40 min.

To use STOPCovid in POC contexts, Zhang and his colleagues developed a simple testing chemistry which was not need sample extraction because of lysis the viral particles with QuickExtract at room temperature (22 ℃) or at one-pot incubation temperature (60 ℃) for 10 min. The commercial availability of loop-mediated amplification (LAMP) enzymes and buffers and systematic optimization with Cas enzymes resulted in using LAMP for amplification of viral nucleic acid. Cas12b belongs to *Alicyclobacillus acidiphilus* (AapCas12b) which maintains sufficient activity in the same temperature range as LAMP (55–65 ℃) was used for detection of N gene of SARS-CoV-2. AapCas12b was combined with scaffold of *Alicyclobacillus acidoterrestris* Cas12b (AacCas12b) as tracrRNA due to the AapCas12 locus lacks of a CRISPR array. The implementation of this platform will significantly help "test-trace-isolate" approach, specifically in low-resource areas, which will be essential for long-term safety of public health and effective for reopening the social and economic activities of the society (Joung et al., 2020).

5.1.2. CREST

To devise a cost-effective, sensitive, and easy handling SARS-CoV-2 detection method, Rauch and her colleagues have designed a CREST platform using Cas13 detection combined with a thermal cycling amplification step (PCR), a linear amplification step (transcription), and enzymatic signal amplification via fluorescence detection. To address the need to expensive equipment, CREST employs affordable, Bluetooth enabled, battery operated and field-ready thermocyclers (DIY-Bio and miniPCR mini16). These thermocyclers work with low-cost solution, enable the amplification of the viral target material and improve the accessibility of COVID-19 testing. For visualization of positive results P51 cardboard fluorescence visualizer (powered by a 9 V battery) has been used as a scalable and easy-to-interpret device.

The LOD of the CREST protocol was up to 10 copies of a target RNA molecule per microliter which unveiled that CREST is as sensitive as the corresponding RT-qPCR. The cost of CREST instrumentation is estimated to be 30–50 times lower than RT-qPCR and also more cost effective and accessible than a variety of Cas13-based protocols that utilize RT-Recombinase Polymerase (Rauch et al., 2020).

Table 1
COVID-19 conventional therapeutics.

Therapeutics	Name	Target/rational for use	Clinical phase	status	Findings
Drugs	Remdesivir	an inhibitor of RNA-dependent RNA polymerase (RdRP)	Phase III	Ongoing	No Result Posted
	Dexamethasone	Inhibition of *Strongyloides* hyperinfection syndrome	Phase IV	Recruiting	No Result Posted
	Hydroxychloroquine	Block viral entry by inhibiting glycosylation of host receptors, proteolytic processing, and endosomal acidification.	Phase III	Completed	No Result Posted
Vaccines	mRNA-1273	S protein	Phase III	Recruiting	No Result Posted
	AZD-1222	S protein	Phase III	Recruiting	No Result Posted
	Ad5- nCoV	S protein	Phase III	Recruiting	No Result Posted
Antibodies	LY-CoV555	S protein	Phase III	Recruiting	No Result Posted
	REGN-COV-2	S protein	Phase III	Recruiting	No Result Posted

Table 2
Properties of CRISPR based COVID-19 detection assays.

platform name	Sample size	Cas protein	Time (min)	lod	Sensitivity (%)	Specificity (%)	Visualization	Target genes	Amplification system	Amplification temperature	Qualitative/ Quantitative
AIOD-CRISPR	28	Cas12a	20	5	N/A	N/A	Naked eye	N	RT-RPA	37	Qualitative
SHERLOCK	534	Cas13a	35	42	flu96/ 88 lateral	100	Fluorescent /lateral flow	S	RRT-RPA	37	Quantitative
SARS-CoV-2 DETECTR	11	Cas12a	30	10	90	100	Lateral flow	E,N	RT- LAMP	62	Qualitative
CRISPR/ Cas12a-NER	31	Cas12a	45	10	N/A	N/A	Fluorescent	E	RT-RAA	39	Qualitative
CRISPR based DETECTR	378	Cas12a	30	50	95	100	Lateral flow	N	RT-LAMP	62	Qualitative
STOPCovid.v1	17	Cas12b	50	100	N/A	N/A	Lateral flow	N	RT- LAMP	60	Quantitative
STOPCovid.v2	402	Cas12b	15–45	40.3	93.1	98.5	Fluorescent /lateral flow	N	LAMP	N/A	N/A
CONAN	25	Cas3	40	1	N/A	N/A	Lateral flow	N	RT-RPA	37	Qualitative
CREST	N/A	Cas13a	120	10	N/A	N/A	Fluorescent	N	PCR	N/A	Quantitative

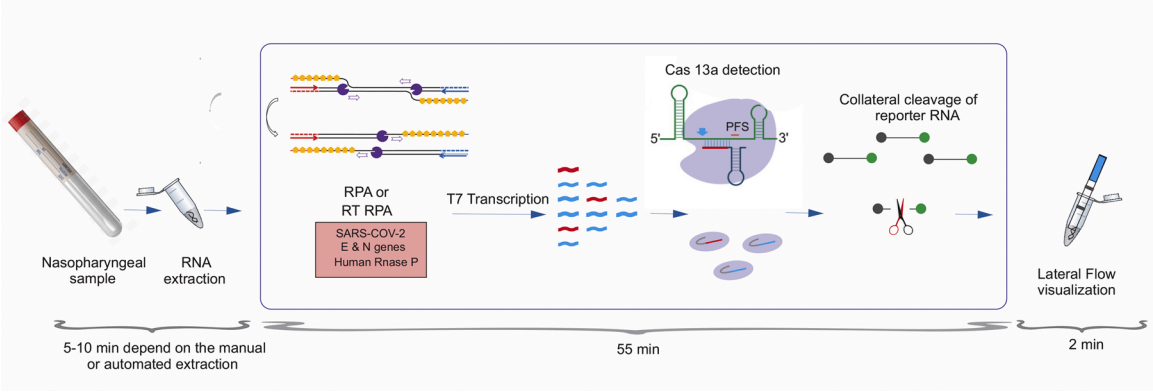

Fig. 2. SHERLOCK system combining RPA pre-amplification, Cas13 detection and colorimetric reporter for visualized readout.

5.1.3. DETECTR

Cas12 mediated SARS-CoV-2 DETECTR was also used to detect the SARS-CoV-2 from RNA extraction of the patient samples. Employing reverse transcription simultaneous with isothermal amplification via RT-LAMP (Panno et al., 2020), this version of DETECTR amplifies the extracted RNA from nasopharyngeal or oropharyngeal patients' sample. Subsequently, Cas12 recognizes and cleaves the predefined coronavirus sequences which are detected by cleavage of a reporter molecule. For this purpose, primers were designed to amplify the E (envelope) and N (nucleoprotein) genes of SARS-CoV-2. The amplified regions have overlaps with the WHO assay (E gene region) and US CDC assay (N2 region in the N gene) (Dastan and Turan, 2004; Rauch et al., 2020) but has observed the requirements for LAMP. The N1 and N3 regions, used by the CDC assay, did not target for amplification, as these regions lack the suitable PAM sites for the Cas12 gRNAs.

SARS-CoV-2 DETECTR can discriminate SARS-CoV-2 with no cross-reactivity for related coronavirus strains. Detection assay was optimized on the E gene, N gene and the human RNase P gene as a control. In this method, a highly sensitive detection can be run in approximately half an hour consisting of an RT-LAMP reaction at 62 °C for 20 min and Cas12 detection reaction at 37 °C for 10 min and visualized on a dipstick. Hence, a positive test output requires to detect both the E and N genes to confirm the SARS-CoV-2 infection. The estimated analytic limits of detection (LOD) for DETECTR assay are ten copies/μL reaction versus for the one copy/μL reaction is the LOD of the CDC assay tested by the California Department of Public Health (Fig. 3) (Broughton et al., 2020).

5.1.4. AIOD-CRISPR

AIOD-CRISPR is a fast, highly sensitive, specific and visual based assay for the detection of nucleic acid. In the state of the art, in this assay, all reagents using for nucleic acid amplification and detection by

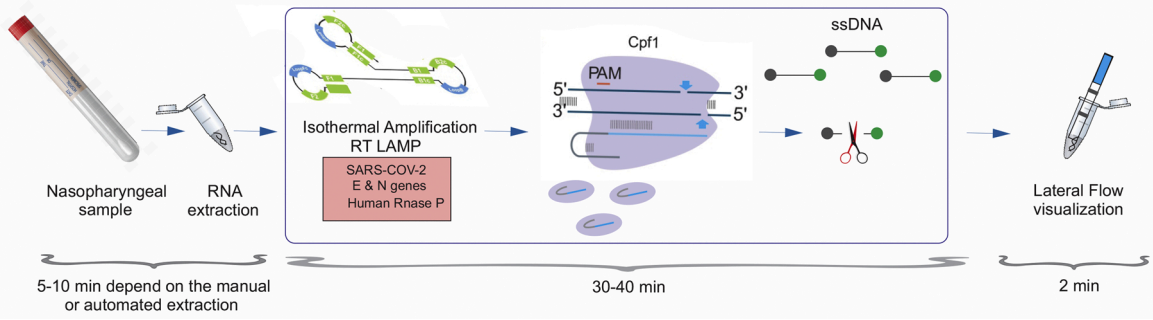

Fig. 3. DETECTR system combining LAMP pre-amplification, Cas12a detection and colorimetric reporter for visualized readout.

CRISPR are entirely mixed in a single reaction tube and incubate at a 37 °C as a single temperature due to using isothermal nucleic acid amplification techniques such as LAMP 8 and RPA. Designing a single pot reaction system eliminate the need for separate transferring of pre-amplification and amplified product. In addition, to improve the detection signals ssDNA-FQ reporters are added to AIOD-CRISPR assay. Real-time detection recruiting fluorescence and color change of reaction solutions lead to using an AIOD-CRISPR assay as a point-of-care detection. Combination of Avian Myeloblastosis Virus (AMV) reverse transcriptase with AIOD-CRISPR assay reagent results in the development of a one-step RT-AIOD-CRISPR to detect RNA targets such as HIV-1 and SARSCoV-2 RNAs. RT-AIOD-CRISPR simplifies the detection of RNA without the preparation of cDNA. Engineered AIOD-CRISPR detects HIV-1 and SARS-CoV-2 without need to preamplification. The estimated LOD of AIOD-CRISPR is as low as 4.6 copies for RNA targets and 1.2 copies for DNA targets in 40 min incubation. Furthermore, ultra-specificity of AIOD-CRISPR is confirmed by detection of HIV-1 with very low background in comparison with reported real-time RPA (Ding et al., 2020).

5.1.5. CRISPR/Cas12a-NER

CRISPR/Cas12a-NER is the other CRISPR based platform that include the Cas12a effector, specific crRNAs designed for SARS-CoV-2 and a ssDNA reporter. In this diagnostic platform, Cas12a in exposure with nucleic acid of SARS-CoV-2 cleaves an ssDNA reporter labelled with a quenched green fluorescent and the results in green fluorescence release which can be seen with the naked eye under 485 nm light. crRNAs deigned for this platform target orf1a, orf1b, N and E genes over the Wuhan-Hu-1 strain. Reverse transcript recombinase aided amplification (RT-RAA) was used to pre-amplify the RNA of SARS-CoV-2 in 30 min at 39 C following by Cas12a-mediated detection which take place in a 15 min, and then completed in the other 15 min. The sensitivity of the CRISPR/Cas12a-NER is variable among the four target genes. Findings unveiled that the crRNAs targeting the E gene is the most sensitive with the LOD of 10 copies of SARS-CoV-2 synthetic nucleic acid. Clinical validation of CRISPR/Cas12a-NER showed that it has 100 % agreement with the results of qPCR assays and confirming the significant performance of this platform (Wang et al., 2020).

5.1.6. CONAN

As well as Cas12 and Cas13effectors, Cas3 protein which belongs to the type I-E CRISPR-Cas system from Escherichia *coli* and Thermobifida *fusca* can also mediate the target-activated, nonspecific ssDNA cleavage. Using this promising property along with isothermal amplification methods provides a rapid, sensitive and instrument-free detection system for SARS-CoV-2 point-of-care tests (POCTs). RT-LAMP (at 62 °C for 30 min), followed by incubation with CONAN for 10 min at 37 °C, specifically detected SARS-CoV-2 using N1 and N2 crRNAs. The LoD of CONAN-LAMP ($<10^2$ copies) compare favorably with DETECTR-LAMP and the CDC qRT-PCR assay for SARS-CoV-2 detection (Yoshimi et al., 2020).

Altogether, it seems that Cas12 based detection methods are more sensitive (1–10 copies/μL) and time effective than Cas13 based platform. However, these platforms need more optimization to achieve the sensitivity of CDC qRT-PCR assay for SARS-CoV-2 detection.

5.2. CRISPR based COVID-19 therapeutic platforms

5.2.1. PAC-MAN

Conventional vaccines work by engaging with the human immune system to detect viral proteins or weakened viruses and reduce viral entry into the cells (Bastola et al., 2017). On the other hand, there is an alternative antiviral approach which was introduced that relies on a CRISPR-based system for detection and cleavage of the intracellular viral genome and its resulting viral mRNAs. Direct targeting of the (+) ssRNA and subgenomic RNAs responsible in viral replication and gene expression seems to be robust in impeding the viral replication (Li et al., 2020).

Recently, a CRISPR-Cas13d system derived from *Ruminococcus flavefaciens* XPD3002 has been used to inhibit RNA viruses in human cells (Konermann et al., 2018). In this method, Cas13d recruits crRNAs that contain a customizable 22 nucleotide (nt) spacer sequence. This spacer sequence directs the Cas13d protein to specific RNA targets to degrade these molecules. The excellent on-targeting ability of Cas13d on mammalian cells leads to the use of this nuclease to target viral resources like SARS-CoV-2 (Gootenberg et al., 2017). The target Cas13d may also possess the capability to cleave the viral-specific RNA genome and thereby, inhibit the viral gene expression. The highly crucial factors like small size, high specificity, and intense catalytic activity anoint Cas13d, rather than other Cas13 proteins, to target and disrupt the invasive RNA viruses including SARS-CoV-2 and Influenza (Yan et al., 2018). In the past few months, PAC-MAN strategy has also been developed as a form of genetic intervention to target SARS-CoV-2, IAV, and potentially all strains of sequenced coronaviruses (Abbott et al., 2020).

By targeting both SARS-CoV-2 and IAV with Cas13d, Abbott and his colleagues found several highly conserved regions within different viral genomes. In the case of SARS-CoV-2, RdRP and Nucleocapsid encoding genome, there have been attempts to target the regions which are necessary for coronavirus replication and function (Abbott et al., 2020). RdRP catalyzes the replication of all viral mRNAs and nucleocapsid as one of the two primary virions with structural proteins that bind to the genomic RNA and thereby protecting it (Bastola et al., 2017). The disruption of these two proteins could have a significant effect on the inhibition of virus function and viral particle production. Hence, the degradation of the viral genome itself reduces the viral load. To confirm this hypothesis, GFP fused with the SARS-CoV-2 proteins as infection reporters was transfected to lung epithelial cells (A549 cell line) which expressed Cas13d and a pool of crRNAs. Targeting different regions of SARS-CoV-2 RdRP gene with pool of crRNA lead to more than 85 % reduction in the GFP reporter signal. Furthermore cleavage of SARS-Cov-2 N protein encoding gene using a pool of crRNAs repressed the GFP signal by more than 70 %.

The main superiority of PAC-MAN is the adaptability of this system to treat pan-coronaviruses using a single cocktail, but it should take into consideration that a combinations of crRNAs should be carefully selected to obtain high efficiency. Since, the CRISPR technology has been used safely in humans, therefore this strategy can be rapidly stablished at very early stages of the next pandemic with minimum changes to the present system. Three approaches can be used to transfer the CRISPR system for working inside animals or humans: (1) transfect the Cas13 and crRNA in the form of DNA to be transiently expressed inside cells, (2) using RNA form of Cas13 and crRNA which could be translated inside the host cells is the second approach, (3) the third approach is introducing the CRISPR components as a ribonucleoprotein complex. Using the third strategy results in high on-target efficiency, limited unintended effects and a short period of time (Nalawansha and Samarasinghe, 2020).

Coupling with an efficient delivery mechanism ensures the delivery of CRISPR components into the cells. Using conventional delivery methods include liposomal, polymer-based, and adenoassociated virus (AAV) vectors appear to be feasible but shows limited efficiency (Pan et al., 2018).

5.2.2. ABACAS

To enable the delivery of Cas13 into infected cells and to target the SARS-CoV-2 viruses specifically, the CRISPR components can be bind to one of the main virus structural proteins such as S protein. Fusion of Cas13 to the specific antibody of SARS-CoV-2 S protein results in the construction of a promising antiviral therapeutics termed ABACAS.

Fused antibody in ABACAS could detect SARSCoV-2 S protein and facilitates the delivery of the Cas13 along with the virus into the infected cell (Joyce et al., 2020; Yuan et al., 2020). Exposure of ABACAS with

viral RNA in the infected cell leads to cleavage of the viral RNA. This innovative approach results in a potent viral inhibition and reduces off-tissue effects due to the selective delivery by virus itself. Since, REGN-COV2 and LY-COV555 tightly bind to S protein of SARS-COV-2 so they would be ideal nominates for the development of ABACAS (Joyce et al., 2020). Findings proposed that developed SARSCoV-1 antibodies could neutralize SARS-CoV-2 due to their cross-reactivity. Among SARS-CoV-1 S antibodies CR3022 and S309 are potential candidates to fuse with Cas13 and using as fascinating CRISPR based antiviral therapeutics (Joyce et al., 2020).

Challenges in the development of monoclonal antibodies such as the high cost of production, and potential resistant mechanisms directed the efforts to use the biologics that can tether to the S protein as an alternative to provide selective delivery of Cas13 and virus particles. ACE2 receptor is a gateway of SARS-CoV-2 S protein, hence a soluble form of recombinant human ACE2 protein (rhACE2) may be a potential competitive inhibitor for blocking the interaction of S protein and ACE2 receptor of the host cell (Lei et al., 2020; Pang et al., 2020). Recently, it is demonstrated that fusion of the Fc region of the human immunoglobulin IgG1 to both the catalytically active and inactive extracellular domains of ACE2 successfully neutralize the SARS-CoV-2 by masking the S protein (Zhang et al., 2020b). In the state of the art, Peptidase Domain of ACE2 and Cas13 (PDCas13) is an alternative for ABACAS in which the antibody piece of ABACAS replaced with the peptidase domain of ACE2 or with a simple peptide of ACE2 (potentially binds to the S protein of SARS-CoV-2). However PDCas13 is unable to mask all S proteins of SARS-CoV-2 but co-delivery of PDCas13 along with virus particles to the infected cells triggers the antiviral activity of PDCas13 by RNA virus degradation (Fig. 4) (Nalawansha and Samarasinghe, 2020).

Noteworthy, in the lake of FDA-approved specific monoclonal antibodies against the S protein of SARSCoV-2, ABACAS containing SARS-CoV-1 nAb and PDCas13 containing rhACE2 or ACE2 peptide are valuable achievements for evaluation of selective and efficient delivery of CRISPR system to the infected cells and acting as an antiviral agents against SARS-CoV-2.

5.2.3. *Deaminase as antiviral against SARS-CoV-2*

As the replication of viruses relies on intracellular mechanisms of susceptible host cell (Safari et al., 2020d), innate immunity of organisms can evolve to detect and tracking the viruses. Among the innate immune defenders, endogenous deaminases mediating RNA and DNA editing can confer a potent resistance against specific viruses. Mammalian deaminase families include the ADARs (adenosine deaminases that act on RNA) and APOBECs (apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like). ADARs target double-stranded RNA (dsRNA) by deamination of adenines and changes it to inosines (A-to-I) (O'Connell et al., 2015). On the other hand, APOBECs act on single-stranded nucleic acids (ssDNA and ssRNA) for deamination of cytosines into uracils (C-to-U) (Di Giorgio et al., 2020).

ADAR1 has a critical role in the triggering of the innate immune response, through nucleic acids sensors, such as the cytoplasmic retinoic acid-inducible gene I (RIG-I) like receptors (RLRs) and the endosomal Toll-like receptors (TLRs). These sensors recognize typical intermediates of virus replication (dsRNA or ssRNA) and induce the production of type I interferons (Koyama et al., 2008). In turn, type I interferons upregulate the expression of interferon-stimulated genes (ISGs), such as ADAR1p150 and members of the APOBEC protein family (Borden and Williams, 2011).

ADAR1p150 acts an antiviral by destabilizing dsRNAs via multiple A-to-G substitutions called hyper-editing. In contrast proviral effects suppress the innate immune response by A-to-I RNA editing of long dsRNAs (Samuel, 2011, 2019). Probing the source of A-to-G changes happening along the SARS-COV-2 genome could be resulted in better understand the host-virus relationships, the evolutionary dynamics of the viral genome and suggest potential targets for therapeutic interventions. Finding suggests that ADAR1 can target SARS-COV-2 double strand structures. In addition, it is reported that the numerous hyper edited reads sites (96 %) are resided in exonic viral regions which comprise 64 % of nonsynonymous events that may have a significant functional impact on the pathogenicity of SARS-COV-2 (Picardi et al., 2020).

Although RNA editing occurs at low extent it could significantly influence the virulence, pathogenicity and host response. Since the virus have propensity to avoid the RNA editing process, it seems that it could have a strong effect on its survival. Hence, RNA editing opens a promising therapeutic approach for different human genetic diseases (Katrekar et al., 2019) as well as the SARS-COV-2 and/or other RNA viruses.

Lesson learned from the role of endogenous deaminases in combating against SARS-CoV-2 paves the way for using CRISPR base editors as a useful approach to fight with RNA viruses by mutations in viral genome. Base editors precisely makes targeted point mutations without need to DSBs or donor DNA templates, and without dependence on HDR (Anzalone et al., 2020). These features introduce CRISPR base editing as a robust strategy to combat COVID-19.

5.3. *The pros and cons of CRISPR based platform for clinical applications*

CRISPR based SARS-CoV-2 diagnostic platforms include SARS-CoV-2 DETECTR and SHERLOCK seem to be rapid, sensitive and specific tests for detection of SARS-CoV-2 but their efficiency in clinical use remains

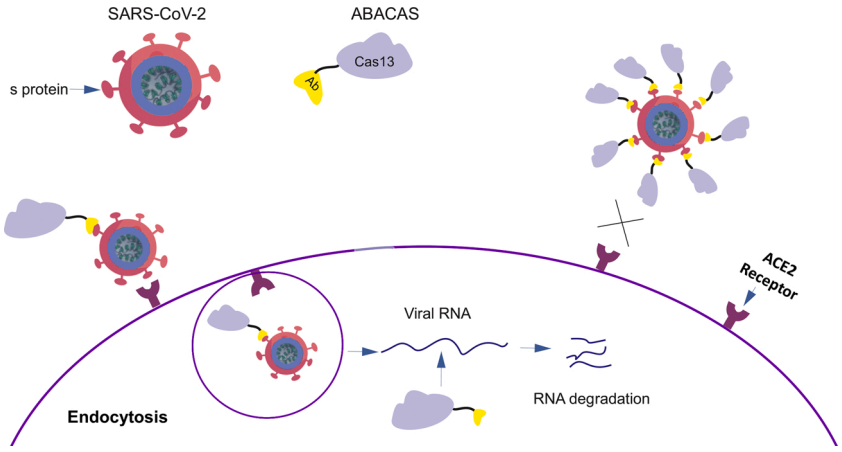

Fig. 4. Schematic description of the ABACAS and SARS-CoV-2 in bound form. Viral infection of a host cell via endocytosis results in the entrance of ABACAS into the infected cell along with the virus. Release of ABACAS activates the Cas13 portion which recognize the viral RNA and mediates its degradation. In addition, masking of virus s protein by ABACAS inhibits the entry of virus to the target cells.

unclear. Hence, more and more investigation of clinical samples should be done to maintain their efficiency. Approving the clinical usage of these platforms facilitates the establishment of rapid, accurate and cost-effective diagnostic tests for the future outbreaks (Zhang et al., 2021).

DETECTR assay is as precise as qRT–PCR but is a more rapid test as well. Thus, DETECTR improves the qRT–PCR assay limitations such as requirement to expert personnel, complex thermocycler, reagents, extraction kits, as well as personal protective equipment (Bauchner et al., 2020). CRISPR based platforms have ability to be quickly reorganized to detect viral diseases mediated by emerging viruses, which confirmed by the rapid development of the DETECTR assay for SARS-COV-2 diagnosis (Jolany Vangah et al., 2020).

The sensitivity of qRT–PCR test during the early phase of infection is low, therefor it could be negative at the time when the viral load is low. But, the CRISPR-Cas -based diagnosis test will be able to detect the viral genome at any stage of infection, particularly in the early stages of the incubation period (Metsky et al., 2020).

Besides various advantages of CRISPR based diagnostic test, one of the main disadvantages of these tests is the off-target effect which results in the poor signaling and misinterpretation of the results (Safari et al., 2020a). Using specialized bioinformatics software to select the most appropriate gRNAs may reduce the unintended effects of CRISPR systems (the list of gRNAs used in each diagnostic and therapeutic platforms are listed in Table 3 (Li et al., 2018b, b). In addition, to diagnose the emerging viral diseases by CRISPR systems, a precise understanding of viral genome is necessary which is time consuming for development of these diagnostic tests.

Standardization is the Achilles Heel of POC based CRISPR which requires obtaining the same results by different users. A potential bottle neck of various CRISPR-based diagnostic approaches is that they are qualitative and only provide absolute positive or negative results but not quantify viral loads (Huang et al., 2020; van Dongen et al., 2020).

The other limitation of fluorescence readout based methods is discrimination of background from positive tests. To address this drawback in DETECTR, fluorescence values were normalized to the highest value (within N gene, E gene or RNase P set) with a positive threshold at five standard deviations above background (Broughton et al., 2020). In SHERLOCK also a threshold has been set to eliminate the side effect of fluorescence background on the accuracy of detection (Patchsung et al., 2020). LAMP and RPA are robust isothermal amplification methods but lack of sufficient commercial resources especially for RPA (only TwistDx Company) makes hurdle in vast employment of these methods in diagnostic platforms. In addition, Cas13 and Cas12 are not widely commercially available which shorten the global utilization of diagnostic platforms based on these two endonucleases (Rauch et al., 2020).

PAC-MAN system as a therapeutic approach can reveal robust inhibition of viral sequences in an in vitro condition. PAC-MAN system exhibits potential superiorities over traditional vaccines. Traditional vaccines are mainly designed to stimulate the immune system via facing viral components such as proteins or peptides which almost derived from surface proteins. The high rate of mutation in these proteins improves the chances of viral escape from the host immune system (Zhu et al., 2020a). However, the PAC-MAN system provides a genetic attack strategy that targets highly conserved regions, which prevents the virus to bypass the immune system by a mutation in genomic regions. In addition, Cas13d has the ability to target the multiple regions (due to the capability of this nuclease to process its own crRNAs from a crRNA array), therefore, leads to reducing the chances of viral evasion (Abbott et al., 2020). The collateral activity of Cas13d which may disrupt the RNAs of the host cells that attacked by viruses is one of the concerns that remain unresolved using of PAC-MAN system in in vivo tests.

The lack of an effective in vivo delivery system for using PAC-MAN in the clinic may be a major bottleneck of this system. To date, several

Table 3
The sequence of crRNAs used in CRISPR-based platforms using for detection and treatment of SARS-CoV-2.

platforms	Target genes	crRNA sequences
RT-PCR	N1-CDC FW/Rev primer	GAC CCC AAA ATC AGC GAA AT TCT GGT TAC TGC CAG TTG AAT CTG
	N2-CDC FW/Rev primer	TTA CAA ACA TTG GCC GCA AA GCG CGA CAT TCC GAA GAA
SHERLOCK	S-crRNA_v1	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACGCAGCACC AGCUGUCCAACCUGAAGAAG
	Orf1ab-crRNA_v1	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACCCAACCUCU UCUGUAAUUUUUAAACUAU
CREST	N1	gaaatTAATACGACTCACTATAgggcgaccccaaaatcagcgaaat
	N2	gaaatTAATACGACTCACTATAgggcttacaaacattggccgcaaa
	N3	gaaatTAATACGACTCACTATAgggcgggagccttgaatacaccaaaa
DETECTR	N-gRNA 1	UAAUUUCUACUAAGUGUAGAUCCCCCAGCGCUUCAGCGUUC
	E-gRNA2	UAAUUUCUACUAAGUGUAGAUGUGGUAUUCUUGCUAGUUAC
	RNaseP gRNA	UAAUUUCUACUAAGUGUAGAUAAUUACUUGGGUGUGACCCU
	N-gRNA 1 other species variant	UAAUUUCUACUAAGUGUAGAUCUCCAAGUGCCUCUGCAUUC
	N-gRNA 2	UAAUUUCUACUAAGUGUAGAUGCAAUGUUGUUCCUUGAGGA
	E-gene gRNA 1	UAAUUUCUACUAAGUGUAGAUUUGCUUUCGUGGUAUUCUUG
CRISPR/Cas12a-NER	orf1a-crRNA1	TTTGGTGGTGCATCGTGTTGTCTGTAC
	orf1a-crRNA2	TTTGTGACTTAAAAGGTAAGTATGTAC
	orf1a-crRNA3	TTTGTACATACTTACCTTTTAAGTCAC
	orf1a-crRNA4	TTTACACTTAAAAACACAGTCTGTACC
	orf1b-crRNA1	TTTCACTCAATACTTGAGCACACTCAT
	orf1b-crRNA2	TTTTTAACATTTGTCAAGCTGTCACGG
	orf1b-crRNA3	TTTTAACATTTGTCAAGCTGTCACGGC
	orf1b-crRNA4	TTTGTCAAGCTGTCACGGCCAATGTTA
	E-crRNA1	TTTTCTTGCTTTCGTGGTATTCTTGCT
	E-crRNA2	TTTCGTGGTATTCTTGCTAGTTACACT
	E-crRNA3	TTTACAAGACTCACGTTAACAATATTG
	E-crRNA4	TTTACTCTCGTGTTAAAAATCTGAATT
	N-crRNA1	TTTCTTGAACTGTTGCGACTACGTGAT
	N-crRNA2	TTTGCTGCTGCTTGACAGATTGAACCA
	N-crRNA3	TTTACCAGACATTTTGCTCTCAAGCTG
PAC-MAN	N1-crRNA N18f	UGAACCAAGACGCAGUAUUAUU
CONAN	N1-crRNA	ATG TCTGGTAAAGGCCAACAACAACAAGGCCAAAC
	N2-crRNA	AAG GAACTGATTACAAACATTGGCCGCAAATTGCA

potential strategies have been introduced for transient in vivo expression of CRISPR ingredients. Polymers or lipid nanoparticles could be employed for Cas13d and its related crRNAs delivery which increases the stability of CRISPR component (Tahamtan and Ardebili, 2020). Moreover, a high-level extended duration gene expression system (HEDGES), as a non-viral DNA-and liposome-based gene delivery platform may be a promising carrier for Cas13 in in vivo applications (Wang et al., 2020). The delivery of ribonucleoprotein complexes comprise of Cas13d proteins assembled with crRNAs is the other strategy for using PAC-MAN system in the clinic (Anderson et al., 2020; Beigel et al., 2020).

ABACAS is proposed as a new antiviral strategy to target emerging viruses such as SARS-CoV-2. This approach recruits the potential of the CRISPR-Cas13 system to cleave the genome of RNA virus. CRISPR-Cas13 tethers itself to S protein of the virus through the antibody fragment and endocytose together with the virus to the infected cells. Hence, the ABACAS strategy has the ability to deliver the CRISPR system selectively into the patient's cells. Therefore, ABACAS provides an effective and safe delivery of CRISPR machinery without requiring to additional delivery agents such as electroporation or nanoparticles.

However ABACAS provides a promising approach for termination of SARS-CoV-2 but this novel treatment should pass several phases in order to be used in clinic. The mechanism of action of this antiviral strategy must be exactly defined including the process of any biological pathway targeting such as single protein, genomic DNA or RNA in the virus or in the host cell. These findings would make a basis to predict any unintended effects related to mentioned therapeutic approach. During the in vitro studies serum binding, cytotoxicity outline, and therapeutic profiles should also be investigated before the clinical evaluation of the antivirals (Nalawansha and Samarasinghe, 2020). Moreover lack of FDA approved antibody to use for selective delivery of Cas13 into the infected cells is the other shortcomings of ABCAS.

6. Conclusion

The worldwide outbreaks of highly contagious viral diseases have led to occasional epidemics and pandemics. This grim reality reveals the need for rapid and real-time diagnostic tests as well as efficient and specific therapeutic approaches. The robustness of novel molecular tools allows for the quick integration of these methods into clinical use. The advances in PCR and DNA sequencing have facilitated the rapid detection and combating of emerging pathogens.

Diagnostics based on CRISPR-Cas technology has been shown to be rapid, specific, and cheap. Although, these techonolgies are not still commercially available, the lateral flow-based diagnostic platforms can be easily use to detect the emerging viral diseases as POCs in areas with limited resources. Large scale utilization of these platforms for screening of infected populations will aid the restriction of the virus spread by detection of carrier individuals.

Nevertheless, for a successful containment of the disease, we need viable antiviral with variable uses. CRISPR based system such as PAC-MAN are perfect examples of these kinds of antiviral tools in which the Cas proteins remain to be constant and only requiring the design of a crRNA which targets the conserved genomic region of the virus. However, this therapeutic faces various challenges in terms of efficiency, delivery, safety, regulatory approval for employing in human subjects. Totally, CRISPR based platforms can be feasibly implemented for the detection and treatment of new viral infections "diagnostic and therapeutic strategies".

Funding

Not applicable.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Consent to participate

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

FS: she was the main author who designed the manuscript template and drew the figures, MA: help in data gathering and writing the COVID-19 pathogenesis section, BR: help in data gathering and writing general aspect of CRISPR, A-BH: he wrote a pros and cons of CRISPR based platforms, AB-B: he supervised the process of data gathering, writing and revising the manuscript.

Declaration of Competing Interest

Not applicable.

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