

To counter the pandemic, clinicians bank on repurposed drugs

Teams are pursuing a dizzying array of therapeutic strategies to stymie COVID-19. It's not yet clear which approach, or combination of approaches, will work best.

****Due to new and rapidly moving developments related to COVID-19 therapies, we updated this article with additional clinical study details on May 1.**

Jyoti Madhusoodanan, Science Writer

In late February, when reports of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) transmission in California started to emerge, Nevan Krogan's molecular biology lab went into overdrive. Everyone in the lab switched to studying how viral proteins affect host cells. The group, at the University of California, San Francisco, came up with a schedule of lab shifts to work around the clock while maintaining at least six feet of space between colleagues. Although a mass spectrometer broke down and the group failed to synthesize three

viral proteins, the last pieces of data—revealing which host proteins interacted with viral proteins—came in just as the lab shut down to comply with the state's shelter-in-place orders.

Last month, the team published a preprint describing 69 potential drugs—24 of them already approved by the US Food and Drug Administration (FDA) for other diseases—that could help treat coronavirus disease 2019 (COVID-19) patients. The team identified the drugs by studying how 26 of the virus's



Armies of researchers around the world have launched coronavirus disease 2019 (COVID-19) clinical trials and completed studies at record pace. Image credit: Shutterstock/Matej Kastelic.

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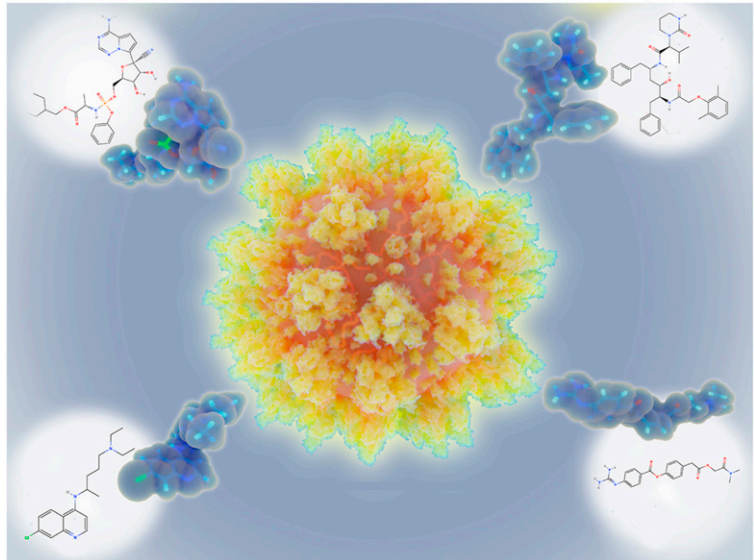
29 proteins interact with host cells (1). In cell cultures, the researchers used synthetic versions of viral proteins to uncover the human proteins that bound to each viral molecule. Then they identified small molecules that bind to the host proteins or otherwise interact with them via cellular pathways.

The host-virus interactions that Krogan's lab identified offer up clues as to why this new coronavirus causes symptoms that range from diarrhea and an inability to smell, to pneumonia and fatal multiorgan failure. "Other coronaviruses are similar but not as virulent," Krogan says. "In terms of protein interactions, this virus seems to hijack and rewire so many different cellular pathways—it gets its fingers into all the key machinery of a cell."

The race is on to stop the virus from wreaking havoc on those many pathways. Armies of researchers around the world have launched COVID-19 clinical trials and completed studies at record pace. Most focus on drugs that are already approved for other indications. Whereas some are studying molecules that target host proteins, others are testing drugs that attack the viral machinery, such as the oft-cited remdesivir from Foster City, CA-based Gilead Sciences. In March, the World Health Organization announced large-scale clinical trials for four promising drugs: the malaria medications chloroquine and hydroxychloroquine, remdesivir, HIV antivirals lopinavir-ritonavir, and a combination of these HIV drugs with interferon-beta, an immune system molecule.

The molecular similarities of SARS-CoV-2 to other pathogens such as SARS have helped accelerate the hunt for treatments. It's also given clinicians much-needed emergency options for helping severely ill patients. Remdesivir has received widespread attention, and based on preliminary evidence from a few phase III trials, the FDA granted emergency use authorization for the drug on May 1. In the case of the much-touted antimalarials chloroquine and hydroxychloroquine—which purportedly act by raising the pH inside the cell's endosomes and lysosomes to attract and trap the virus, and thus block it from proliferating—the evidence is more tenuous. The primary article suggesting some efficacy against COVID-19 recently received an "expression of concern" from the society that publishes the journal in which it appeared (2). And researchers and physicians are wary of potentially dangerous cardiac side effects. Although the drugs are still available for off-label use, an NIH panel recommended against the combination treatment of hydroxychloroquine and azithromycin because of these side effects. The Infectious Diseases Society of America also recommended that chloroquine and hydroxychloroquine should only be given to patients as part of a clinical trial.

Of course, doctors and patients are clamoring for some therapeutic hope. "As of now, clinicians do everything they can to keep severely ill patients breathing," says biochemical virologist Matthias Gotte of the University of Alberta in Canada. "There's no standard drug or antiviral treatment that has been approved." Still, the many potential candidates being pursued by Krogan's lab and others offer the possibility that one or more effective drugs, using multiple strategies, could be approved for widespread use in the coming months.



Remdesivir, lopinavir, camostat, and chloroquine (clockwise from upper left) are among the drugs being investigated to fight the novel coronavirus. Image credit: Shutterstock/Juan Gaertner.

From One Virus to Another

There are more than 3 million confirmed COVID-19 cases around the world. Although the virus was first reported in late 2019, the first randomized, controlled trials to test drugs for effectiveness against the infection were only announced in March. To date, Gilead's remdesivir, an antiviral originally developed to combat Ebola, is among the most widely used, under the FDA's compassionate use guidelines. The first person in the United States to be diagnosed with COVID-19 was treated with intravenous remdesivir and improved shortly after (3). On April 4, the company announced an "expanded access" program so hospitals can apply for the drug to treat multiple patients.

Remdesivir mimics adenosine nucleosides, which constitute one of the building blocks of genetic material. "It's a very logical target," Gotte explains. "The most compelling argument in its favor is that most currently approved antivirals for HIV, hepatitis C, and even DNA viruses are such nucleotide analogs," suggesting that they are key to targeting viruses.

As the viral RNA polymerase enzyme builds a new copy of its RNA, remdesivir competes with the nucleotide ATP, inserts itself into the viral genome, and halts the virus from replicating its genetic material. This inhibition is only effective if the enzyme frequently substitutes its natural substrate with the pharmaceutical analog. "But these are analogs, so the logic is that all these viral enzymes always prefer the natural substrate," Gotte says.

In a recent study, Gotte and his colleagues tested how remdesivir might work on Middle East respiratory syndrome (MERS) coronavirus, a pathogen related to SARS-CoV-2 (4). The team expressed the MERS polymerase enzyme in insect cells and found that remdesivir was incorporated three times more often than its

natural counterpart, suggesting that the drug might prove to be a potent viral inhibitor.

Once remdesivir was inserted into a growing RNA chain, the MERS enzyme halted after adding just three more nucleotides, whereas the Ebola polymerase, in contrast, did not accept the drug as efficiently and allowed the RNA chain to grow longer before it stopped viral replication. This *might* be an early hint that the drug is more effective against MERS than Ebola. "The nuances of inhibition are different," Gotte explains.

On April 13, Gotte's team published results testing remdesivir with the SARS-CoV-2 polymerase (5). "We obtained almost identical results as previously reported with the MERS enzyme," Gotte says. "SARS-CoV, SARS-CoV-2, and MERS use remdesivir with the same high efficiency." Apart from preliminary clinical evidence, this work is the first direct mechanistic evidence that remdesivir can act against the new pathogen.

Trial and Error

Right now, precisely what a doctor will prescribe to someone with symptoms of COVID-19 depends on several factors, including the patient's symptoms, age, or other illnesses, explains Neera Ahuja, division chief of hospital medicine at Stanford University in Palo Alto, CA. Those with milder symptoms quarantined at home may receive oral medications such as chloroquine, whereas remdesivir, which must be given intravenously, is usually used in more severe cases.

But researchers are frantically hunting for more evidence of what will work to treat the greatest number of patients. Ahuja is leading one of several randomized trials to test remdesivir in COVID-19 patients around the world. At 65 clinics, global collaborators aim to evaluate the drug in patients with varied symptoms. The trial is an "adaptive" one, so the team can alter strategies such as patient eligibility or measures of drug effectiveness as data roll in each day.

In preclinical studies with Ebola infections in monkeys, remdesivir didn't appear to have serious side effects and seemed most effective at treating disease when used within the first few days of infection. "Animal models often set the stage to understand how these drugs will work in humans," says Kari Nadeau, professor of medicine and pediatrics at Stanford University and co-investigator on the trial. "But it's not as if this drug has ever been tested in monkeys that were experiencing severe respiratory distress or a cytokine storm," Nadeau adds, alluding to the deadly out-of-control immune system reaction that's killed some patients.

Several other remdesivir trials are underway, including some by drug manufacturer Gilead. Two weeks ago, the company's phase III trial criteria were expanded to include thousands more participants and to include patients who are on mechanical ventilation. The company also reported preliminary results from 53 patients who received the drug via the compassionate use program; 36 of the 53 showed signs of clinical improvement (6). But the data were not from a randomized clinical trial and hence lacked a

control to gauge how patients would have done without the drug.

On April 16, media outlet STAT News reported that, according to a leaked video from one study site at the University of Chicago, several patients treated with remdesivir in the company's phase III trials showed significant improvements. The leaked video contained no information about a placebo-controlled group. A statement from the University of Chicago, quoted by STAT News, said: "drawing any conclusions at this point is premature and scientifically unsound." An April 10 statement from Gilead stated that investigations into remdesivir must not only elucidate safety and efficacy but also demonstrate "in which patients it shows activity, how long should they receive treatment, and at what stage of their disease would treatment be most beneficial."

Then, on April 29, two further reports were published: A Gilead-sponsored trial in China of 237 patients found that remdesivir offered no clinically significant benefits compared to a placebo (7). But early results from the NIH-sponsored trial, where Ahuja and Nadeau are co-investigators, reported that compared to a placebo, remdesivir shortened the time to recovery from 15 days to 11, and also appeared to decrease mortality rates from 11.6% to 8%.

Because remdesivir aims at viral enzymes it's unlikely to be toxic; the drug shouldn't, in theory, interfere with human versions of RNA polymerases. "But many of these small molecule drugs can also be processed by the kidney or liver, so we do have to watch out for side effects," Nadeau says.

Targeting Host Proteins

Concerns about side effects are greater with another group of medications: those that target host proteins that the virus needs to enter cells and cause disease. One such drug, the antimalarial chloroquine, was found to block SARS-1 from entering primate cells by modifying the host cell receptors that the virus needs to bind (8).

To understand how similar SARS-CoV-2 is to SARS-1, Stefan Pohlmann of the University of Göttingen in Germany and his colleagues studied how the new virus binds to host cells and initiates infections. They found that the two viruses used the same surface receptor, called Ace2, to enter cells and the same protein-cutting enzyme, known as a protease, to become infectious. "Coronaviruses need to be activated by having their surface proteins cleaved by a host cell enzyme," Pohlmann says. "Earlier, it was believed that viruses used several different proteases for their activation."

Recent studies have found that many clinically relevant viruses, including MERS, SARS, influenza A, and the new SARS-CoV-2, all rely on one particular enzyme, known as TMPRSS2 (9). "If this enzyme is hit, these viruses have a problem," Pohlmann says. Identifying broadly applicable targets such as TMPRSS2, says Pohlmann, is "exactly what's needed to prevent pandemics like this."

His team found that camostat mesylate, a molecule that inhibits TMPRSS2, could prevent SARS-CoV-2

from infecting cultured human lung cells. The drug is currently approved to treat chronic pancreatitis in Japan and might prove useful in patients who display clear symptoms but aren't in critical condition, Pohlmann says.

Krogan and his collaborators are also moving several of the drugs identified in their screen into clinical tests for COVID-19. Twenty-four of these drugs are approved for other indications, such as the common diabetes drug metformin, as well as others used to treat cancer, Parkinson's disease, and hypertension.

Immune Interventions

As is often the case with infectious diseases, the influence of the novel coronavirus on the human immune system is at times more deadly than the pathogen itself. COVID-19 patients, particularly those with severe infections, often experience a runaway immune response; inflammatory molecules cause a dangerous cytokine storm. Some drugs currently being tested, such as interferon- β or an interleukin-6 inhibitor (named tocilizumab), are immune regulatory molecules that may work to dial down this immune response.

Other trials are looking to recruit the help of the immune system. Recent trials have turned to antibodies generated by people who have successfully recovered from COVID-19 infections. Some hospitals have begun using plasma donated by such survivors to treat patients, and researchers at several hospitals have initiated trials to evaluate convalescent plasma as a treatment. It's not a new idea. This treatment was first attempted during the 1918 flu pandemic, and doctors have turned to it as a last-ditch means to counter measles, pneumonia, and other infections.

The treatment relies on antibodies that target the pathogen in question. Although it's not a cure—or a sustainable way to combat infections—it can serve as a stopgap measure to help critically ill patients. Early results suggest that the method may prove effective against COVID-19 (10). At least two startups, Chinese-US firm Bria Biosciences and south San Francisco-based Centivax, are isolating and developing antibody-based treatments.

These immune-targeting medicines may prove more effective in severe cases, because "by the time patients develop serious respiratory distress, an antiviral alone may be insufficient," says Paul Goepfert, infectious diseases researcher at the University of Alabama in Birmingham. But these immunosuppressants can also increase the risk of infection from other pathogens—a possibility that will need further tests, he adds.

Multiple Strategies

Thus far, evidence to support—or undermine—clinicians' use of remdesivir, lopinavir, and other drugs has only emerged from preclinical studies or very small

studies of human patients. Only data from randomized trials will reveal whether and how any of these medications should be recommended to COVID-19 patients. In the future, researchers may also be able to look back to examine clinical records and identify drug combinations or patients' responses to different treatment regimens.

One reason for the lack of data on these medications is that drugs developed for SARS never reached clinical trials, Goepfert notes. "When the SARS epidemic died down, ideally we should have kept going with drug development," he says. "But nobody was willing to fund it so the research died down too."

Now, Gilead has also rushed to scale-up remdesivir production to match global needs. The process usually requires a sequence of sensitive chemical reactions, many needing novel substrates. Because the drug is given intravenously, production has to occur in specialized sterile conditions. On April 4, the company announced that they had increased available amounts and reduced production time by months.

Eventually, clinicians will likely use both drugs that block the virus from multiplying as well as medications that inhibit host proteins that viruses hijack. Although virus-directed therapeutics are less likely to interfere with human metabolic pathways—and thus have fewer side effects—viruses can develop evasive mutations. This is less of a problem with therapies targeting host proteins.

However, host-directed drugs, such as chloroquine, must minimize toxic side effects that can arise from inhibiting cellular enzymes. These issues commonly occur when drugs that target human metabolism are used to treat chronic conditions, such as autoimmune diseases. But these drugs may be less problematic "if someone just needs a few days of treatment to help fight off an acute infection," Krogan explains. "In the short term, these drugs may help individuals who are in the most desperate need," he adds, "while we develop prophylactics and longer-term solutions such as vaccines."

Therapeutics aimed at host enzymes offer another perk: Their targets appear to be used by many viruses and, because they're essential human proteins, don't mutate quickly. So they don't just offer a path out of the current crisis—they might be the solution to avoiding the next one.

"As we've looked at maps of how these different viruses interact with human proteins, we see similar host machinery coming up again and again," Krogan says. It's a promising sign. "If we had a nontoxic treatment that targeted the human protein," Krogan adds, "this could be a treatment not just for COVID-19 but for something else that comes up down the line, including other viruses that we don't even know yet."

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