



CRISPR/Cas System: A Revolutionary Technique Applied Potentially from Diagnosis to Therapeutics in COVID-19

Roshan Roy¹, Ashish Srivastava^{1,2*}, Sonal Srivastava¹, Taruna Gupta¹,
Tripti Singhal¹, Prashant Kumar¹, Sunny Dhir¹ and Narayan Rishi¹

¹Amity Institute of Virology and Immunology, Amity University Uttar Pradesh,
Noida, India

²Department of Entomology and Plant Pathology, University of Arkansas,
Fayetteville 72701, USA

*Corresponding Author: Ashish Srivastava, Department of Entomology and Plant
Pathology, University of Arkansas, Fayetteville 72701, USA.

Received: November 30, 2021

Published: December 23, 2021

© All rights are reserved by Ashish
Srivastava, et al.

Abstract

The emergence of Coronavirus disease-2019 caused by SARS-CoV-2 has also brought new challenges to the researchers to develop novel therapeutic and diagnostic methods for the pathogen. The preventive vaccines are approved for the public but their role in the development of herd immunity is not clear due to the emergence of new variants of SARS-CoV-2. Therefore, the prime requirement to cope up with the current situation is to develop highly sensitive diagnostics and effective therapeutics to restrict the spread of SARS-CoV-2 among human population. CRISPR/Cas, a talk of the town, is one of the tools that has been used to develop sensitive, rapid and cost-effective diagnostics that may be made available even in the remote areas of the country. The ability of different Cas proteins viz. Cas9, Cas12, and Cas13 in binding and cleaving of dsDNA, ssDNA, and ssRNA, respectively, have been explored for these purposes. The Cas13 based Prophylactic Antiviral CRISPR in huMAN cells (PAC-MAN) and the Specific High-Sensitivity Enzymatic reporter unlocking (SHERLOCK) and Cas 12 based FALUDA technology have already been developed for diagnosis of COVID-19. This review summarizes the role of CRISPR/Cas based approaches for reliable, rapid, and ultrasensitive diagnostics development, and explore the possible therapeutic alternative for combating COVID-19.

Keywords: Coronavirus; SARS-CoV-2; Cas13; Diagnosis; Therapeutic; PAC-MAN

Key Points

- This MS describes information of the emergence of Coronavirus disease-2019 caused by SARS-CoV-2.
- It summarizes and discusses in details about the novel therapeutics and diagnostics developed to date to control this disease.
- Role of CRISPR/Cas is discussed in detail for novel therapeutics and diagnostics development.

Abbreviations

SHERLOCK: Specific High-Sensitivity Enzymatic Reporter Unlocking; SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2; PAC-MAN: Prophylactic Antiviral CRISPR in Human Cells

Introduction

A devastating disease, the COVID-19 has completed one year of its revelation to the world while the scientists and administrators are still making the strategies to get rid of this disease. SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) is the causative

agent of the disease which emerged in Wuhan province of China in late 2019 and was declared as a pandemic in February 2020 by World Health Organization. Phylogenetic analysis of the viral proteins reveal that the virus has evolved from the bat coronavirus, RaTG13, which is evolutionarily linked with Pangolin-CoVs. More than six SARS-CoV-2 strains have been recorded till date with the double mutant virus being the latest circulating strain having one of the most devastating capability. COVID-19 is associated with diverse symptoms including fever, dry cough, tiredness, diarrhea, running nose, bodyache etc which lead to some serious issues such as difficulty breathing or shortness of breath, chest pain, or pressure [1]. So far around 145M cases of COVID-19 disease have been recorded with 3.07M deaths around the globe. United States, India, and Brazil are the top presenters with around 50% of total cases. Most of the individuals infected with SARS-CoV-2 are either asymptomatic or present mild to moderate symptoms which go away on its own while only a small percentage of affected individuals become critically ill especially if they have other co-morbidities like hypertension, diabetes, cancer etc. (Table 1).

S. No.	Event	Date	Country
1	Pneumonia cases were confirmed at Wuhan City, Hubei province, China	December 20, 2019	China
2	The Huanan seafood market was suspected to be the source of the disease	January 01, 2020	China
3	First death of a 61-year-old male due to this disease	January 09, 2020	China
4	The Genetic sequence of the novel coronavirus were released	January 12, 2020	China
5	First imported case of COVID-19 in Thailand	January 13, 2020	Thailand
6	Imported case of COVID-19 in Japan	January 16, 2020	Japan
7	First case of COVID-19 in South Korea	January 20, 2020	South Korea
8	First case of COVID-19 in USA	January 20, 2020	USA
9	WHO confirms human-to-human transmission of the virus.	January 21, 2020	Switzerland
10	First case of COVID-19 in Nepal	January 24, 2020	Nepal
11	First case of COVID-19 in Australia	January 25, 2020	Australia
12	First case of COVID-19 in France	January 25, 2020	France
13	First case of COVID-19 in Malaysia	January 25, 2020	Malaysia

14	First case of COVID-19 in Canada	January 25, 2020	Canada
15	WHO Director-General declared COVID-19 an outbreak a public health emergency of international concern	January 30, 2020	Switzerland
16	First COVID-19 death outside China	February 02, 2020	Philippines
17	COVID-19: the official name of novel coronavirus was announced	February 11, 2020	Switzerland
18	A global research and innovation forum started in Geneva	February 11, 2020	Switzerland
19	First case of COVID-19 at Egypt	February 14, 2020	Egypt
20	First case of COVID-19 at Iran	February 19, 2020	Iran
21	First case of COVID-19 at Israel	February 21, 2020	Israel
22	First case of COVID-19 at Lebanon	February 21, 2020	Lebanon
23	First case of COVID-19 at Kuwait, Bahrain, Afghanistan, Iraq, and Oman	February 24, 2020	-
24	First case of COVID-19 at Switzerland, Croatia, and Austria	February 25, 2020	-
25	First case of COVID-19 at Brazil, Greece, Georgia, North Macedonia, Norway, Romania, and Pakistan	February 26, 2020	-
26	First case of COVID-19 at Ecuador, Qatar, Monaco, and Luxembourg	February 29, 2020	-
27	First case of COVID-19 at Czech Republic, Iceland, and Armenia	March 01, 2020	-
28	First case of COVID-19 at Indonesia, Senegal, Portugal, Andorra, Latvia, Jordan, Morocco, Saudi Arabia, and Tunisia	March 02, 2020	-
29	First case of COVID-19 at Ukraine, Argentina, and Chile	March 03, 2020	-
30	First case of COVID-19 at Poland	March 04, 2020	Poland
31	First case of COVID-19 at Bosnia and Herzegovina, Slovenia, South Africa, and Palestine	March 05, 2020	-
32	First case of COVID-19 at Slovakia, Bhutan, Peru, Costa Rica, Columbia, Cameroon, and Togo	March 06, 2020	-
33	First case of COVID-19 at Malta, Moldova, Paraguay, and the Maldives	March 07, 2020	-
34	First case of COVID-19 at Bulgaria and Bangladesh	March 08, 2020	-

35	First case of COVID-19 at Albania, Cyprus, Burkina Faso, and Panama	March 09, 2020	-	50	First case of COVID-19 at Saint Kitts and Nevis, Guinea-Bissau, British Virgin Islands, and Mali	March 25, 2020	-
35	First case of COVID-19 at Brunei Darussalam, Democratic Republic of Congo, Bolivia, Jamaica, and Mongolia	March 10, 2020	-	51	First case of COVID-19 at Saint Kitts and Nevis, Guinea-Bissau, British Virgin Islands, and Mali	March 25, 2020	-
36	First case of COVID-19 at Turkey, Côte d'Ivoire, Cuba, Guyana, Honduras, Saint Vincent, and the Grenadines	March 11, 2020	-	52	Johnson and Johnson announced the selection of a lead COVID-19 vaccine candidate.	March 30, 2020	USA
37	First case of COVID-19 at Trinidad and Tobago, Ghana, and Gabon	March 12, 2020	-	53	COVID-19 ELISA IgG Antibody Test approved	April 15, 2020	USA
38	First case of COVID-19 at Kazakhstan, Sudan, Puerto Rico, Venezuela, Antigua and Barbuda, Ethiopia, Guinea, Kenya, Uruguay, Guatemala, St. Lucia, Suriname, and Mauritania	March 13, 2020	-	54	CRISPR-based strategy for RNA-guided viral RNA inhibition and degradation (PAC-MAN)	May 14, 2020	USA
39	First case of COVID-19 at Namibia, Central African Republic, Republic of Congo, Equatorial Guinea, Rwanda, Seychelles, and Eswatini	March 14, 2020	-	55	RT-LAMP AQ-TOP COVID-19 Rapid Detection Kit approved	May 21, 2020	USA
40	New York SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic kit approved and First case of COVID-19 at Uzbekistan and the Bahamas	March 15, 2020	USA	56	SARS-CoV-2 DETECTR Reagent Kit	August 31, 2020	USA
41	First case of COVID-19 at Somalia, Benin, Liberia, and Tanzania	March 16, 2020	-	57	The Russian Direct Investment Fund submitted applications for WHO prequalification Sputnik V vaccine,	October 27, 2020	Russia
42	First case of COVID-19 at Montenegro, Barbados, Gambia, and Montserrat	March 17, 2020	-	58	U.K. government approves first COVID-19 vaccine candidate from Pfizer and BioNTech for domestic use	December 02, 2020	United Kingdom
43	First case of COVID-19 at Kyrgyzstan, Djibouti, Zambia, El Salvador, and Nicaragua	March 18, 2020	-	59	The U.K. begins to roll out the Pfizer-BioNTech vaccine use	December 08, 2020	United Kingdom
44	First case of COVID-19 at Mauritius, Fiji, Chad, Niger, and Haiti	March 19, 2020	-	60	The Pfizer-BioNTech COVID-19 vaccine receives regulatory approval in Canada	December 09, 2020	USA
45	First case of COVID-19 at Papua New Guinea, Cape Verde, Zimbabwe, and Madagascar	March 20, 2020	-	61	U.S. FDA gives emergency use authorization to the Pfizer-BioNTech COVID-19 vaccine	December 11, 2020	USA
46	First case of COVID-19 at East Timor, Angola, and Eritrea	March 21, 2020	-	62	The U.S. begins to roll out a mass COVID-19 vaccination campaign with the Pfizer-BioNTech vaccine.	December 14, 2020	USA
47	First case of COVID-19 at Uganda, Grenada, Mozambique, Dominica, and Syria	March 22, 2020	-	63.	The COVID-19 vaccine was launched for the first group includes healthcare and frontline workers.	January 16, 2021	India
48	First case of COVID-19 at Myanmar and Belize	March 23, 2020	-	64.	After over a year, Israel declared mask free through successful vaccination program.	April 20, 2021	Israel
49	21 days lockdown in India and First case of COVID-19 at Libya and Laos.	March 24, 2020	India	65.	India recorded highest COVID-19 cases (314,835) in a day.	April 23, 2021	India
				66.	India recorded 14 million COVID-19 vaccine doses in a day.	August 31, 2021	India
				67.	India become first country to administered 7.3 billion doses	September 11, 2021	India
				68.	Omicron (B.1.1.529): SARS-CoV-2 Variant of Concern	November 24, 2021	South Africa

Table 1: Showing the various Timeline of Covid-19.

Although several vaccines have been developed against SARS-CoV-2 with efficacy ranging from 65% to more than 90%, their efficacy is compromised due to emergence of new variants of the virus. With the emergence of these new variants, the virus is spreading at an alarming rate with a very high reproductive number and infectivity rate of almost 25% in India. Since most of the cases are asymptomatic, the best way to contain the spread of this virus is early detection and prevention of its spread at individual level in the community. Simultaneously, availability of effective and specific antiviral therapy is also important for reducing the mortality rate among critically ill individuals. The popular clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) proteins-based techniques are playing important roles in the development of point-of-care-testing (POCT) and therapeutics for COVID-19 disease [5]. The need for surveillance of SARS-CoV-2 accelerates research in the field of Nucleic acid-based testing (NAT) based diagnostics to develop inexpensive, rapid, and ultrasensitive

kits. NAT tools proved ultrasensitive and accurate diagnostics for multiplexed virus infection thus major attempts being performed to convert typical diagnostics into nucleic acid POCT tools. From the earliest case of COVID-19 disease, the use of qRT-PCR (quantitative, real-time reverse-transcription polymerase chain reaction) was recommended worldwide, however, the testing requires expensive infrastructure and trained manpower. These limitations proved insufficient to surveillance the spread of the disease. The popular POCT techniques such as Recombinase Polymerase Amplification (RPA) and Loop-Mediated Isothermal Amplification Based Assay (LAMP), could not be proved as efficient as qRT-PCR. Therefore, multiplexing of CRISPR/Cas was studied along with these isothermal amplification techniques to improve the reliability of the diagnosis [6,7]. This review article deals will explain the overall advances of the CRISPR/Cas system towards COVID-19 disease surveillance and therapeutics (Figure 1).

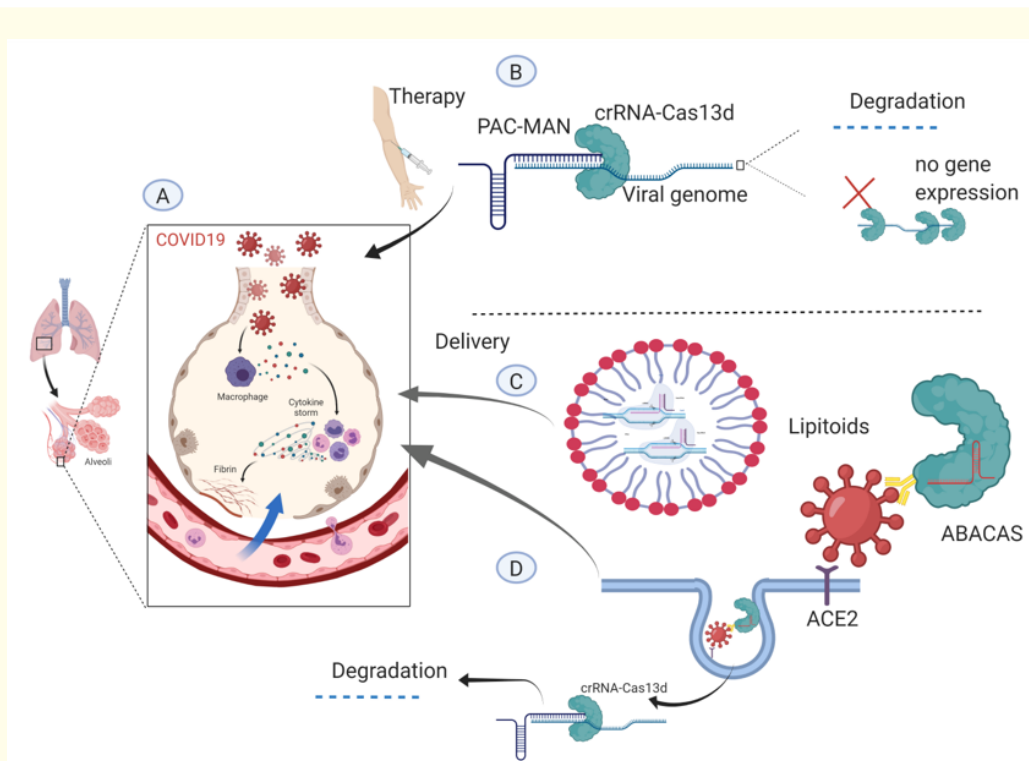


Figure 1: Overview of CRISPR based therapeutics and their delivery systems for COVID19 (Created with BioRender.com)

(A). SARS-CoV-2 upon entry to pulmonary niche via ACE2 triggers cytokine storm that leads to acute respiratory distress syndrome (ARDS). (B). Prophylactic Antiviral CRISPR in the human cells (PAC-MAN) is a CRISPR-Cas13d-based intervention developed by Abbott, et al. is capable of degrading viral genome or blocking the expression of essential genes with dead Cas13 (C). Lipitoid 1 mediated delivery of PAC-MAN is highly efficient solution to delivery problems. (D). Novel delivery system suggestions like fusion of Cas13 with antibody fragment specific to S protein of virus [AntiBody And CAS fusion (ABACAS)] can also be very efficient in delivering CRISPR components to specific infected locations.

Coronavirus disease 2019 (COVID-19)

The 21st century is known for the advances in technologies of communications such as smartphones, and medical devices such

as ultrasound, advanced diagnostic systems, and genetic engineering which made human beings safer. However, the emergence of new diseases in this century and the inability of the human being

to handle them shows that we are still far behind to control an emerging or re-emerging disease. Since the first report of human coronavirus infection in 1960s, seven viruses have emerged which have the potential to cause mild to severe disease in human population. In the past 20 years itself, human beings have witnessed the emergence of highly pathogenic coronaviruses (CoV) viz. SARS-CoV, MERS-CoV and SARS-CoV-2. The Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) emerged in 2003 in Guangdong, China while the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) emerged in 2012 in Saudi Arabia [8,9]. Although SARS-CoV and MERS-CoV didn't cause pandemic, they had a very high mortality rate of 10% and 35% respectively which is much higher as compared to the mortality rate observed in the ongoing COVID-19 pandemic.

Taxonomy of SARS-CoV-2

SARS-CoV-2 belongs to subfamily *Orthocoronavirinae* in the *Coronaviridae* family under the order *Nidovirales*. There are four genera viz. *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus* and *Deltacoronavirus* in this subfamily and SARS-CoV-2 belongs to *Betacoronavirus* genus. The genus is further subdivided into five subgenera viz. *Embecovirus*, *Hibecovirus*, *Merbecovirus*, *Nobecovirus*, and *Sarbecovirus* and SARS-CoV-2 forms a discrete lineage in *Sarbecovirus* subgenus.

Structural features of SARS-CoV-2

SARS-CoV-2 has a crown-shaped appearance due to uniform distribution of spike proteins on the virion surface as observed under electron microscope. It is an enveloped virus having a nonsegmented positive sense ssRNA genome of ~30kb. The genome encodes four structural proteins viz. spike protein (S), envelope protein (E), Nucleoprotein (N) and Matrix membrane protein (M) in addition to six accessory and sixteen non-structural proteins. The trimeric spike glycoprotein is responsible for host cell tropism and entry into the target cells via ACE2 receptor. The spike glycoprotein has two subunits (S1 and S2) and shows a structural difference from the spike protein of other similar coronaviruses in having a typical furine like cleavage site (S1/S2) rich in basic amino acid residues (SPRRARSVAS) which favors its efficient entry of virus into host cells.

Transmission

The incubation period for SARS-CoV-2 ranges from two to fourteen days with a median incubation period of 5-6 days but an infected individual starts shedding the virus before the onset of symptoms or even if he or she is asymptomatic. Transmission of SARS-CoV-2 primarily occurs via aerosols exhaled by an infected individual leading to an airborne transmission. The virus can also be transmitted to a healthy individual via fomites carrying the virus or by direct contact between an infected and healthy individual.

During the first wave of pandemic in 2020, individuals above 65 years of age or those having co-morbidities like hypertension, diabetes, cancer, asthma etc were more prone to infection with SARS-CoV-2 but the second wave of pandemic in India has revealed a different scenario. The double mutant reported in the country along with Brazilian strain, UK strain and South African strain equally affects the young adults and children. The transmissibility rate has almost doubled with the R_0 value upto 5.4.

Clinical symptoms

Individuals infected with SARS-CoV-2 may be asymptomatic carrier or may exhibit mild, moderate or severe ARDS. The patients usually show symptoms like high fever above 102°F, headache, dry cough, sore throat, chest congestion, chest pain, shortness of breath, loose motion, running nose, redness in eyes etc. In severe cases, the patients may show the respiratory rate above 30/min, oxygen saturation in blood below 95% and in this critical stage, severe pneumonia, septic shock, respiratory failure, cardiac arrest and multiple organ failure may occur leading to death of individual. The fatalities during SARS-CoV-2 infection could be attributed to ARDS which occurs primarily due to cytokine storm resulting from abnormal immune response and excess release of pro-inflammatory cytokines in lungs.

Diagnosis of COVID-19

Molecular and serological assays are available for the diagnosis of COVID-19. Though the specimen required for these tests depends on the clinical presentations in affected individuals but respiratory specimens are the most common specimen for the diagnosis of SARS-CoV-2 infection. Nucleic acid amplification test (NAAT) is the most accurate test available for the diagnosis of COVID-19 and it involves procedures like sample collection, sample processing, RNA extraction, real time RT-PCR and its analysis. As far as possible, the patients suspecting SARS-CoV-2 infection should get diagnosed based on rRT-PCR. Amplification of more than one target on SARS-CoV-2 genome is needed for accurate diagnosis of the viral infection. The commonly used rRT-PCR targets in SARS-CoV-2 include the Envelope gene (E), Nucleoprotein gene (N), RNA dependent RNA Polymerase (RdRP) gene and Spike protein (S) gene. Owing to enhanced rate of mutation in the viral genome, at least three of the mentioned targets must be included in the diagnosis to avoid any false negative results. Recently, fully automated NAAT system has been developed that integrates the processes like sample processing, RNA extraction, amplification and reporting. These automated systems can be installed in remote resource limited areas with limited numbers of high performance trained staffs.

Additionally, other amplification methods for detection of SARS-CoV-2 are under development or under the process of commercialization. These methods involve the technologies like Re-

verse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP), Clustered Regularly Interspersed Short Palindromic Repeats (CRISPR) and molecular microarray assays.

The serological assay includes the detection of viral protein in respiratory tract specimen. These are rapid tests which are based on Lateral Flow Immunoassays (LFI) and can be completed within 30 minutes, however, the sensitivity of LFI based rapid tests are lesser as compared to NAAT. Moreover, chances of false positive results also exist with the rapid tests if the antibodies on test strip cross-reacts with the antigen on viruses than SARS-CoV-2.

Vaccines

Considerable efforts have been put for the development of ef-

fective vaccines against SARS-CoV-2 infection. Vaccines that are being developed to tackle COVID-19 are inactivated vaccines and subunit vaccines including mRNA and DNA vaccines targeting the spike protein of the virus. According to World Health Organization (WHO), seven different vaccines have been released in various countries (Table 2) and vulnerable populations are being given priority for vaccination. Apart from this, approximately 200 vaccine candidates are under development, out of which 60 are under clinical evaluation. Though vaccination, as a prophylactic measure, is the best way to protect the population against SARS-CoV-2 infection, mutation in the viral genome is a big challenge to the vaccination drive. However, the vaccinated individual is always at a lower risk as compared to unvaccinated individuals in terms of the disease severity.

Name of Vaccine	Vaccine Type	Primary Developers	Country of Origin
Comirnaty (BNT162b2)	mRNA-based vaccine	Pfizer, BioNTech; Fosun Pharma	Multinational
Moderna COVID19 Vaccine (mRNA-1273)	mRNA-based vaccine	Moderna, BARDA, NIAID	US
COVID-19 Vaccine AstraZeneca (AZD1222); also known as Vaxzevria and Covishield	Adenovirus vaccine	BARDA, OWS	UK
Sputnik V	Recombinant adenovirus vaccine (rAd26 and rAd5)	Gamaleya Research Institute, Acellena Contract Drug Research and Development	Russia
COVID-19 Vaccine Janssen (JNJ-78436735; Ad26.COV2.S)	Non-replicating viral vector	Janssen Vaccines (Johnson and Johnson)	The Netherlands, US
EpiVacCorona	Peptide vaccine	Federal Budgetary Research Institution State Research Center of Virology and Biotechnology	Russia
Covaxin	Inactivated vaccine	Bharat Biotech, ICMR	India

Table 2: Vaccines approved for SARS-CoV-2.

Therapeutics for COVID-19

Pathogenesis of SARS-CoV-2 infection involves two processes viz. replication of SARS-CoV-2 in the tissues of host organisms in early stage of infection and abnormal/exaggerated host immune response in late stage of infection. Therefore, the therapeutics to treat COVID-19 depends on the stage of disease. Antibody based therapy may be more suitable when the patient displays mild or moderate symptoms and disease progression needs to be stalled to prevent hospitalization during early stage. Two monoclonal antibodies viz. Bamlanivimab and the combination of casirivimab and imdevimab have received Emergency Use Authorization by FDA for treatment of outpatients suffering from COVID-19.

In the advanced stage of disease, Remdesivir, which is an anti-viral agent, along with Dexamethasone can be used to treat hospi-

talized patients. Other combination of drugs prescribed for SARS-CoV-2 infected patients include Paracetamol, Hydroxychloroquine, Ivermectin, and Doxycyclin.

Establishment of the CRISPR/Cas system:

The CRISPR-Cas (Clustered Repeat Interspaced Short Palindromic Repeats/CRISPR Associated Proteins) system is derived from the adaptive immune response which is commonly found in bacteria and archaea to combat the bacteriophage infection. The prokaryotic immune system is adapted upon the presence of foreign viral genome material entering into the cell. As a memory of the victory, their system incorporates the parts of the genome of the invading bacteriophage into their specific loci and transcribes the resulting loci into the guide RNA to ultimately destroy the virus on re-entry [14]. The CRISPR/Cas system contains an endonucle-

ase and a CRISPR array that carries a dual-RNAs, tracrRNA:crRNA, for site-specific and programmable DNA cleavage. The CRISPR array of genes consists of the leader sequence (an AT-rich sequence), repeats, and spacer sequences (crRNA). The Cas9 protein has six domains, the remodeling of the recognition (REC) I and II, Bridge Helix, PAM Interacting region, HNH, and RuvC domains [15]. The largest Rec I domain works as a site for binding guide RNA while the REC II domain in off-target discrimination [16]. The PAM-Interacting domain is held responsible for the binding to target DNA while the bridge helix help in the initiation of the cleavage post target DNA binding. The HNH and RuvC domains are the nuclease domains that create nicks in the single-stranded DNA [17].

The CRISPR Cas systems are majorly classified into two classes; the Class 1 system (type I, III, IV) and the Class 2 system (type II, V, VI). The Class 1 system (type I, III, IV). The class 1 system imparts immunity by forming an effector complex of multi-subunit crRNA and the Class 2 CRISPR Cas system (type II, V, VI) imparts immunity by a single subunit of crRNA. The major Cas proteins used for therapies and diagnosis of SARS-CoV-2 are Cas9, Cas12, and Cas13 [5]. Cas9 requires a trans-activating CRISPR RNA (tracrRNA) and guided by single-guide RNA (sgRNA) and in the presence of PAM sequence in the host, this protein creates a double-stranded break in the dsDNA [5,18]. The most popular CRISPR-associated protein 9 is the *Streptococcus pyogenes* Cas9 (SpCas9) which recognizes the 5' NGG 3' as PAM sequence in a host and the *Francisella novicida* Cas9 (FnCas9) which recognizes 5' NGG 3' as PAM sequence [19,20]. Since the activated Cas9 works by cleaving the target and cause silencing of the genes to the next generation, therefore, development of a new Cas9 with mutated nuclease domains that is dCas9 (nuclease deficient Cas9) can be a good step to bind at the transcription site of the desired section of a gene. These two features of Cas9 and dCas9 were explored for the diagnosis of SARS-CoV-2 [21].

The activity of cleaving the target DNA creates double-stranded breaks which need to reseal for normal replication to occur. The sealing of the DNA usually occurs through two pathways (i) non-homologous end joining (NHEJ) and the homology directed repeat (HDR) [22]. These two *in vitro* mechanisms are studied to edit genomes either by knocking out of genes or knocking in sequences of interest. Another class of Cas protein (Class 2 type V) is Cas12 (Cpf1), which is known to target and create cuts in both dsDNA

as well as the ssDNA. Cas12 works with a CRISPR RNA (crRNAs) and possesses the cis and trans cleavage activity of ssDNA which is being used for the SARS-CoV-2 diagnostic kit preparations. The research on RNA phages leads to the discovery of a new class of CRISPR-associated protein, Cas13 (C2c2) nuclease which has a specific recognition and cleavage activity for complementary RNA and once activated also cleaves nearby trans RNA [23]. This feature is first utilized to develop diagnostic played an important role in the diagnosis of viruses and other mRNAs *in vitro*.

CRISPR/Cas based methods for SARS-CoV-2 diagnosis

At the onset of COVID-19 in the globe, the biggest limiting factor to treat the disease was the unavailability of the easy and POCT molecular diagnostic kits. qRT-PCR based kits were majorly recommended by the WHO (World Health Organization) for early diagnosis of the disease, however, the suitability of such kits is restricted due to the requirement of specialized instruments and trained manpower. Simultaneously, the serological POCT kits were having limitations with their sensitivity and accuracy [24]. Therefore, the major research groups around the world were focused on CRISPR/Cas based rapid and ultrasensitive diagnostic kits for the SARS-CoV-2 and recommended their suitability for surveillance of SARS-CoV-2 strains. Researchers have explored different properties of Cas9, Cas12, and Cas13 proteins in addition to various isothermal amplification technology to develop reliable, ultrasensitive, and rapid diagnostics.

The story of CRISPR/Cas-based diagnostics was started with Nucleic acid Sequence-based Amplification based CRISPR cleavage (NASBACC) with the use of Cas9 to detect Zika virus strains *in vitro*. In this method, the isolated RNA of ZIKA virus was amplified by isothermal amplification technique, NASBA, and detected through paper-based sensors [25]. In India, the first dCas9 based kit for SARS-CoV-2 diagnostic kit 'Feluda' was developed and approved for commercial use by the Drugs Controller General of India (DGCI). The FnCas9 Editor Linked Uniform Detection Assay (FELUDA) kit was developed by the use of dCas9 which selectively binds to target DNA without nicking it. To detect a SARS-CoV-2 genome, the Fluorescein amidites (Fam)-tracr RNA-sgRNA and anti-FAM antibody conjugate with nanoparticles and a paper-based Lateral flow assay device was developed for the field-based diagnosis of SARS-CoV-2 outbreaks [26].

The type V CRISPR associated effector protein, Cas12 is commonly explored for CRISPR based diagnostics development. Cas12a protein needs one CRISPR RNA (crRNA) without tracrRNA and cleaves dsDNA by RuvC domain, and once activated, it can cleave nearby ssDNA and RNA in trans, therefore, most suitable for diagnosis (Table S1) [27]. The system was assembled with some isothermal systems and the lateral flow devices as well as the fluorimeter-based detection system. The Cas12a-based system utilizes ssDNA probes in place of RNA probes, therefore, are more stable in field conditions. With the identification of the role of Cas12 in collateral cleavage of ssDNA, the HOLMES (one-Hour Low-cost Multipurpose highly Efficient System) and the DETECTR (DNA Endonuclease Targeted CRISPR Trans Reporter) techniques were established for pathogen detection [28]. The CRISPR/Cas12-based detection was demonstrated for SARS-CoV-2 by utilizing DETECTR assay and it was claimed as a visual and faster alternative to the COVID-19 qRT-PCR assay [29]. In the past few months, several new techniques were established for the detection of the SARS-CoV-2 genome in nasal swab samples with the role of Cas12 proteins. The All-in-One Dual CRISPR-Cas12a (AIOD-CRISPR) and iSCAN (*in vitro* Specific CRISPR-based Assay for Nucleic acids detection) are two important systems that were explored for the diagnosis of the SARS-CoV-2 [30,31]. These techniques utilize the collateral cleavage activity of ssDNA probes to cleave nearby DNA probes. AIOD-CRISPR operates with a pair of crRNAs that initiate dual CRISPR-Cas12a detection, therefore, it improves the detection sensitivity of the COVID-19 pathogen. While the AIOD-CRISPR is a one-pot LAMP-based system, which detects the SARS-CoV-2 genome in an ultrasensitive and reliable manner. Since Cas12 targets amplified DNA sequences and do not requires extra RNA transcription steps, therefore, this system gets popular over all the available techniques. A new system, ENHanced Analysis of Nucleic acids with CrRNA Extensions (ENHANCE) is also introduced for SARS-CoV-2 genome detection, which is based on an extended 3' or 5'-ends of the crRNA with different lengths of ssDNA, ssRNA, and phosphorothioate ssDNA. This detection system can measure up to the femtomolar range without any target pre-amplification step [32]. Recently, Srivastava et al (2022) has proposed a new Collateral Cleavage Independent CRISPR/Cas12a based detection system (CCI-CRISPR) for plant viruses [50].

The use of CRISPR/Cas as a detection system was popularized after the successful introduction of the specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) system which har-

nesses the quality of collateral cleavage of RNA by Cas13 for the diagnosis of viral genomes [33]. The SHERLOCK system showed its potential during the outbreak of the SARS-CoV-2 and a modified

S. No.	Bacterial Strain	Cas12a
1.	<i>Francisella tularensis</i>	FnCas12a
2.	<i>Acidaminococcus</i> sp.	AsCas12a
3.	<i>Lachnospiraceae</i> <i>bacterium</i>	LbCas12a, Lb5Cas12
4.	<i>Oribacterium</i> sp	OsCas12a
5.	<i>Helcococcus kunzii</i>	HkCas12a
6.	<i>Thiomicrospira</i> sp	TsCas12a
7.	<i>Bacteroidales</i> sp.	BbCas12a, BoCas12a
8	<i>Lachnospiraceae</i> <i>bacterium</i>	LbCas12a

Table S1: List of bacterial strains standardized for HOLMES (one-Hour Low-cost Multipurpose highly Efficient System) based diagnostic assay (Li., *et al.* 2018).

protocol COVID-19 diagnosis was established [34]. Another Cas13 based method for SARS-CoV-2 diagnosis, Rugged, Equitable, Scalable Testing (CREST) is introduced which is claimed to be a low-cost technique without sacrificing detection sensitivity [35]. The Combinatorial Arrayed Reactions for Multiplexed Evaluation of Nucleic acids (CARMEN) technique is also based on Cas13, which is a highly multiplexed method for SARS-CoV-2 molecular detection [36] (Table 3).

CRISPR/Cas based methods for COVID-19 therapeutics

Unlike vaccines which in principle train the immune system to fight the invading pathogen and unlike drugs that generally develop resistance, especially in case of highly mutating viruses. The CRISPR based therapeutics directly target the viral genome or mRNA and perform degradation or inactivation of effector functions. Therefore, the outcome of the CRISPR based therapeutic interventions highly rely upon the identification of pathogen-specific conserved genome or coding sequence responsible for important pathogenesis mechanisms. Utilizing in-depth knowledge of these basics, Abbott., *et al.* have developed Prophylactic Antiviral CRISPR in the huMAN cells (PAC-MAN) approach by identifying a pool of six CRISPR RNAs bio-informatically that can target 90% coronaviruses with 90% efficiency. Derived from *Ruminococcus flavefaciens* XPD3002, this CRISPR-Cas13d-based intervention effectively in-

Nuclease	Platform	Isothermal Amplification method	Detection method	sgRNA target	Mechanism	References
Cas9	FELUDA	RPA/ PCR	Paper-based LF device	Genome of SARS-CoV-2	gRNA-dFnCas9 RNP complexes with target DNA and detected colorimetric LF device	Azhar., <i>et al.</i> 2020
Cas12	DETECTR	LAMP	Paper-based LF device	Nucleoprotein and Envelop gene of SARS-CoV-2	Cas12a-crRNA complex binds and cleave a dsDNA, and detects fluorescence in collateral cleaved probe DNA	Broughton et a., 2020
	AIOD-CRISPR	PCR	LED blue light illuminator	complete N gene of SARS-CoV-2	One-pot collateral cleavage reaction system and colorimetric detection	Ding., <i>et al.</i> 2020
	iSCAN	LAMP	Fluorescence visualization in UV light and LF device	Nucleoprotein and Envelop gene of SARS-CoV-2	CRISPR-Cas12a based collateral cleavage and fluorescence-based detection	Ali., <i>et al.</i> 2020
	ENHANCE	LAMP	fluorescence-based and a paper-based lateral flow assay	SARS-CoV-2 N-gene	the modified crRNAs in a paper-based LFA that can detect the target with up to 23-fold higher sensitivity.	Nguyen., <i>et al.</i> 2020
Cas13	SHERLOCK	RPA	Fluorescence spectrophotometer/Paper based LF device	SARS-CoV-2 N-gene	crRNA/ Cas13 targets an ssRNA and cleaved fluorescent ssRNA probe collaterally	Joung., <i>et al.</i> 2020
	CREST	RPA/ PCR	Fluorescence spectrophotometer/Paper based LF device	SARS-CoV-2 nucleocapsid gene	Cas13 activation followed by visualization with blue LED and orange filter.	Rauch., <i>et al.</i> 2020
	CARMEN	PCR	Fluorescence spectrophotometer	SARS-CoV-2 genomic parts	SHERLOCK method in one array enables more than 4500 nucleic acids.	Ackerman., <i>et al.</i> 2020

Table 3: Various CRISPR associated protein-based diagnostic platforms for SARS-CoV-2.

hibits the infection by degrading two conserved regions coding for RNA-dependent RNA polymerase (RdRP) gene and Nucleocapsid (N) gene of synthetic SARS-CoV-2 infected human lung epithelial cells in in-vitro settings by measuring pathogen-associated gene expression and GFP reporter signal repression. Since live strains of COVID-19 were not available, the group tested the proof of concept by targeting conserved regions of neuraminidase (NA) of H1N1 IAV live infection in human lung epithelial cells and found the results showing similar trends in reduction of viral load [37]. In a similar direction, Nguyen., *et al.* have designed 10,333 guide RNAs targeting ORF1a/b which codes for the RdRP gene and the spike (S) gene

[38]. In-effective delivery systems, however, make these studies lack data from in-vivo studies, possible immune responses, side effects, and clinical trials.

CRISPR-Cas13 is a Class 2 type VI CRISPR-associated RNA-guided RNA nuclease, this version of Cas is independent of PAM and thus offers higher flexibility. Studies show that CRISPR-Cas13 is capable of effective suppression of gene expression at the post-transcriptional level in about 24 h of time [39], thus marking the potential of Cas13 to inhibit the coronavirus infection at the post-transcriptional level also. Further, a smaller size of 967

amino acids helps Cas13 to get easily packed in gene therapy vector AAV (Adeno-associated virus) over conventional methods like electroporation, lipofection, and microinjection. With a sgRNA size requirement of 20bp and AAV, this system is capable of targeting more than one region of SARS-CoV-2 very efficiently [38]. In comparison to Cas9 which generates lethal off-target effects like cancer by generating DNA breaks that are repaired by Non-Homologous End Joining (NHEJ) repair, Cas13 is safe, since it functions at the RNA level. Further, usage of catalytically inactive dead Cas13 can help to block the activation of essential polyproteins without cutting the RNA. However, this easy-to-use, highly specific, sensitive, and the cost-effective tool has lacked the attention of clinicians due to side effect concerns and the unavailability of efficient delivery systems. Since it is now possible to cultivate SARS-CoV-2, utilizing the virus only expressing S and N proteins as the delivery vectors can also have potential benefits [40].

Major issues with even highly efficient delivery methods like AAV are that they are less efficient in delivery and deliver components non-specifically to both infected and uninfected cells, increasing chances of off-target activity. Thus, there is a need for selective and efficient CRISPR component delivery systems. In this line, Dhanusha and Kusal, *et al.* suggest a fusion of Cas13 with antibody fragment specific to S protein of virus [AntiBody And CAS fusion (ABACAS)] can be an approach for specific delivery of component to infected cell. In addition to this, studies have also shown that lipid means like lipofection and peptide means like lipitoids can act as effective delivery systems for CRISPR. Lipitoids are synthetic peptide mimics (peptoid) capable of encapsulating the nucleotides in nanoparticles that have a size similar to a virus. In this direction, a group of researchers from Stanford have conducted studies by combining lipitoid 1 and PAC-MAN technologies together, which proved to be highly effective in human lung cells, this combination further reduced the concentration of synthetic SARS-CoV-2 by greater than 90%. Moreover, the group now aims to replicate this approach in animal models and if proven successful they further aim to scale up the experiments for pre-clinical procedures [41].

Similar to gene therapy, cell therapy is also a novel and advanced therapeutic intervention in the CRISPR era. Mesenchymal Stem Cells (MSCs) are a highly popular choice for cell therapy with their easy source availability, less reported adverse effects, high proliferative index, few invasive procedures, and free from ethical issues. Further, COVID19 is associated with cytokine storm which is dam-

aging by its inflammatory nature, MSCs can be highly beneficial in this case, with their regeneration, repair, and immune-modulatory properties, which can help in repairing or regenerating the alveolar epithelial cells and cure lung dysfunction [42]. Since they can be isolated from many sources and stored for longer periods, they make into list of potential COVID19 therapeutics. Clinical studies show that MSCs cell therapy has shown remarkably positive recovery of lung condition in H5N1 infection, thus increasing the hopes for similar results for COVID infection, with many MSCs cell therapy candidates permitted clinical trials [43]. Early results from this cell therapy are very promising for COVID19 with significant improvement in health and an increase in CD4+ and CD8+ T cell population [44]. Further, MSCs are ACE2- and TMPRSS2- [42], which means they are free from infection, thus making them safe and effective candidates. The ability of CRISPR to regulate the cell behavior at the genome level by genetic and epigenetic modifications, at the transcriptional level by editing and silencing mechanisms, makes it a very favorable tool in the cell therapy field. In this line, MSCs can be edited by CRISPR to act as immune modulators to repair the pathological damage, for instance, induced expression of anti-inflammatory genes in MSCs used for arthritis cell therapy [45]. Likewise, CRISPR can help delay telomere shortening by epigenetic modifications and delay the senescence of these stem cells, thereby prolonging the therapeutic effect [46]. These immune-modulation and prolonged therapeutic activities can be highly beneficial for MSCs used for SARS-CoV-2 therapy (Figure 1).

Since viruses are highly evolving species, there is a high need for an antiviral platform that is self-adaptable. Cas13-assisted Viral Expression and Readout (CARVER) is one such platform that combines mechanisms of Cas13 nuclease and Cas 13 based SHERLOCK diagnosis, this development is programmed to kill the RNA viruses in human cells. The effectiveness of this strategy was tested on LCMV, VSV, and IAV infections, which marked CARVER as a strong diagnostic and therapeutic tool [47].

Conclusion and Future Prospects

The twenty-first century is considered the golden time of Medical sciences research, however, SARS-CoV-2 emerged as a big challenge to researchers from the whole world. The limitations with the diagnostics infrastructure and therapeutics remained insufficient to prevent the spread of the disease. However, the emergence of the CRISPR/Cas technique in parallel proved as a blessing in dis-

guise. The technique proved equally powerful in the development of molecular diagnostics as well as potential therapeutics. Unlike the qRT-PCR based techniques where a well-established infrastructure and trained manpower required, the CRISPR/Cas based technique such as SHERLOCK, DETECTR, FELUDA, CARMEN showed their strength in the field of reliable and rapid POCT detection of SARS-CoV-2. Approval of Cas9 based 'Feluda' and Cas13 based 'SHERLOCK' for public use suggests that the CRISPR/Cas system may prove the better alternative for qRT-PCR based kits.

With successful applications of CRISPR in gene therapy and the FDA approval for clinical trials for Retinal gene therapy, researchers find a good future for CRISPR based technologies in the future [48]. With more and more cutting-edge technologies like CARVER, PAC-MAN, and delivery systems like Lipitoids being developed by research society, there have been continuous efforts to overcome concerns over the efficiency of CRISPR therapeutics. Looking ahead to the future, these novel strategies are advanced alternatives to fight pandemics like COVID19 than traditional drugs and vaccines, which some-times fails with cases of resistance and re-infections or short-lived immunity [49], respectively.

Funding

Department of Science and Technology, New Delhi (DST-IN-SPIRE-Faculty scheme, IFA-17-LSPA-100) to AS; Indian Council of Medical Research, New Delhi (ICMR- File No. 5/3/8/29/ITR-F/2020) to AS, TG; and the University Grant Commission, New Delhi, India (UGC) to SS (795/CSIR- UGC NET JUNE 2017).

Conflicts of Interest

There is no competing interest.

Availability of Data and Material

Data is available with the communicating authors and can be presented on demand.

Code Availability

Not applicable.

Ethics Approval

This review article doesn't require Ethics approval. All the participants have agreed to this publication.

Authors' Contributions

AS and NR developed the concept. RR, AS, SS, PK and TG have written the MS. RR and SS prepared the figure. AS and PK finalized the draft.

Consent to Participate

All the authors have given consent for the participation of this MS.

Consent for Publication

All the authors have given consent for the publication of this MS.

Bibliography

1. K Dhama., *et al.* "An update on SARS-CoV-2/COVID-19 with particular reference to its clinical pathology, pathogenesis, immunopathology and mitigation strategies". *Travel Medicine and Infectious Disease* 37 (2020): 101755.
2. S Zaim., *et al.* "COVID-19 and Multiorgan Response". *Current Problems in Cardiology* 45 (2020): 100618.
3. KG Andersen., *et al.* "The proximal origin of SARS-CoV-2". *Nature Medicine* 26 (2020): 450-452.
4. YS Malik., *et al.* "Emerging novel coronavirus (2019-nCoV)-current scenario, evolutionary perspective based on genome analysis and recent developments". *Veterinary Quarterly* 40 (2020): 68-76.
5. P Kumar., *et al.* "CRISPR-Cas System: An Approach with Potentials for COVID-19 Diagnosis and Therapeutics". *Frontiers in Cellular and Infection Microbiology* 10 (2020): 639.
6. Srivastava T., *et al.* "Next-Generation Rapid Advanced Molecular Diagnostics of COVID-19 by CRISPR-Cas". *Diagnostic Strategies. COVID-19 Other Coronaviruses. Medical Virology from Pathogenesis to Disease Control* (2020): 175-187.
7. S Srivastava., *et al.* "Next-Generation Molecular Diagnostics Development by CRISPR/Cas's tool: rapid detection and surveillance of viral disease outbreaks". *Frontiers in Molecular Biosciences* (2020).
8. MA Shereen., *et al.* "COVID-19 infection: Origin, transmission, and characteristics of human coronaviruses". *Journal of Advanced Research* 24 (2020): 91-98.

9. R-H Xu, *et al.* "Epidemiologic clues to SARS origin in China". *Emerging Infectious Diseases* 10 (2004): 1030-1037.
10. F Li. "Receptor Recognition Mechanisms of Coronaviruses: a Decade of Structural Studies". *Journal of Virology* 89 (2015).
11. M Pal, *et al.* "Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2): An Update". *Cureus* 12 (2020): e7423.
12. E Hartenian, *et al.* "The molecular virology of coronaviruses". *Journal of Biological Chemistry* 295 (2020): 12910-12934.
13. R Wölfel, *et al.* "Virological assessment of hospitalized patients with COVID-2019". *Nature* 581 (2020): 465-469.
14. Y Ishino, *et al.* "History of CRISPR-Cas from Encounter with a Mysterious Repeated Sequence to Genome Editing Technology". *Journal of Bacteriology* 200 (2018).
15. M Jinek, *et al.* "A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity". *Science* 337 (2012): 816-821.
16. K Sung, *et al.* "Target Specificity of Cas9 Nuclease via DNA Rearrangement Regulated by the REC2 Domain". *Journal of the American Chemical Society* 140 (2018): 7778-7781.
17. HY Kim, *et al.* "Chimeric crRNAs with 19 DNA residues in the guide region show the retained DNA cleavage activity of Cas9 with potential to improve the specificity". *Chemical Communications* 55 (2019): 3552-3555.
18. Z Liu, *et al.* "Application of different types of CRISPR/Cas-based systems in bacteria". *Microbial Cell Factories* 19 (2020): 172.
19. B Wiedenheft, *et al.* "RNA-guided genetic silencing systems in bacteria and archaea". *Nature* 482 (2012): 331-338.
20. J Murovec, *et al.* "New variants of CRISPR RNA-guided genome editing enzymes". *Plant Biotechnology Journal* 15 (2017): 917-926.
21. W Zhou, *et al.* "A CRISPR-Cas9-triggered strand displacement amplification method for ultrasensitive DNA detection". *Nature Communications* 9 (2018): 5012.
22. H Yang, *et al.* "Methods Favoring Homology-Directed Repair Choice in Response to CRISPR/Cas9 Induced-Double Strand Breaks". *International Journal of Molecular Sciences* 21 (2020).
23. OO Abudayyeh, *et al.* "C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector". *Science* 353 (2016): aaf5573-aaf5573.
24. M Lisboa Bastos, *et al.* "Diagnostic accuracy of serological tests for covid-19: systematic review and meta-analysis". *BMJ* 370 (2020): m2516.
25. K Pardee, *et al.* "Rapid, Low-Cost Detection of Zika Virus Using Programmable Biomolecular Components". *Cell* 165 (2016): 1255-1266.
26. M Azhar, *et al.* "Rapid, field-deployable nucleobase detection and identification using FnCas9". *BioRxiv* (2020).
27. N Rusk. "Spotlight on Cas12". *Nature Methods* 16 (2019): 215.
28. S-Y Li, *et al.* "CRISPR-Cas12a-assisted nucleic acid detection". *Cell Discovery* 4 (2018): 20.
29. JP Broughton, *et al.* "CRISPR-Cas12-based detection of SARS-CoV-2". *Nature Biotechnology* 38 (2020): 870-874.
30. Z Ali, *et al.* "An RT-LAMP-coupled CRISPR-Cas12 module for rapid, sensitive detection of SARS-CoV-2". *Virus Research* 288 (2020): 198129.
31. X Ding, *et al.* "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 - The Preprint Server for Biology* (2020).
32. LT Nguyen, *et al.* "Enhancement of trans-cleavage activity of Cas12a with engineered crRNA enables amplified nucleic acid detection". *Nature Communication* 11 (2020): 4906.
33. MJ Kellner, *et al.* "SHERLOCK: nucleic acid detection with CRISPR nucleases". *Nature Protocols* 15 (2020): 1311.
34. FZhang, *et al.* "A protocol for detection of COVID-19 using CRISPR diagnostics". *Bio Archive* (2020): 1-8.
35. JN Rauch, *et al.* "A Scalable, Easy-to-Deploy, Protocol for Cas13-Based Detection of SARS-CoV-2 Genetic Material". *BioRxiv* 59.4 (2020): e02402-02420.
36. CM Ackerman, *et al.* "Massively multiplexed nucleic acid detection with Cas13". *Nature* 582 (2020): 277-282.
37. TR Abbott, *et al.* "Development of CRISPR as an Antiviral Strategy to Combat SARS-CoV-2 and Influenza". *Cell* 181 (2020): 865-876.

38. TM Nguyen., *et al.* "Virus against virus: a potential treatment for 2019-nCov (SARS-CoV-2) and other RNA viruses". *Cell Research* 30 (2020): 189-190.
39. U Unniyampurath., *et al.* "RNA Interference in the Age of CRISPR: Will CRISPR Interfere with RNAi?" *International Journal of Molecular Science* 17 (2016): 291.
40. M Lotfi and N Rezaei. "CRISPR/Cas13: A potential therapeutic option of COVID-19". *Biomedicine and Pharmacotherapy* 131 (2020): 110738.
41. J Straiton. "CRISPR vs COVID-19: how can gene editing help beat a virus?" *Biotechniques* 69 (2020): 327-329.
42. Z Leng., *et al.* "Transplantation of ACE2 (-) Mesenchymal Stem Cells Improves the Outcome of Patients with COVID-19 Pneumonia". *Aging and Disease* 11 (2020): 216-228.
43. Golchin E., *et al.* "Mesenchymal Stem Cell Therapy for COVID-19: Present or Future". *Stem Cell Reviews and Reports* 16 (2020): 427-433.
44. Liang J., *et al.* "Clinical remission of a critically ill COVID-19 patient treated by human umbilical cord mesenchymal stem cells: A case report". *Medicine (Baltimore)* 99 (2020): e21429.
45. C-C Wu., *et al.* "CD146+ mesenchymal stem cells display greater therapeutic potential than CD146- cells for treating collagen-induced arthritis in mice". *The Journal of Stem Cell Research and Therapy* 7 (2016): 23.
46. DM Filho., *et al.* "Enhancing the Therapeutic Potential of Mesenchymal Stem Cells with the CRISPR-Cas System". *Stem Cell Reviews and Reports* 15 (2019): 463-473.
47. CA Freije., *et al.* "Programmable Inhibition and Detection of RNA Viruses Using Cas13". *Molecular Cell* 76 (2019): 826-837.
48. AL Askou., *et al.* "Retinal gene therapy: an eye-opener of the 21st century". *Gene Therapy* (2020).
49. J Parry. "Covid-19: Hong Kong scientists report first confirmed case of reinfection". *BMJ* 370 (2020): m3340.
50. A Srivastava., *et al.* "Development of a new Collateral Cleavage-independent CRISPR/Cas12a based easy detection system for plant viruses". *Journal of Virological Methods* 300 (2022).

Assets from publication with us

- Prompt Acknowledgement after receiving the article
- Thorough Double blinded peer review
- Rapid Publication
- Issue of Publication Certificate
- High visibility of your Published work

Website: www.actascientific.com/

Submit Article: www.actascientific.com/submission.php

Email us: editor@actascientific.com

Contact us: +91 9182824667