REVIEW

Development and application of sensitive, specific, and rapid CRISPR-Cas13-based diagnosis

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Abstract

Nucleic acid detection is a necessary part of medical treatment and fieldwork. However, the current detection technologies are far from ideal. A lack of timely and accessible testing for identifying cases and close contacts has allowed severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the causative virus of the ongoing coronavirus disease-2019 (COVID-19) pandemic, to spread uncontrollably. The slow and expensive detection of mutations—predictors for chronic diseases such as cancer—form a barrier to personalized treatment. A recently developed diagnostic assay is ideal and field-ready—it relies on CRISPR-Cas13. CRISPR-Cas13 works similarly to other CRISPR systems: Cas13 is guided by a crRNA to cleave next to a specific RNA target sequence. Additionally, Cas13 boasts a unique collateral cleavage activity; collateral cleavage of a fluorescent reporter detects the presence of the target sequence in sample RNA. This system forms the basis of CRISPR-Cas13 diagnostic assays. CRISPR-Cas13 assays have >95% sensitivity and >99% specificity. Detection is rapid (<2 h), inexpensive ($0.05 per test), and portable—a test using lateral flow strips is akin to a pregnancy test. The recent adaptation of micro-well chips facilitates high-level multiplexing and is high-throughput. In this review, we cover the development of CRISPR-Cas13 assays for medical diagnosis, discuss the advantages of CRISPR-Cas13-based diagnosis over the traditional reverse transcription polymerase chain reaction (RT-PCR), and present examples of detection from real patient samples.

KEYWORDS
clustered regularly interspaced short palindromic repeats (CRISPR)-associated genes 13, coronavirus disease 2019, nucleic acid detection, severe acute respiratory syndrome coronavirus-2, specific high-sensitivity enzymatic reporter unlocking

1 | INTRODUCTION

Diagnostics are a necessary part of medical treatment and field work. One approach to diagnostics is the detection of nucleic acids. In particular, the detection of pathogen DNA or RNA in patient samples, such as by reverse transcription polymerase chain reaction (RT-PCR) tests, informs if a patient is infected.[1] A clear determination of infection allows for the treatment and isolation of a patient as well as the quarantine of their close

contacts. Effective contact tracing—accomplished via early and widespread detection of infection—is necessary to stop the spread of a pathogen. A lack of robust testing programs for the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has allowed the virus to spread around the world, causing the ongoing coronavirus disease-2019 (COVID-19) pandemic; testing issues are most pronounced in countries with the most infections.[2,3] The COVID-19 pandemic proves that nucleic acid detection is urgently needed to control the spread of emerging and remerging

pathogens. Recent pandemics were caused by the related coronaviruses Middle East respiratory syndrome-related coronavirus (MERS, 2012) and severe acute respiratory syndrome coronavirus-1 (SARS-CoV-1, 2003), as well as the H1N1 strain of influenza A virus (IAV, 2009).[4,5]

Nucleic acid detection is also needed to personalize treatment against chronic diseases. In the case of HIV, the virus can acquire many types of drug-resistant mutations that affect various drugs. As a result, patients must be tested for mutations by the individual so that each patient can have their drug regimen adjusted to their specific mutation.[6] Humans also naturally develop nucleotide polymorphisms (SNPs). SNPs require personalized detection and treatment—they are unique to each patient's DNA. SNPs serve as predictors for cancer and also tell which immunotherapy is an effective treatment.[7,8] In addition, SNPs tell which immunosuppressant is an effective treatment for autoimmune diseases or after organ transplanations.[9]

Detection of mutations is most commonly performed by high-throughput sequencing of DNA or RNA.[10] The benefit of high-throughput sequencing is that the technology provides genome-wide information. However, sequencing the entire genome costs thousands of dollars per patient, and sequencing large amounts of information means that the required equipment is complex and not available at all treatment centers.[10] Detection for mutations needs to be made more accessible, flexible, and cost-effective so that every patient can benefit from personalized treatment.

While nucleic acid detection tells whether a patient has a disease-related mutation or is infected with a pathogen, it is important to acknowledge that detection is not a complete medical diagnosis. A complete diagnosis also takes into account a patient's condition, such as the stage of viral infection or tumor progression, as well as a patient's symptoms and medical history. During a pandemic, widespread testing for a pathogen is used without doctor input. However, this strategy is again to help doctors by buying them time to attend to more serious cases, the triage of which still considers symptoms. Along these lines, nucleic acid detection guides the decision-making for a complete diagnosis.

Medical diagnosis covers a wide-range of fields—there are no existing assays that can detect pathogen messenger RNA (mRNA) but also find single-nucleotide mutations out of an entire genome, all while meeting the needs and holding up to the inadequacies of any clinical environment. In the search for a holistic solution, much progress has been made in developing clustered regularly inter-spaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) protein 13 as a diagnostic assay. CRISPR-Cas13 diagnostic assays are sensitive, specific, inexpensive, rapid, and portable. CRISPR-Cas13 assays are also field-ready. In this review, we cover the development of CRISPR-Cas13 diagnostic assays for medical diagnosis. We discuss the advantages of CRISPR-Cas13-based diagnosis as compared to traditional RT-PCR. Finally, we present examples in which pathogen infection and chronic disease-related mutations are detected in real patient samples.

2 | DEVELOPMENT OF CRISPR-CAS13 FOR DIAGNOSTICS

2.1 | Cas9 and Cas13 work on the same underlying principles

The CRISPR-Cas system was first discovered in action with the Cas9 protein, and CRISPR-Cas9 has since been harnessed for genome editing of DNA in vitro and in vivo.[10,11,13] Cas9 works in tandem with a gRNA (guide RNA). The gRNA comprises a CRISPR RNA (crRNA) that has its 3′ end based paired to a tracrRNA.[14] The free 5′ end of the crRNA is a target sequence from DNA.[14] Cas9 grabs onto the tracrRNA, forming a binary complex. This allows Cas9 to guide the crRNA target sequence to the complementary region (protospacer) on a DNA molecule. The pairing of the crRNA to the protospacer will separate the matching second DNA strand and forms the Cas9-gRNA-protospacer ternary complex. Dependent on the presence of the protospacer and a protospacer adjacent motif (PAM),[15] the crRNA target sequence serves as a guide for Cas9 to cleave both DNA strands within the protospacer sequence.[16] For convenience with genome editing, the crRNA and tracrRNA are programmed into a single chimera, called a sgRNA (single guide RNA), where the two molecules are connected by a loop in addition to base pairing.[17]

CRISPR-Cas13 is a variant of CRISPR-Cas9 where Cas13 cleaves RNA instead of DNA.[18] CRISPR-Cas12 is works similarly but with DNA; several key differences between CRISPR-Cas12 and Cas13 are summarized in Table 1. Since this review is focused on RNA detection, here we describe the function of CRISPR-Cas13. The system uses a crRNA that is comprised of a target sequence (spacer) and an adjacent stem-loop, called a direct repeat (DR).[18] Cas13 grabs the DR, and guides the crRNA such that the spacer matches and base pairs with the complementary sequence on sample RNA.[18] In contrast to Cas9, Cas13 then cleaves distal to the RNA target sequence that is paired with a spacer.[18]

Cas13 has specific sequence requirements for cleavage. First, cleavage can occur at any distance along with the RNA that is away from the target sequence, though Cas13 cleaves only where the RNA is single-stranded.[18] Second, Cas13 is tolerant to single base pair mismatches in spacer-RNA pairing but not double base pair mismatches.[18] Based on this property, the synthetic addition of a single base pair mismatch in the spacer means that a lack of targeting by Cas13 identifies any additional mutations that occur in the sample nucleic acid.[20,24,25] Lastly, Cas13 has specific base preferences for cleavage: most orthologs prefer Us, a combination of bases, though more rarely, As.[20]

The target cleavage activities of Cas9 and Cas13 act in *cis*. Cas13 has an additional *trans* (collateral) cleavage activity that works on surrounding nontarget ssRNA; collateral cleavage is dependent on target cleavage.[18] Collateral cleavage occurs with specific dinucleotide preferences that vary greatly by Cas13 ortholog.[20] The collateral cleavage activity of Cas13 forms the basis of Cas13 diagnostic assays.

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TABLE 1 Comparison of CRISPR-Cas12 versus Cas13 nucleic acid detection

Cas12	Cas13
Cis cleavage	
ssDNA and dsDNA[19]	ssRNA[20]
At specific bases[19]	At specific bases[20]
Distal to target sequence[21]	Distal to target sequence[20]
Target or bystander ssDNA[21]	Bystander ssRNA[20]
Trans cleavage	
Across entire ssDNA (shredding, *Lachnospiraceae bacterium*, LbCas12a)[21]	At specific bases[20]
Thermostable (*Alicyclobacillus acidiphilus*, AapCas12b)	Works at room temperature[22]
Features	
nM limit of detection (LoD)	nM LoD[20]
aM LoD after PCR[21,23]	aM LoD after PCR[20]
>90% sensitivity[21]	>95% sensitivity[17,20,24–27]
~100% specificity[21]	>99% specificity[17,20,24–27]
Pioneering diagnostic assays	
One-hour low-cost multipurpose highly efficient system (HOLMES)[23]	SHERLOCK[20,25]
Specific high-sensitivity enzymatic reporter unlocking (SHERLOCK)[25]	
DNA endonuclease-targeted CRISPR trans reporter (DETECTR)[21]	

2.2 | Development of diagnostic assays based on Cas13 collateral cleavage activity

Gootenberg et al.[20] were the first to develop CRISPR-Cas13 as a diagnostic assay. A detection mix is used that consists of sample RNA, Cas13a, designed crRNA, and reporter RNA (RNA sensor) that has a fluorophore and quencher on opposing ends.[20] If sample RNA is present that contains the target sequence, Cas13 will cleave the sample RNA and collaterally cleave the RNA sensor to relieve the fluorophore.[20] Thus, fluorescence is the output of whether the target is present or not. The fluorescence level is quantitative for the amount of target in the sample.[20,24–26,28] This assay is termed specific high-sensitivity enzymatic reporter unlocking (SHERLOCK).[20]

The process of SHERLOCK involves two-steps. In the first step, DNA purified from the sample undergoes recombinase polymerase amplification (RPA) (or reverse transcription [RT]-RPA for RNA) and the amplified DNA undergoes T7 RNA polymerase transcription to RNA. In the second step, the resulting amplified RNA is added to the

CRISPR-Cas13 target sequence detection reaction.[20] A comprehensive SHERLOCK protocol is published by Kellner et al.[27]

2.3 | Modifications to SHERLOCK improve the original assay

One of the major inconveniences to SHERLOCK is the need to perform initial nucleic acid purification. Early experiments showed that detection without purification only works for synthetic nucleic acids spiked in up to 2% human serum,[18] but these sample conditions are not so favorable in real patient samples. However, modifications to the original protocols have now allowed for direct detection from raw patient samples. One procedure, called heating unextracted diagnostic samples to obliterate nucleases (HUDSON), uses chemical reduction from bench-stable reagents along with heat to inactivate nucleases and virus.[24] Raw patient samples—such as blood, saliva, and urine—are added into one tube for HUDSON and then transferred into a second tube for SHERLOCK.[24]

Still, it is cumbersome to use separate tubes for HUDSON and SHERLOCK. A new procedure called STOP (SHERLOCK testing in one pot) accomplishes nucleic acid purification and detection into one tube/pot.[28] The initial extraction is simplified to a single step by using magnetic beads that concentrate RNA.[28] The second set of reagents are added: loop-mediated isothermal amplification (LAMP) makes and amplifies DNA that is detected in a SHERLOCK reaction.[28] The ssDNA-detecting Cas12b is used because the enzyme is heat-stable (*Alicyclobacillus acidiphilus*; AapCas12b) to survive the LAMP.[20] A similar, also new, procedure, called diagnostics with coronavirus enzymatic reporting (DISCoVER), combines HUDSON with LAMP amplification and T7 RT for detection.[29]

Another recent modification that improves the speed and convenience of SHERLOCK is skipping the PCR amplification step. To get SHERLOCK to work with less input, the researchers employed two strategies. First, the researchers chose optimal crRNAs that when tested yielded the highest fluorescence levels.[30] Second, multiple crRNAs were combined into a single reaction so that more Cas13 that can bind to the target and induce more detectable fluorescence.[30]

CRISPR-Cas13 has also been improved for multiplexed detection. For separate target specificities, Cas13 orthologs are matched to crRNAs based on their DR base preferences.[25] For separate readouts, the orthologs are also matched to RNA sensors based on their base preferences for collateral cleavages.[25] These strategies allow for one Cas13a ortholog, two Cas13b orthologs, and one Cas12a ortholog (cleaves dsDNA) to be mixed with four crRNAs and four RNA sensors to detect three RNA targets and one DNA target.[25]

The problem with this early method of multiplexing is that each Cas13 ortholog only works with one crRNA; the number of multiplexed targets is limited by the number of crRNA-ortholog pairs that can be added to a tube before having molecules stops the detection reaction. Moreover, most orthologs cannot be used together because

they share target sequence, DR, and RNA sensor base preferences. Lastly, each sample must undergo multiplexed detection in a separate tube. Thus, the number of samples that can be tested is limited by the number of tubes that can be handled—having too many tubes likely result in some human error.

To improve on SHERLOCK multiplexing, Ackermen et al.[31] recently developed combinatorial arrayed reactions for multiplexed evaluation of nucleic acids (CARMEN). CARMEN uses detection mixes containing crRNAs that differ only by target sequence; all mixes use the same Cas13a and RNA sensor.[31] Solution-based fluorescent dyes are used to identify the detection mixes and amplified samples, which are added to a massive-capacity chip (mChip) for SHERLOCK fluorescence detection. Since the chip forms all possible droplet combinations of detection mixes and amplified sample, CARMEN can perform paired detection of M samples × N crRNAs – each mCHIP can handle 4500 unique pairs/tests.[31]

Taken together, CRISPR-Cas13 relies on the principle workings of the original CRISPR-Cas9 system. However, Cas13's collateral cleavage activity has allowed for the development of CRISPR-Cas13 as a diagnostic assay. Modifications, including direct detection from raw patient samples, skipping PCR amplification steps, and high-level multiplexing make the assays field-ready. Still, the improvements were each completed in individual studies. Ideally, the improvements can be combined to further boost the speed and convenience of the assays.

3 | COMPARISON OF CRISPR-CAS13 DIAGNOSTIC ASSAYS TO TRADITIONAL PCR ASSAYS

3.1 | CRISPR-Cas13 assays are highly sensitive and specific

CRISPR-Cas13 diagnostic assays provide several benefits over traditional RT-PCR tests that detect pathogens. RT-PCR tests that detect SARS-CoV-2 mRNA have low sensitivity of about 63%–78%, which means that false negatives are common.[32] False negatives are usually a result of low viral load from patients in the early or late stages of infection.[32] Nevertheless, the virus still contagious and dangerous—a more sensitive test is urgently needed to identify whether a patient is infected.

SHERLOCK detection has both high sensitivity and high specificity. Sensitivity is the percent of detected positives out of true positives; a higher percentage means less false negatives. Specificity is the percent of detected negatives out of true negatives; a higher percentage means less false positives. For brevity, we review SHERLOCK's sensitivity and specificity only in studies involving real patient samples. The following studies used RT-qPCR as a gold-standard to establish true positives and negatives. An early study found that SHERLOCK has 100% sensitivity and 100% specificity when detecting purified Zika virus (ZIKV) RNA.[24] More recently, SHERLOCK had 100% sensitivity and 96% specificity when detecting

SARS-CoV-2 mRNA from COVID-19 samples.[26] STOP can detect SARS-CoV-2 mRNA with 93.1% sensitivity and 98.5% specificity.[28] The latter two studies showed more sensitivity errors—false negatives. Indeed, the errors arise from samples that have low viral load and thus RT-qPCR CTs above 33.5[26] or 37.[28] In fact, optimization of SHERLOCK's RPA step (see next paragraph) improves sensitivity and resolves detection.[26] When SHERLOCK is modified so that PCR amplification can be skipped, the assay maintains 100% sensitivity and 100% specificity.

Another measure of an assay's performance is its lower limit of detection (LoD). Comparable to PCR, SHERLOCK has an LoD at single copy/ml, attomolar (aM; 10^{-18} M) range.[20] Optimization of SHERLOCK's RPA step pushes the LoD down to 8 zeptomolar (zM; 10^{-21} M).[25] This high sensitivity holds for multiplexed detection using Cas13a orthologs or CARMEN.[26,31] Skipping PCR amplification results in a 480 fentomolar (fM; 10^{-15} M) LoD.

3.2 | CRISPR-Cas13 assays are portable, rapid, and inexpensive

RT-PCR tests are labor-intensive because they require individual processing of each sample. As a result, these tests are prone to human errors,[33] demand long return times, and carry high costs. Furthermore, PCR machines can only be found in lab environments. On the other hand, SHERLOCK is more convenient in several aspects.

First, SHERLOCK is portable: a commercial lateral flow strip can be used for single target detection.[25] A generic SHERLOCK detection reaction is applied to the strip, though fluorescein (FAM)-biotin RNA sensor is used. A negative test means that the full-length RNA sensor accumulates at the first streptavidin line. A band is visualized because anti-FAM gold nanoparticle-conjugated antibodies are added to the detection reaction. A positive test means that the RNA sensor is cleaved, and the FAM antibodies can flow down the strip where they bind to the second protein-A line; a second band is instead visualized. In sum, test results are instrument-free and read as easily as a pregnancy test.

SHERLOCK is also convenient because it is rapid. The first steps of RPA and T7 RT are completed in 30–45 min. The second detection step is set up in less than 15 min, and provides fluorescence results in 1 h or lateral flow results in 1.5 h.[27] Thus, the entire SHERLOCK is completed in about 2 h. HUDSON (< 30 min) or 10 min rapid gDNA extraction saves time in the nucleic acid purification steps before SHERLOCK.[24,25] When STOP is used, the purification steps are included in the workflow and only take 15 min.[27] The versality of SHERLOCK is further improved by the fact that the assay can be performed at room temperature (25°C).[22]

Lastly, a SHERLOCK test is inexpensive. CARMEN brings the cost of detection down to $0.05 per test (one sample against one target sequence). This is because CARMEN scales up SHERLOCK detection and reduces reagent waste by miniaturizing detection to an mChip. Thus, the low cost is achieved when 200 samples × 100 crRNAs = 20,000 tests are combined across multiple mChips.[31]

The original SHERLOCK in a 96-well plate is also inexpensive—$5.52 for a single test.[31] A paper test (not lateral flow) is as cheap as $0.61.[20]

Taken together, sensitive, specific, rapid, inexpensive, and portable nucleic acid detection cannot all be accomplished via PCR tests but can be accomplished via CRISPR-Cas13 diagnostic assays. These strengths further validate that these assays are field-ready.

4 | APPLICATIONS OF CRISPR-CAS13 DIAGNOSTIC ASSAYS

4.1 | Initial validation using pathogens and disease-related mutations

In early studies, SHERLOCK was validated on synthetic DNA and RNA designed by the researchers.[20] SHERLOCK was first developed during the ZIKV pandemic (2016) and dengue epidemic, so the first tests involving real patient samples were samples from ZIKV patients and involved detection of ZIKV mRNA.[20] Validation of HUDSON was performed by detecting directly from the ZIKV raw samples.[24]

To validate SHERLOCK's specificity, several studies performed detection down to single-nucleotide mutations. For example, SHERLOCK detected cancer mutations in cell-free DNA (cfDNA) fragments.[20] SHERLOCK also detected 16S rRNA sequences differentiating bacterial strains, as well as SNPs differentiating human subjects or ZIKV strains.[20] In a later study, SHERLOCK could again detect ZIKV SNPs and HIV drug-resistant mutations. This time, the researchers started from zero reagents—the crRNAs were designed, Cas13 was purified, and the assay was working within 1 week.[24] This study proved that SHERLOCK is ready for real patient samples.

4.2 | Detection of SARS-CoV-2 mRNA during the COVID-19 pandemic

Several studies have applied SHERLOCK in the field, particularly in the detection of SARS-CoV-2 mRNA. Patchsung et al.[26] recently used both SHERLOCK fluorescence and lateral flow strips to detect SARS-COV-2 mRNA in 154 COVID-19 clinical samples. The samples were from Siriraj Hospital in Thailand and 81 were COVID-19 positive mRNA.[26] A similar study applied to STOP to detect SARS-CoV-2 mRNA from 202 COVID-19 positive and 200 COVID-19 negative samples.[28] Another study tested five clinical samples using SHERLOCK that skipped the PCR amplification. Taking the validation even further, Patchsung et al. applied SHERLOCK to the screening of COVID-19 patients. The patients of all surgical operations after May 2020 at Siriraj Hospital were tested for SARS-CoV-2 mRNA using both SHERLOCK fluorescence and lateral flow strips.[26] In total, there were 380 clinical samples.[26] The sensitivities and specificities of detection found in all referenced studies were higher than that of traditional RT-PCR tests (see above).

Patchsung et al.[26] also multiplexed detection of RNase contamination with SARS-CoV-2 mRNA on the same lateral flow strip. The ability to detect RNAse contamination eliminates false negatives from the degradation of input RNA and eliminates false positives from the degradation of the RNA sensor. SHERLOCK will be reliable in the field, even where clean facilities and equipment are not available, and particularly in low-resource environments.

Although SHERLOCK has been thoroughly tested in real-world settings and with COVID-19 cases, the current assay does not have the throughput necessary to significantly improve the lack of testing during this pandemic. All of the aforementioned studies could only test one sample per tube. Moreover, the winter encompassing 2020–2021 will involve both the yearly flu season and COVID-19 pandemic. There is a need for high-throughput testing for SARS-CoV-2—this will allow for the differentiation of IAV versus SARS-CoV-2 infection. At the patient level, treatment can then be targeted against IAV or SARS-COV-2 so that it is more effective. At the population level, detailed contact tracing, vaccination plans, and where to send drugs are all told by where infections are occurring.

4.3 | Widespread testing and surveillance for pathogens

High-level multiplexing via CARMEN increases the throughput of SHERLOCK. CARMEN simultaneously detects 169 human-associated viruses (HVs).[31] 184 consensus sequence oligos, each belonging to one HV species, were paired with 184 crRNAs—30,912 tests (including repeats) were performed across eight mChips.[31] The detected species matched the actual species an impressive 94% of the time (no gold-standard needed); this percentage is called "concordance", which combines sensitivity and specificity. Applying the same crRNA panel to real patient samples maintained 99.7% concordance when next-generation sequencing (NGS) was used as a gold-standard.[31] CARMEN is a relevant ongoing COVID-19 pandemic—the assay simultaneously detected SARS-CoV-2 mRNA and other coronaviruses, including SARS-CoV-1 from the SARS pandemic (2003) and MERS from the MERS pandemic (2012). CARMEN also detected against numerous influenza strains and subtypes, and HIV drug-resistant mutations.[31]

An important part of stopping pandemics is through the robust surveillance of the numerous species and strains of zoonotic pathogens. Surveillance involves identifying which pathogen variant presents a threat of jumping to humans. Advance action can be taken against that pathogen to prevent a pandemic. Both a literature review and a global panel of infectious disease experts concluded that current surveillance efforts are insufficient and that detection involving individual species and strains must be expanded to prevent another pandemic.[34,35] Moreover, surveillance must take across world, particularly in low-resource areas that would not be able to manage a pandemic.[35]

Surveillance performed by RT-PCR is low throughput, and faces the same problems are the original multiplexed SHERLOCK. High-level

multiplexing CARMEN again increases throughput to the level needed for surveillance to be effective. Hundreds of samples can be paired to hundreds of target sequences via mChips.[31]

Research and clinical validation of CRISPR-Cas13 assays have certainly proven that they are field-ready. Of course, the sample size of the presented studies is still small. Additionally, no studies have used CRISPR-Cas13 assays for pathogen surveillance in real samples collected in the wild, or for the detection of mutations in real patient samples. Therefore, the next step is to expand the use of the assay into more zoonotic and clinical settings. The assays would help increase testing during this COVID-19 pandemic and aid in ramping up surveillance efforts. Additional will allow for more rigorous assessments about the assay's sensitivity and specificity.

5 | CRISPR-CAS13 AS A FUTURE RNA-BASED THERAPEUTIC

Another promising development involving CRISPR-Cas13 is its use as a therapeutic for chronic diseases. CRISPR-Cas13 can make genetic changes to RNA to fix mutations implicated in chronic diseases. RNA editing for programmable A to I replacement (REPAIR) uses adenosine deaminase acting on RNA type 2 (ADAR) fused to a catalytically inactive Cas13 so that ADAR is guided by the CRISPR-Cas13 ternary complex to change an A to I at the target sequence. REPAIR reverses mutations on dsRNA, mutations involved in X-linked nephrogenic diabetes insipidus (878G→A, W293X in AVPR2) and Fanconi anemia (1517G→A, W506X in FANCC).[36] In a later study, CRISPR-Cas13 was applied to a mimic of the full clinical process of diagnosis and treatment. The researchers used HEK293FT (human embryonic kidney) cells with the APC mutation (APC:c.1262G>A); the mutation is linked to familial adenomatous polyposis 1. SHERLOCK has detected the mutation in the mRNA, REPAIR was edited the mRNA in vitro, and SHERLOCK confirmed mRNA editing.[25]

Another system called Cas13-assisted restriction of viral expression (CARVER) uses Cas13 to cleave viral genomes (vRNA) or mRNA, and thus inhibit RNA viruses.[37] CARVER reduced vRNA levels of several RNA viruses: IAV in MDCK (Madin-Darby Canine Kidney) or A549 (adenocarcinomic human alveolar basal epithelial cells); LCMV (lymphocytic choriomeningitis virus) in HEK 293FT cells; and VSV (Indiana vesiculovirus) in HEK 293FT cells.[37] To mimic the clinical process, HUDSON and SHERLOCK were used before and after CARVER to validate the vRNA and mRNA levels.[37] Both CARVER and REPAIR would be particularly convenient as therapeutics because the same crRNAs can be used in SHERLOCK detection.[37]

The advantage of CRISPR-Cas13 as a therapeutic over other systems is that Cas13 and crRNA can be packaged in adeno-associated viral (AAV) vectors.[36] In fact, AAV vectors have already been used for drug delivery in humans. CRISPR-Cas13's proven strengths as a diagnostic and development as a therapeutic form a comprehensive system against pathogen infection and chronic diseases.

6 | CONCLUSION

CRISPR-Cas13 finally provides a holistic solution for all areas of medical diagnosis. The assays combine high sensitivity of > 95% with high specificity of > 99%. CRISPR-Cas13 assays are also rapid, low cost, and portable. The high-level multiplexing of CARMEN increases throughput and has the potential to greatly expand testing during the COVID-19 pandemic. In fact, SHERLOCK and CARMEN have already been applied to real-world screening scenarios involving COVID-19 patient samples.

CRISPR-Cas13 diagnostic assays can also guide personalized treatment against chronic diseases. By knowing the mutations that are specific to each patient, doctors can determine whether a patient is at risk for a disease. Treatments can be adjusted so that they are effective in the context of the patient's mutations, such as drug-resistant mutations or onco-mutations. The studies presented here have shown that SHERLOCK and CARMEN can in fact detect single nucleotide mutations, including those implicated in chronic disease such as diabetes. While detection of disease-related mutations has only involved synthetic DNA and RNA, SHERLOCK has been used with real samples to detect SNPs differentiating humans, bacteria, and viruses.

The studies presented in this review have applied SHERLOCK and CARMEN to real-world situations and serve as proof-of-concept. Expanding these field-ready assays to a variety of zoonotic and clinical settings will ultimately show that the assays drastically improve the speed and accuracy of a doctor's diagnosis. CRISPR-Cas13's eventual application as both a nucleic acid detection assay and a therapeutic means will make the system a swiss army knife against disease. All told the CRISPR-Cas system has been greatly enhanced since it was first discovered and used to edit the genome. The rapid progress in genome technologies has shown that they are the future of medicine.

CONFLICT OF INTERESTS
The authors declare that there are no conflict of interests.

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Conceptualization, literature reading and analysis, writing, editing, and revisions: David Gao. *Topic selection, feedback, and revisions*: Xiaodong Zhu. *Management, supervision, feedback, and revisions*: Binfeng Lu.

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