A CALL TO ARMS

Researchers are testing an arsenal of weapons against the pandemic coronavirus

By **Robert F. Service**

In March 2020, as the scope of the COVID-19 pandemic was coming into view, Jen Nwankwo and colleagues turned a pair of artificial intelligence (AI) tools against SARS-CoV-2. One newly developed AI program, called SUEDE, digitally screens all known druglike compounds for likely activity against biomolecules thought to be involved in disease. The other, BAGEL, predicts how to build inhibitors to known targets. The two programs searched for compounds able to block human enzymes that play essential roles in enabling the virus to infect our cells.

While SUEDE sifted through 14 billion compounds in just hours and spit out a hit, BAGEL made equally fast work of designing a lead. Nwankwo, CEO of a Massachusetts biotech startup called 1910 Genetics, asked a chemical company partner to synthesize the compounds. A week or so later, her team received the orders, added each compound in turn to human cells, and learned that each blocked its target and prevented viral entry into cells. 1910 Genetics is now looking to partner with antiviral drug developers to pursue animal and human trials. "It shows that AI can massively accelerate drug design," Nwankwo says.

ILLUSTRATION: STEPHAN SCHMITZ/FOLIO ART

Designing and developing a medicine is almost always painfully slow, regularly taking at least a decade. Many steps—such as animal studies, tweaking molecules to avoid side effects, and clinical trials—can't be accelerated. But the race toward new treatments against COVID-19 is off to a blistering start as researchers accelerate other parts of the search, deploying supercomputers, robots, synchrotrons, and every other tool they have to find and lab test possible medicines at speed. According to a biotech industry drug tracker, some 239 antiviral molecules against COVID-19 are under development, targeting multiple parts of the viral life cycle.

Antivirals have proved critical in fighting other infections such as HIV and hepatitis C. Such drugs will be vital in the struggle against the pandemic coronavirus, too, despite the ongoing rollout of COVID-19 vaccines. "We know not everyone will be able to take the vaccine or respond to it," says Mark Denison, a virologist at Vanderbilt University. Vaccines may also lose effectiveness as immune protection wanes or viral variants emerge. "So, continuing development of antivirals is critical," Denison says.

To date, most of that search has centered on "repurposed" compounds, antivirals originally developed to combat other diseases. (Other repurposed drugs, such as the steroid dexamethasone, target the body's reaction to infection rather than the virus itself.) "Drug repurposing made sense as the first thing to try," Nwankwo says. Many repurposed antivirals have shown promise against SARS-CoV-2 in cell and animal studies and are now in clinical trials. One, remdesivir, has already proved to speed recovery by a few days in very ill people. But several other repurposed antivirals have failed to prove effective.

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As a result, says Francis Collins, director of the National Institutes of Health (NIH), "We really, really need a bunch more [antivirals]." Buoyed by almost daily advances in understanding SARS-CoV-2, the rapidly growing list of new compounds that might block it, and ongoing clinical trials—some of them late stage—Denison and others hope to deliver effective drugs this year. Says Andrew Mesecar, a structural biologist from Purdue University: "I am confident we will have more treatments for coronavirus."

AS VIRUSES GO, SARS-CoV-2 is a behemoth, with some 30,000 letters of RNA in its genetic code. Those letters encode 29 viral proteins that enable the virus to infect cells, reproduce, escape, and spread. "We're fortunate this virus has provided us with so many targets, so many opportunities for intervention," says Sandra Weller, a molecular biologist at UConn Health.

The 29 proteins come in three main categories: structural proteins that make up the outer coat; nonstructural proteins (NSPs), most of which help the virus replicate; and accessory proteins, several of which appear to subdue the host's immune response. Thus far, drug hunters have taken aim mainly at the structural and replication proteins, concentrating on molecules similar to those that have paid off in fighting other viruses.

SARS-CoV-2 has just four structural proteins. The envelope and membrane proteins make up the virus' spherical shell, and the nucleocapsid protein shields its genome. The fourth protein, spike, protrudes from the shell, creating the crown of thorns that gives the virus its name and enables it to bind to angiotensin-converting enzyme 2 (ACE2) receptors, its main entry point into cells.

Spike is the primary target of many vaccines and antivirals. However, small molecules, the typical focus for drug discovery programs, won't work because they aren't bulky enough to prevent spike from binding to the ACE2 receptor.

David Baker, a computational biologist at the University of Washington, Seattle, and colleagues turned instead to miniproteins, each with about 60 amino acids, customized to block protein-protein interactions. In late 2020, Baker's team described miniproteins tailormade to bind tightly to the virus' spike protein and block it from attaching to the ACE2 receptor (*Science*, 23 October 2020, p. 426). The tiny proteins kept the virus from infecting human cells in a test tube, and Baker says miniproteins could make ideal drugs because they are far more stable than conventional protein therapeutics, such as antibodies, which must be refrigerated. Baker is in discussions with drug companies to pursue his leads.

Other researchers have a different strategy for interfering with viral binding. They are designing ACE2 look-alikes to serve as decoys, drawing SARS-CoV-2 away from cells. Researchers at Neoleukin Therapeutics and their partners, for example, reported creating a miniprotein, CTC-445.2d, that mimics ACE2, binding tenaciously to spike (*Science*, 4 December 2020, p. 1208). The compound protected human cells from infection in vitro. When given to hamsters in a nasal spray, the decoy also prevented them from getting severe disease after they received a normally lethal dose of the virus. Another "receptor trap" molecule, described in November 2020 in the *Proceedings of the National Academy of Sciences*, also diverted SARS-CoV-2, keeping it from infecting cells in the test tube.

AFTER ENTERING A CELL, a virus transforms its host into a virus factory. That's where SARS-CoV-2's NSPs come in. The viral proteins are made by the host cell's own protein factories, the ribosomes, which translate the viral RNA into two long "polyprotein" chains. The chains spin off two smaller proteins, NSP3 and NSP5, protein-cutting protease enzymes that then chop up the rest of the polyproteins into independent, functioning proteins.

"These are absolutely critical functions that are highly conserved and should be very, very vulnerable" to antivirals, Denison says.

Drugs that block proteases have successfully fought HIV and hepatitis C and have been among the most popular candidate antivirals for SARS-CoV-2. Two repurposed protease inhibitors for treating HIV, lopinavir and ritonavir, showed promise in vitro against SARS-CoV-2, but in October 2020, the United Kingdom's large Recovery clinical trial reported they offered no benefit.

Researchers at the drug giant Pfizer are pursuing an inhibitor that may work better

because it is designed to target NSP5, a protease that is specific to SARS-CoV-2 and its coronavirus relatives. Pfizer scientists developed the drug in 2003 to block the molecule, also known as main protease (Mpro), in severe acute respiratory syndrome (SARS), the deadly coronavirus that emerged the previous year. That work was set aside when the SARS epidemic died out. Now, Pfizer has pulled the compound off the shelf and found that it stops SARS-CoV-2 from reproducing inside human cells. Pfizer researchers tweaked the structure to make a more soluble version, known as PF-07304814. They showed that it sharply reduced viral load in mice; in other animals, high concentrations of the drug could reach tissues.

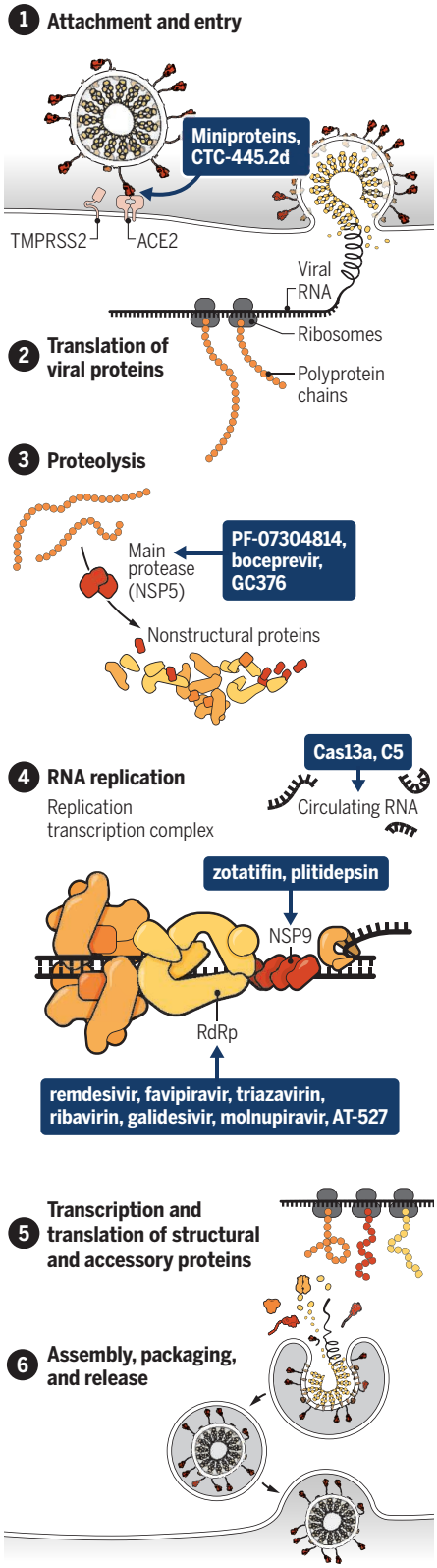
"It's a promising lead," says Celia Schiffer, a molecular biologist with the University of Massachusetts Medical School. In September 2020, Pfizer launched a small clinical trial to test the safety of PF-07304814, delivered intravenously. But Annaliesa Anderson, who leads Pfizer's antiviral program, says recruiting volunteers has been difficult. "Patients are either very sick, and it might be too late [to inhibit viral replication], or they might not feel that bad," making an intravenous therapy less appealing. Given the slow recruitment, she expects results toward the end of this year.

Other researchers are also working on Mpro inhibitors. In *Nature Communications* in September 2020, researchers in China reported on two repurposed drugs, boceprevir and GC376. Boceprevir is a hepatitis C drug, whereas GC376 was designed to target a feline coronavirus. Both compounds slowed SARS-CoV-2 replication in cells. On 5 February, U.S. researchers reported in a bioRxiv preprint that most mice given GC376 after receiving a lethal dose of the pandemic virus survived. And in August 2020 in *Science Translational Medicine*, U.S. researchers described a GC376 analog that dramatically boosted survival rates in mice infected with Middle East respiratory syndrome and showed potent antiviral effects against SARS-CoV-2 in cells.

Other molecules designed specifically to inhibit SARS-CoV-2's Mpro remain at an earlier testing stage. In November 2020, for example, Charlotte Lanteri, a research microbiologist at the Walter Reed Army Institute of Research, reported discovering 807 Mpro inhibitors through an AI screen of 41 million compounds. Her team has identified seven as particularly promising, but turning one or more of those into drugs remains at least a couple of years away, says Lanteri, who reported the findings at an antiviral drug summit at NIH. She and colleagues are also looking for antiviral drugs effective against all coronaviruses. "We want to be prepared as much as possible for the next emerging threat," she says.

Antivirals take aim

As it infects cells, reproduces itself, and spreads, the pandemic coronavirus relies on dozens of viral and host proteins. They offer an array of targets for candidate drugs (blue boxes) that aim to block proteins key to different stages in SARS-CoV-2's life cycle.



AFTER SARS-COV-2'S proteases have freed coronavirus proteins from the original chains, 15 of them come together to form the replication transcription complex (RTC), which copies the virus' RNA genome to make new viruses (see graphic, left). Central to that machinery are NSP9, which locks onto the virus' RNA strand, and the RNA-dependent RNA polymerase (RdRp) that copies the RNA.

The RTC's critical role has made it, and RdRp in particular, the most popular treatment bull's-eye of all. It's where remdesivir does its work. The drug is a nucleoside analog, a sort of imitation RNA building block that resembles adenosine (A), one of the four letters that make up RNA. The imposter tricks the RdRp into inserting remdesivir molecules instead of A's into growing RNA strands, jamming RdRp and stopping viral replication.

Researchers hope other repurposed nucleoside and nucleotide analogs will be better at fooling the coronavirus' RdRp. (Nucleotides are nucleosides with one or more phosphate groups added.) The candidates include favipiravir and triazavirin, both originally designed to combat flu viruses; ribavirin, a treatment for respiratory syncytial virus and hepatitis C; and galidesivir, which can block replication of Ebola, Zika, and yellow fever viruses.

Researchers are guardedly optimistic about molnupiravir, a nucleoside analog that can be taken as a pill and was originally developed to combat influenza. Last year, concerns swirled around the drug after a whistleblower criticized what he saw as an improper effort to steer federal funding to it. (*Science*, 13 May 2020). But positive results have kept progress on track.

Early work showed molnupiravir inserts itself into RNA in place of the nucleoside cytidine, prompting errors in the copying process and causing a lethal buildup of mutations in the virus. That mechanism has raised worries that the drug might cause similar mutations in host cells. But Richard Plemper, a cell biologist at Georgia State University, says such problems have not been seen in animal studies.

In April 2020 in *Science Translational Medicine*, Denison and colleagues reported that in mice, molnupiravir sharply reduced replication of multiple coronaviruses, including SARS-CoV-2; the drug also cut SARS-CoV-2 replication in human airway epithelial cells. A *Nature* paper added to the encouraging data in February, showing the compound decreased viral replication 100,000-fold in mice engineered to have human lung tissue.

In December 2020, Plemper and colleagues reported in *Nature Microbiology* that molnupiravir might do more than just prevent symptoms. The researchers gave the drug to ferrets, which readily spread the coronavirus,

GRAPHIC: V. ALTOUNIAN/*SCIENCE*

and transmission fell to zero within 24 hours. "This is the first demonstration of an orally available drug to rapidly block SARS-CoV-2 transmission," Plemper says. That's crucial to slowing the spread of disease. Because it is a pill, molnupiravir can be given early in the disease cycle, just when SARS-CoV-2 replication typically peaks, in contrast to injectable drugs such as remdesivir. "We want to start treatment early and prevent people from ever showing up in a hospital," Plemper says.

The same month, a medRxiv preprint reported that a small safety trial showed the drug was well tolerated, with no serious side effects in healthy volunteers. Molnupiravir is now in phase 2/3 clinical trials run by Merck and Ridgeback Biotherapeutics; in March, scientists reported at a meeting that molnupiravir reduced patients' viral levels.

AT-527, another oral nucleoside analog developed to treat hepatitis C by Atea Pharmaceuticals and Roche, is also in a phase 2 clinical trial against COVID-19.

Pharmaceutical researchers use high-speed robotics and other tools to speed the search for antivirals.

Scientists are trying to shut down other RTC proteins, too. In recent results, two compounds—zotatifin and plitidepsin—appear to block viral replication by interfering with NSP9, the RNA-grabbing enzyme. Plitidepsin is in a phase 2/3 trial by the Spanish drug company PharmaMar. At least three other NSPs are considered good targets, says Tomáš Cihlář, a virologist with Gilead Sciences, maker of remdesivir.

Eventually, drugs could target the coronavirus' RNA, not just its proteins. In *Nature Biotechnology* in February, Emmeline Blanchard, a biomedical engineer at the Georgia Institute of Technology, and colleagues reported creating a polymer-encased formulation of a gene-editing enzyme called Cas13a that seeks out and chops up snippets of SARS-CoV-2 RNA. The team's Cas13a enzyme targets highly conserved regions of two viral genes encoding the RdRp enzyme and the nucleocapsid protein. When hamsters infected with SARS-CoV-2 inhaled a vaporized formulation of the drug, it reduced viral replication and disease symptoms.

And in September 2020 in *ACS Central Science*, Matthew Disney, a chemist at the Scripps Research Institute, and colleagues reported discovering a compound called C5 that blocks a short, hairpin-shaped segment of RNA involved in SARS-CoV-2 replication. "We have other segments of the viral genome we think we can target as well," he says.

BECAUSE SARS-COV-2 relies on a host cell's proteins to reproduce, disrupting those proteins could be another avenue to treatments—with the advantage that not targeting the virus directly could lower its odds of becoming resistant to the drugs. Their targets include host cell proteases TMPRSS2 and furin, which the candidate drugs from Nwankwo's team block. Last month, NIH announced it was launching a phase 2/3 trial for camostat mesilate, another TMPRSS2 inhibitor.

Another target is a protein called dihydroorotate dehydrogenase (DHODH). It's the linchpin in a pathway that cells use to make two of RNA's four bases when they need extra RNA—for example, when proliferating. Viruses hijack that pathway to replicate. In cell studies, blocking DHODH has halted cancer and viral diseases, such as influenza and cytomegalovirus. And DHODH blockers have thus far proved safe when tested in hundreds of patients.

Two biotech companies, PTC Therapeutics and Immunic Therapeutics, are trying the same strategy against SARS-CoV-2. Rapidly reproducing viruses "have a great need for RNA," says Marla Weetall, vice president of pharmacology with PTC Therapeutics. The company's compound, PTC299, was originally designed as an oral drug to halt cell proliferation in acute myeloid leukemia. In an August 2020 preprint on bioRxiv, Weetall and colleagues reported that PTC299 sharply inhibited SARS-CoV-2 replication in cells. The compound also blocked the production of immune molecules that cells build using RNA bases, hinting that PTC299 might help tame the immune overreaction seen in severe COVID-19.

Daniel Vitt, CEO of Immunic Therapeutics, says his company has also seen promising results in human trials of its oral compound, IMU-838, developed to treat inflammatory and autoimmune diseases. In February, the company reported preliminary results suggesting hospitalized patients on the drug had less need for ventilators. Trials continue for both companies.

ULTIMATELY, no one compound is likely to deliver a knockout punch to the pandemic coronavirus, in part because drug-resistant viruses are likely to emerge. Collins and others argue that the best strategy takes a page from the treatment for HIV and hepatitis C: mixing and matching antivirals aimed at several proteins, making it harder for the virus to evolve multiple workarounds at once. "We really need an arsenal," says Lillian Chiang, CEO of Evrys Bio, which is working on antivirals against host-cell proteins.

"This is going to take time," says Michael Sofia, a chief scientific officer with Arbutus Biopharma, a Canadian antiviral company. And money. According to recent estimates, bringing a new drug to market costs between $985 million and $2.8 billion. Anderson says Pfizer, for one, is committing company resources to defeating the pandemic without expectation of profit. Other companies say the same. But during previous lulls in infectious disease outbreaks, many drug companies abandoned work on antivirals. "As soon as this stops being a hot area, people will move on," Nwankwo says.

In another disincentive, antiviral treatments for SARS-CoV-2 might be given for only a week or two, giving drugmakers a narrow window to reap returns. As a result, Denison and others argue that more government support is needed to keep stocking the antiviral arsenal. Any lull in the battle against SARS-CoV-2 and its kin is likely to be temporary, they say.

"We are going to have another coronavirus," Mesecar says. "We just don't know what it will look like." ■

PHOTO: SCRIPPS RESEARCH

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