ANTOINE DORÉ

THERAPEUTICS

A bigger arsenal

Understanding how the influenza virus replicates inside the body is helping researchers develop a wider range of antiviral drugs.

BY NEIL SAVAGE

In 2004, Rick Bright was looking for a new project. As an immunologist then at the US Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, he had learned about a new, faster method of sequencing viral genomes. He decided to use it to test whether the influenza A virus was developing resistance to adamantanes, which at the time were the main antiviral drugs used to treat flu.

Bright collected samples of the flu virus and tested them for an altered amino-acid sequence known to confer resistance. To his surprise, every virus in his sample had the mutation. Bright took his results to the CDC's director, Julie Gerberding, who was sure he must be mistaken and told him to run the tests again.

Some 25,000 samples later, Bright came to a sobering conclusion. Nearly all the viruses in circulation around the globe had a mutation that rendered amantadine and rimantadine — the two adamantanes used to treat flu, which work by blocking a particular step in viral replication — completely useless. In January 2006, Bright and Gerberding held a press conference to issue new guidelines: do not use adamantanes to treat flu because they will not work.

Fortunately, by that time a second class of flu antivirals had been introduced that attack a different mechanism used by the virus to reproduce. These drugs — oseltamivir, zanamivir and, more recently, peramivir — remained the only drugs for treating flu until 2018 when the United States and Japan approved baloxavir, which targets a third part of the viral life cycle. But the arsenal of drugs to combat flu remains limited and there has been evidence of resistance to all of them, although it is not yet widespread. To be effective, each drug must be given within two days of symptoms appearing.

Researchers around the globe are working to develop further antiviral therapies for flu. They are searching for drugs that attack different parts of the virus's reproductive cycle, and are exploring whether the combination of two or more drugs might lead to faster recovery, reduce the development of resistance, or both. They hope that by the time the next pandemic comes around, they will have better weapons to fight this deadly disease.

VITAL ANTIVIRALS

Much of the attention paid to fighting flu is aimed at vaccination (see pages S50 and S60) but antiviral drugs such as baloxavir have a crucial role in reducing illness and death from flu, says Bright, who now directs the Biomedical Advanced Research and Development Authority (BARDA). BARDA funds research into treatments for various diseases and health threats, including flu. "Vaccines get all the marquee lights," Bright says, "but we can't vaccinate everyone, and the vaccines don't offer full protection to everyone. So there's a lot of room for effective therapeutics."

The first antiviral drug, amantadine, was approved by the US Food and Drug Administration (FDA) back in 1966. It works — or rather, it used to until viruses developed resistance — by blocking the virus's M2 proton channels, which the virus uses to release its RNA for replication by a host cell.

M2 blockers were the only way to interfere with the flu virus until 1999, when the oral drug oseltamivir and the inhaled drug zanamivir won FDA approval. These drugs inhibit neuraminidase, an enzyme that allows viruses to escape from one cell and spread to others. Oseltamivir, marketed as Tamiflu, has become the standard flu treatment in most countries. Another neuraminidase inhibitor, peramivir, which is administered intravenously, has been

approved for use in the United States, Japan and South Korea.

The latest addition to the antiviral arsenal, baloxavir, targets a third component of viral reproduction: the enzyme polymerase, which controls the transcription and replication of viral RNA. Baloxavir inhibits transcription by preventing the virus from commandeering the host cell's manufacturing facilities. Normally, in a process known as cap snatching, the virus steals a short string of the host cell's RNA and attaches it to its own RNA, tricking the cell into duplicating it. Baloxavir blocks the part of the polymerase that assists in this cap snatching.

Although baloxavir is available in Japan and the United States, it has yet to be approved by the European Medicines Agency. One appealing aspect of baloxavir is that it requires just one oral dose compared with ten doses over a five-day period for oseltamivir.

FRESH TARGETS

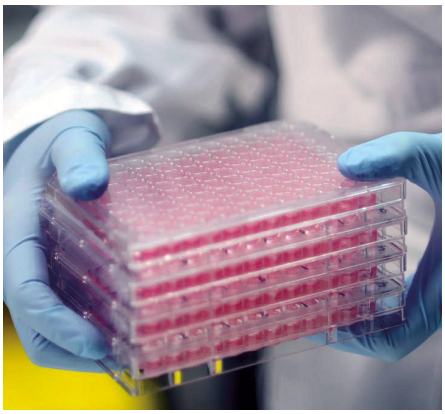
To expand the treatment options, researchers are broadening their search to find a range of different targets. Jun Wang, a pharmacologist at the University of Arizona in Tucson, has his eyes on several. His main approach has been to target the mutation in the M2 channel that created resistance to amantadine and rimantadine. One particular mutation, dubbed AM2-S31N, confers resistance in more than 95% of influenza A viruses. Amantadine blocks the process by which viral RNA is released into the host cell, and the mutation provides a new channel through which the virus can release its RNA.

"We know the mutation," Wang says. The question now is whether new drugs can be developed to target it. "If we can do that then we can treat current viral infections," he adds. So far, Wang has found a molecule that blocks the new channel in cells in his laboratory. He now aims to study it in mice.

Another one of Wang's projects, which is still at an early stage, also focuses on viral polymerase but has a different target to baloxavir. Polymerase consists of three parts that must work together. Wang has found several compounds that seem to block the assembly of the enzyme, rendering it useless and stopping the virus in its tracks. The beauty of this approach, he says, is that the virus is unlikely to get around the blockage with a single mutation.

Wang's drug candidates bind to one component of the polymerase, PA_C, and prevent it from binding to a second component, $PB1_N$. A single mutation could be enough to stop the drug binding to the target, Wang explains, but that mutation would probably mean that the enzyme's components would no longer fit together. "It still will not be able to assemble," he says, because there would need to be a second mutation to allow the reshaped piece of the enzyme to bind to the other parts.

The polymerase complex is an attractive target for antivirals because it is highly conserved — it does not change much as the virus evolves. Being highly conserved is usually a clue that something is vital to the functioning of an organism, as it is less likely to successfully mutate. In addition, Wang's compounds and baloxavir target different parts of the polymerase complex, so together they might be able to cripple the virus more effectively than either could alone.



MATTHIEU YVER, EQUIPE VIRPATH, UNIV. CLAUDE BERNARD

Plates of cells infected with the influenza virus are used to test antiviral drugs.

A third project in Wang's lab that is at an early stage focuses on haemagglutinin, a surface protein that allows the virus to bind to a cell. "It's an easy target, but it's also a really difficult one," Wang says, because its main part, the head, mutates readily, letting it evade attackers. As a result, drugs targeting haemagglutinin might be most effective when used in combination with other drugs.

Different groups of researchers have tried to target the stem of haemagglutinin, as this is more conserved than the head. Scientists at Scripps Research Institute in La Jolla, California, and the pharmaceutical company Janssen Research and Development, based in Raritan, New Jersey, found a small molecule that, like an antibody, could bind to the stem of haemagglutinin. When they gave it to mice that had been infected with 25 times the lethal dose of flu, all of them survived. But Jason Chien, who leads Janssen's research and development team for respiratory infections, says that although the project was scientifically useful, the molecule was effective only against type A influenza, not type B, so the company will not be pursuing it.

"At least in theory it's a very interesting and very quick strategy to propose new drugs."

Chien says that teams at Janssen are studying other potential antivirals in the lab but he declined to disclose details. The company is, however, conducting two phase III clinical trials on pimodivir — one using hospitalized patients and one involving outpatients at high risk of complications. Pimodivir inhibits yet another aspect of the polymerase complex, and if approved it will expand the class of drugs now dominated by baloxavir.

CHECKING THE MEDICINE CABINET

Instead of developing new drugs to target flu, researchers in France are scouring databases of known compounds to see whether any might make effective treatments. "At least in theory it's a very interesting and very quick strategy to propose new drugs," says Olivier Terrier, a virologist at the International Centre for Infectiology Research in Lyon.

Terrier and his colleagues used a database known as the Connectivity Map (CMap), developed by the Broad Institute of Massachusetts Institute of Technology and Harvard University in Cambridge, Massachusetts. The CMap contains gene-expression profiles that are produced when cells are exposed to various drugs. First, the Lyon team developed a profile of how a cell's gene expression is affected by a flu virus — "a fingerprint of infection", as Terrier calls it. Then they combed through CMap looking for drugs that produce a mirror image of that fingerprint. If, for example, the virus causes a particular gene to express less of a certain protein, they looked for a drug that leads it to express more. They hope that a drug that produces an effect opposite to that of the virus could potentially be used to counteract the flu.

The team screened 1,309 FDA-approved molecules and found 35 that looked promising. Of these, 31 showed antiviral activity in viruses swabbed from the nasal passages of people with flu. Studies in mice narrowed the search to just one candidate, the calcium-channel blocker diltiazem, which is normally used to treat hypertension. The researchers founded a company in Lyon, Signia Therapeutics, which is running a phase II clinical trial on the drug. The drugs are already FDA approved, Terrier says, which could shave years off the process for getting them to flu patients.

Other researchers are trying to use antibodies to fight flu. A group at the Liverpool School of Tropical Medicine (LSTM), UK, and Imperial College London attached extra sialic acids to part of an antibody. The flu virus normally infects cells in the lungs by binding through its haemagglutinin and neuraminidase proteins to sialic acid on the surface of lung cells. But when the virus encounters antibodies covered in sialic acids, it binds to those instead, stopping it attaching to the lung cells. Richard Pleass, a virologist at LSTM, says that a treatment based on these antibodies could act as a prophylactic for hospital staff, slowing the spread of flu.

Despite the number of approaches to new flu treatments, it can take years to take a drug from the lab to the clinic. But Wang is confident that an expanded array of antivirals is on the horizon. "We're getting there," he says. "Within the next few years we will definitely see a few other new flu drugs on the market." ■

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