First CRISPR therapy dosed

An adult with congenital blindness is the first person to receive an in vivo CRISPR-based therapy, according to the sponsors of the clinical trial: Editas Medicine and Allergan. The trial is testing whether EDIT-101 (also known as AGN-151587) can remove a point mutation in the *CEP290* gene, which causes type 10 of the retinal degenerative disease Leber congenital amaurosis (LCA). The IVS26 mutation leads to a functional loss in the CEP290 protein, which causes defects in retinal photoreceptors and severe vision loss. To correct the IVS26 mutation, EDIT-101 uses a construct containing the adenovirus vector AAV5 with two guide RNAs to identify the location of the IVS26, combined with DNA encoding the Cas9 enzyme under a promoter specific to photoreceptor cells. Another gene therapy for LCA is Luxturna (voretigene neparvovec-rzyl) from Spark Therapeutics, now owned by Roche. Both Editas and Spark use an AAV vector, but whereas Editas's genome editing corrects the mutation, Spark's agent introduces a correct copy of the affected gene. Luxturna is approved for treating specifically the form caused by mutation of the retinal pigment epithelial 65 (*RPE65*) gene.

In the Editas trial, 18 adult and pediatric participants will receive subretinal injection in a single eye, with any vision improvements anticipated within four weeks of treatment. Also developing a therapeutic for LCA is ProQR Therapeutics. Early results from a phase 1/2 trial of sepofarsen (QR-110), an RNA-based antisense oligonucleotide therapy, showed about 60% of participants improving.

Although EDIT-101 is the first trial using a CRISPR agent inside the body, Sangamo's zinc finger nuclease therapy SB-913 was the first gene therapy used in vivo, to treat patients with the inherited metabolic disorder mucopolysaccharidosis type II. Until now, clinical trials using CRISPR-based agents had been ex vivo: patients' cells are removed, edited and then returned to the body. Initial results from a trial of CRISPR Therapeutics and Vertex's CTX001 in patients with hemoglobin-related blood disorders suggested potentially curative responses in patients with β-thalassemia and sickle cell disease.

Published online: 7 April 2020
https://doi.org/10.1038/s41587-020-0493-4

Coronavirus and the race to distribute reliable diagnostics

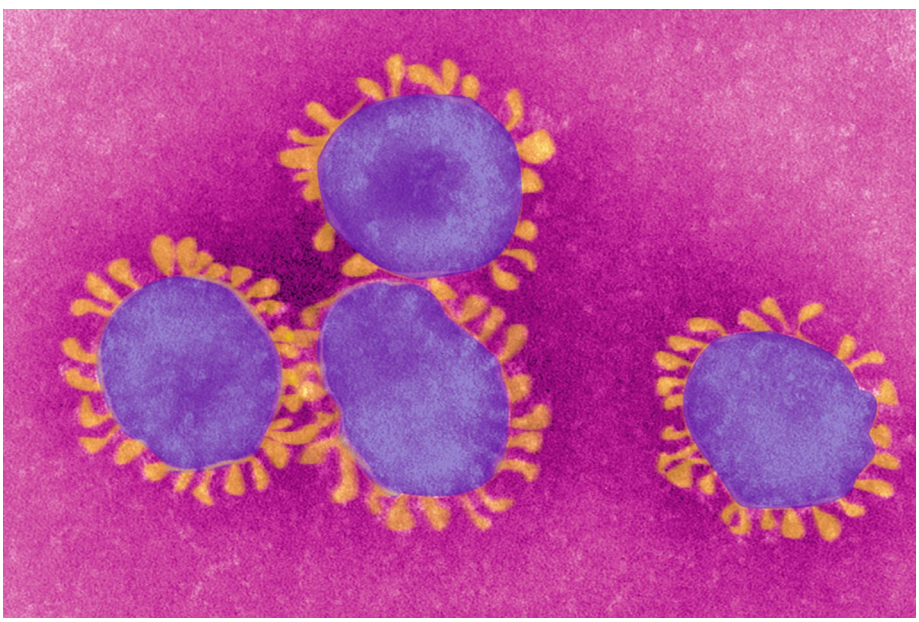
International teams worked at speed to make tests for the virus available in record time.

The medical community is rallying to develop a set of rapid and reliable molecular diagnostic tests for the new human coronavirus that appeared in China — now dubbed sudden acute respiratory syndrome coronavirus-2 (SARS-CoV-2).

This follows a breakneck effort by several research groups to identify and sequence the virus, sharing several viral genomes in open databases over recent weeks. As infections continue to mount — 74,185 people were reported to be infected across China as of midnight local time on 18 February — and authorities seek to accurately diagnose and document infections, the need for rapid and reliable diagnostics increases. Although many clinics still lack access to a robust, accurate and sensitive SARS-CoV-2 test, impressive progress has been made in a matter of weeks. In contrast, it took almost six months to identify and to establish assays for the coronavirus responsible for the 2002–2003 SARS outbreak.

After clusters of pneumonia cases of no known cause were epidemiologically linked to a seafood and 'wet' animal wholesale food market in Wuhan, China, three groups worked tirelessly to identify the culprit. First, researchers from Shanghai, Wuhan, Beijing and Sydney used metagenomic RNA sequencing to identify the previously unknown coronavirus in a sample from a single patient who had worked at the market. This team deposited a draft genome sequence in the publicly available GenBank sequence repository on 10 January; the current version was deposited on 17 January.

Around the same time, a rapid-response team dispatched by the Chinese Center for Disease Control and Prevention reported that they had isolated and cultivated a novel coronavirus from bronchoalveolar lavage fluid of three patients and identified it as the probable source of the outbreak. Electron microscopy observations uncovered a typical coronavirus morphology; light microscopy work demonstrated it had cytopathic effects on human airway epithelial cells. Genome sequencing revealed the virus shared over 85% sequence identity with a known SARS-like coronavirus found in bats. On 12 January, this group deposited three more genome sequences in the open access database Global Initiative on Sharing All Influenza Data (GISAID).



Coronaviruses are so called because the projections that encircle the capsid resemble a monarch's crown when viewed under a microscope. Credit: BSIP SA / Alamy Stock Photo

A third group of scientists, located in Wuhan and Beijing, identified and characterized the same virus in a further five patients with severe pneumonia and deposited five more genomes with GISAID. They also showed that the virus uses angiotensin-converting enzyme II (ACE2) to gain entry to host cells, as did SARS-CoV, the strain that caused the 2002–2003 outbreak, which infected 8,096 people and caused 774 deaths.

Several international groups have been working from these sequence data to design primers for polymerase chain reaction (PCR) tests to support global public health laboratories in the absence of a commercial test for SARS-CoV-2 (formerly 2019-nCoV). The lab of Christian Drosten, of the Institute of Virology, Charité University Hospital, Berlin, along with academic collaborators in Europe and Hong Kong, published details of a real-time PCR (RT-PCR) diagnostic test and workflow on 23 January, which detects SARS-CoV-2 and distinguishes it from SARS-CoV. The group verified the test in the absence of SARS-CoV-2 isolates or patient samples but confirmed its specificity against 297 clinical samples from patients with various other respiratory infections. This formed the basis of shipments of 250,000 kits, which the World Health Organization (WHO) dispatched to 159 laboratories across the globe in recent weeks.

Meanwhile, a group at Hong Kong University have developed two one-step quantitative RT reverse transcription PCR tests targeting both the open reading frame 1b (*ORF1b*) and the *N* regions of the viral genome based on the first sequence deposited at GenBank; these two test have been validated using two clinical specimens obtained from patients infected with SARS-CoV-2. The tests are explicitly designed to identify multiple viruses in the sarbecovirus subgenus to which SARS-CoV-2 belongs, given a lack of data on the genetic diversity of SARS-CoV-2 in humans and animals. As no other sarbecoviruses are known to be circulating in humans, a positive test can be considered as confirmation that a subject is infected with SARS-CoV-2 or a related animal virus. The *N* gene assay is recommended as a screening test and the *ORF1b* test is recommended as a confirmatory test. The diagnostic algorithm is similar to that followed for the Middle East respiratory syndrome (MERS) coronavirus, which was first identified in Saudi Arabia in 2012. "We basically just tried to adopt the same strategy," says Leo Poon, professor in the school of public health at Hong Kong University. His group has shared the test details — and in some cases reagents — with the WHO and with over 30 labs in Asia, Africa, the Middle-East and South America.

The PCR test is highly sensitive, if used correctly. "In our hands, we have a sensitivity of ten copies per reaction," says Poon. However, false negatives — the failure to detect virus in infected patients — can be a significant problem in high-throughput settings operating under severe pressure. The correct operation of the test is crucial. What's more, there are still uncertainties around the kinetics of SARS-CoV-2 viral shedding, so the timing of the test may affect the result. It is also unclear still which types of clinical specimen are optimal. One recent study from Wuhan, which evaluated the performance of a fluorescence-based RT-PCR kit distributed by the Chinese Center for Disease Control and Prevention, suggests that nasopharyngeal swabs offer greater consistency than sputum samples.

In the United States, the CDC (Centers for Disease Control and Prevention) rolled out its own PCR tests to all 50 US states as well as to 30 international locations after it obtained Emergency Use Authorization from the US Food and Drug Administration (FDA) on 4 February. But state testing labs uncovered a quality issue with one element of the three-part assay. "If you scale up fast that may happen," says Marion Koopmans, head of viroscience at Erasmus Medical Center in Rotterdam, the Netherlands, who was involved in the development of the Berlin test.

These early lab tests buy time. They give public health authorities some diagnostic tools before commercial products and kits become available at scale (Table 1). In China, genome sequencing firm BGI Group, of Shenzhen, is already up and running, producing such commercial tests. By the end of January, it had already distributed over 50,000 test kits across China. On 5 February it opened an emergency test laboratory in Wuhan that can process 10,000 samples per day. Other companies are developing their tests more slowly, but initial rollouts are imminent. Not all are relying on primer designs and protocols specified by academic laboratories or public health authorities. "We came to the conclusion that these tests are not optimal for us, in our hands," says Stephan Ölschläger, head of marketing at Hamburg, Germany-based Altona Diagnostics. The company has its own assay platform and is developing a two-part test targeting the *E* gene of lineage B betacoronaviruses and a region of the *S* gene specific to SARS-CoV-2.

Rather than develop bespoke PCR tests, IDbyDNA is one of a handful of companies employing metagenomic nucleic acid analysis as a routine diagnostic and surveillance tool. Its existing Explify Respiratory test, which is a laboratory-developed (or 'home brew') test, can identify over 900 respiratory pathogens, including viruses, bacteria, fungi and parasites, by comparing unbiased metagenomic data obtained from patient samples with a large repository of sequence data. It can already detect the SARS-CoV-2 strain. "We have now updated our data with the new coronavirus and are in the process of revalidating," says cofounder and chief medical officer Robert Schlaberg. "The modifications are all on the data analysis side." That is, he says, an easier process than updating a physical assay. The actual data analysis takes less than an hour; the turnaround time from receipt of sample to test result is 36 hours. Although more expensive than PCR testing, the falling costs of sequencing could help to democratize this approach. The company is selling the technology as well as its testing services. "Traditional diagnostic testing and surveillance are two separate efforts," he says. "It's much faster having real-time monitoring of what is in circulation."

Andrew Rambaut, of the University of Edinburgh, in Scotland, is already doing this, tracking the outbreak by analyzing the phylogenetic relationships between different SARS-CoV-2 genomes. RNA viruses have an error-prone replication process, and the genetic variations that are introduced constitute a molecular clock that can provide insights into the initial emergence and ongoing evolution of the virus. "The molecular clock provides a way of changing the genetic changes into time," he says. An initial analysis, based on 90 publicly available genomes, suggests that SARS-CoV-2 emerged not long before the first cases of pneumonia in Wuhan occurred. "It doesn't predate the timing of the disease detection that much," says Koopmans.

Epidemiologists have also adapted digital surveillance tools to track the new coronavirus outbreak by automatically monitoring structured and unstructured electronic information from official and unofficial sources. The Center for Systems Science and Engineering at Johns Hopkins University has developed a web-based dashboard to visualize and track reported cases of SARS-CoV-2 in real time. The underlying data are freely available to scientists through a GitHub repository. The dashboard's primary source is DXY, an online resource run by members of the Chinese medical community, who aggregate official and media reports on the development of the epidemic. HealthMap.org, developed more than a decade ago to track infectious disease outbreaks, has also released an interface specific for SARS-CoV-2. Crowdsourcing and automated information retrieval have

Table 1 | Selected diagnostic tests for SARS-CoV-2

Developer	Test	Description	Current status
Chinese National Institute for Viral Disease Control and Prevention	New coronavirus nucleic acid assay	Primers and probes for detecting the novel coronavirus with RT-PCR	In widespread distribution in China
US Centers for Disease Control and Prevention (CDC)	CDC 2019-nCoV real-time reverse transcriptase PCR diagnostic panel	PCR test that runs on Applied Biosystems 7500 Fast Dx RT-PCR instrument with SDS 1.4 software	Emergency Use Authorization (EUA) granted by the FDA on 4 February
University of Hong Kong	Real-time reverse transcriptase PCR assays	Two single-step quantitative RT reverse transcription PCR assays for *N* gene and *ORF1b* of sarbecovirus subgenus	Reference test shipped to WHO and to over 30 labs globally
Amoy Diagnostics (Xiamen, China)	Coronavirus gene detection kit	PCR-based rapid detection kit	Seeking emergency approval from China's National Medical Products Administration
Altona Diagnostics (Hamburg, Germany)	Real-time PCR assay	Rapid detection of coronavirus RNA from respiratory samples	In development; shipping expected end of February
BGI Group (Beijing)	Real-time fluorescent RT-PCR kit for detecting 2019-nCoV	Test results delivered in several hours	Emergency approval granted by China's National Medical Products Administration
BGI Group	2019-nCoV nucleic acid detection kit using combinatorial probe-anchor synthesis method	Metagenomic sequencing kit for monitoring novel coronavirus mutations	Emergency approval granted by China's National Medical Products Administration
Hong Kong University of Science and Technology	On-site rapid molecular diagnostic system based on Shenzhen Shineway Technology	Integrated microfluidic PCR test employing silicon-based micro-heater module for rapid heating and processing of test samples; delivers test results in 40 minutes	Platform has European Union CE approval
Novacyt, Primerdesign	Novel Coronavirus Strain 2019-nCoV	Runs on portable genesig16 RT-PCR instrument; delivers test results in less than two hours	CE-marked research-use-only test launched 17 February; seeking FDA EUA
Thermo Fisher Scientific	TaqMan 2019-nCoV Assay Kit	Lab PCR test that runs on Applied Biosystems 7500 RT-PCR system; identifies sequences found in initial 44 SARS-CoV-2 genomes	Research use only
Qiagen (Hilden, Germany)	QIAstat-Dx Respiratory 2019-nCoV Panel	Integrated sample prep and RT-PCR detection of 21 respiratory pathogens; samples and reagents delivered in assay cartridges and analyzed in desktop QIAstat-Dx Analyzer; results delivered in one hour	Prototype panel including COVID-19 test shipped on 11 February for clinical performance assessment in China and Europe
Biomeme	Biomeme COVID-19 Go-Strips	Integrated sample prep and RNA detection test that runs on Biomeme's mobile handheld quantitative PCR device	Research use only
Agency for Science, Technology and Research (A*STAR), MiRXES (both Singapore)	Fortitude Kit 2.0	RT-PCR SARS-CoV-2 assay	100,000 tests produced; provisional authorization received from Health Sciences Authority, Singapore
TIB Molbiol (Berlin, Germany) also via Roche Diagnostics	SARS-CoV-2 E, RdRP or N gene CE-IVD 7 virus Respiratory Panel multiplex RT-PCR	1-step RT PCR	Launched January. 20,000 kits shipped to over 70 countries

https://www.nejm.org/doi/full/10.1056/NEJMoa030747

also been harnessed to develop a shared electronic line list for tracking the SARS-CoV-2 outbreak, case by case. As of 19 February, the list contained demographic, geographic and clinical information on over 7,800 individual cases, which allows researchers to conduct analyses to understand patterns of spread. "A lot of groups are using the data, so that's one encouraging thing," says Moritz Krämer, research fellow at the Department of Zoology, Oxford University, who set up the resource. The present crisis is, he says, characterized by a much stronger data-sharing ethos than previous epidemics. "I think we've learned."

Myriad uncertainties still surround the trajectory of the epidemic. On the positive side, containment efforts outside of China appear, so far at least, to be successful. The number of confirmed cases in other countries remains low, and there is little evidence at this point of widespread community transmission. In China, however, and particularly in Hubei province — the epicenter of the outbreak, which remains in lockdown — the picture is still unclear. The majority of cases have not yet resolved. Moreover, the size of the 'iceberg' is undefined: it is still too early to know whether further undetected reservoirs of infection remain. There is no immediate end in sight to the extraordinary ordeal millions of people are enduring in the cities of Hubei. ❐

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Published online: 19 February 2020
https://doi.org/10.1038/d41587-020-00002-2