Credit: NiseriN/iStock/Getty Images Plus

A tale of two antiviral targets — and the COVID-19 drugs that bind them

The FDA is considering authorizations for Pfizer's paxlovid and Merck & Co.'s molnupiravir, the first two oral COVID-19 antivirals.

Megan Cully

COVID-19, for all of the problems it has created, has shown the unprecedented speed with which drug developers can move. Vaccines and bespoke antibodies were among the first responders on the COVID-19 scene, authorized just under 11 months from the release of the SARS-CoV-2 sequence. Now oral antivirals, from Pfizer and Merck & Co., are set to make their mark.

Pfizer's contender, paxlovid, went from a compound in a freezer and optimization ideas on a drawing board to a regulatory submission in just 20 months. "It was a quick journey," says Mikael Dolsten, Pfizer's chief scientific officer. "In the normal development of a small-molecule programme, that would take 8–10 years," he notes.

Merck's molnupiravir was already a preclinical candidate when COVID-19 struck, but also a beneficiary of a development sprint.

The FDA is now considering both drugs for emergency use authorization (EUA). An FDA advisory committee voted 13 to 10 in favour of an EUA for molnupiravir on 30 November, preparing the way for an approval decision in the coming weeks. The EMA is reviewing molnupiravir too, and an application from Pfizer there is imminent.

Preliminary data suggest that both offer benefits over Gilead's remdesivir — an intravenously injected antiviral that secured an EUA in May 2020 and full FDA approval in October 2020 for hospitalized patients. This antiviral had already been derisked in clinical trials before the pandemic started. It is not widely used outside the USA.

The arrival of oral antivirals that can be used in non-hospitalized patients is raising the hopes of infectious diseases experts.

"It's great to see that the antivirals have proven to be effective," says Peter Horby, co-lead on the multi-drug UK RECOVERY trial, which has examined multiple potential COVID-19 therapies. Oral antivirals could provide a cheaper and easier-to-administer option than the antibodies that have already been authorized for non-hospitalized patients. Shortages of anti-COVID-19 antibodies have caused political tension in the USA, where the government doled out about 2.4 million doses last year. The oral antivirals may also be less susceptible to resistance-causing mutations, adds Horby. Antibodies target Spike, the protein that SARS-CoV-2 uses to gain access to human cells, which is tolerant of mutations. The antivirals target components of the viral replication process that are less tolerant to change, he explains. The recently identified Omicron variant, for example, has more than 30 mutations in Spike, but only one in each of the proteins targeted by paxlovid and molnupiravir.

Vaccines, these new antivirals, and existing therapies for hospitalized patients will complement one another. "We always thought that having both a highly effective vaccine and a very accessible, convenient, time-effective oral would offer unprecedented opportunity" to prevent COVID-19-related deaths, says Dolsten.

Primed and ready to go

As soon as the SARS-CoV-2 genome was published, researchers began analysing the virus's 29 proteins for vulnerabilities. Following the lead from other viral diseases, many researchers started by focusing on the viral replication machinery. Pfizer's paxlovid is the most advanced of the main protease (Mpro) inhibitors.

During viral replication, SARS-CoV-2 synthesizes long polypeptides that must be cleaved into its constituent viral proteins. Mpro carries out most of this cleavage. Inhibiting Mpro thus prevents the virus from generating the proteins it needs to replicate.

"Mpro is a great target because it's kind of the Achilles heel of the virus," says Rolf Hilgenfeld, an antiviral researcher from the University of Lübeck, Germany.

Decades of work on viral proteases positioned the community to move quickly. "We had all the reagents ready to clone the main protease when the sequence was published on January 10, 2020," recalls Hilgenfeld, who had previously solved the crystal structures of Mpros from various coronaviruses including SARS-CoV-1. Within a month of the release of the SARS-CoV-2 genome, his team had solved the crystal structure of SARS-CoV-2's Mpro, alone and bound to an antiviral they called 13b.

Pfizer also leveraged previous work on SARS-CoV-1, making the most of an antiviral called PF-00835231. This antiviral

> It was a quick journey. In the normal development of a small-molecule programme, that would take 8–10 years

is based on rupintrivir, a compound that mimics the peptide substrate of the human rhinovirus protease. Rhinovirus and coronavirus proteases aren't particularly similar, but both cleave their substrate after Gln residues. Part of rupintrivir mimics Gln. Pfizer had modified rupintrivir to make it a better fit for SARS-CoV-1's Mpro; the resulting PF-00835231 binds covalently to the protease's active site, inactivating the enzyme.

Luckily, SARS-CoV-2 Mpro and SARS-CoV-1 Mpro are highly homologous, a 96% match.

Pfizer was therefore able to quickly advance both oral and intravenous SARS-CoV-2 Mpro inhibitors. PF-07304814, the intravenous contender, is the phosphate prodrug form of PF-00835231. PF-07321332, the oral candidate, has added substructures from the HCV protease inhibitor boceprevir and additional optimizations for oral bioavailability. To make paxlovid, Pfizer combined PF-07321332 with ritonavir, an HIV drug that inhibits cytochrome P450 and slows the metabolism of protease inhibitors.

This work was done by a "nimble SWAT team," says Dolsten. "600 compounds, 80 co-crystals, structure-based drug discovery, and a candidate 8 months later," Dolsten summarizes. Pfizer launched a phase I trial of paxlovid in March 2021, and a phase II/III trial was underway by July. Interim results from this phase II/III trial form the basis of Pfizer's EUA submission. The trial will finish in early 2022. The company is also pressing on in people with low risk of progressing to hospitalization, and for prophylactic use.

In a top-line interim analysis of paxlovid in 1,219 patients, treatment reduced hospitalization or death by 89% when administered within 3 days of symptom onset. These results form the basis of Pfizer's EUA application.

Other SARS-CoV-2 Mpro-targeting compounds are also in development, several explicitly designed for this pandemic (TABLE 1). Like Pfizer's compounds, most of these mimic the peptides cleaved by Mpro, covalently binding to cysteine residues in the active site to inhibit the enzyme.

Broad-spectrum benefits

Merck focused on the viral RNA-dependent RNA polymerase (RdRp), a viral enzyme that synthesizes RNA — both for translation into viral proteins and for generating copies of itself. RdRp "offers more opportunities for broad-spectrum antivirals," says Hilgenfeld. Their structures are comparatively well conserved across viral classes, he explains.

Gilead's remdesivir, an RdRp inhibitor, tapped this broad-spectrum potential. Discovery work on this programme started in 2009, during a hunt for antivirals for hepatitis C virus and respiratory syncytial virus. Gilead first advanced it into clinical trials for Ebola virus in 2015, but it failed there. With the arrival of COVID-19, it found new life.

Table 1 | **Selected COVID-19 antivirals in development**

Drug	Company	Delivery	Status	Origin
RdRp inhibitors				
Remdesivir	Gilead	IV	Approved	Repurposed EBV candidate
Molnupiravir	Merck & Co.	Oral	EUA submitted	Repurposed VEEV candidate
Clevudine	Bukwang	Oral	Phase II	Repurposed HBV drug
JGL-2020	Gilead/Jubilant	Oral	Phase I done	Oral remdesivir
ODBG-P-RVn	UCSD	Oral	Preclinical	Oral remdesivir
GS-621763	Gilead/Georgia State University	Oral	Preclinical	Oral remdesivir
Mpro inhibitors				
PF-07321332 (paxlovir)	Pfizer	Oral	EUA submitted	SARS-CoV-2 optimized
S-217622	Shionogi	Oral	Phase III	SARS-CoV-2 optimized
PF-07304814	Pfizer	IV	Phase I done	SARS-CoV-1 candidate prodrug
PBI-0451	Pardes	Oral	Phase I	SARS-CoV-2 optimized
EDP-235	Enanta	Oral	Phase I planned	SARS-CoV-2 optimized
13b	University of Lübeck	Inhaled	Preclinical	SARS-CoV-2 optimized

EBV, Ebola virus; HBV, hepatitis B virus; UCSD, University of California San Diego; VEEV, Venezuelan equine encephalitis virus.

> 600 compounds, 80 co-crystals, structure-based drug discovery, and a candidate 8 months later

Not all RdRp inhibitors work the same way. In most cases, including with remdesivir, viruses incorporate the drug into the elongating RNA, and this brings the elongation process to a halt. Molnupiravir's mechanism is different: elongation doesn't stop when the enzyme incorporates the drug into viral RNA. Instead, the virus reuses molnupiravir-containing RNAs as template strands, and incorporates the wrong bases into new viral RNA when it re-encounters molnupiravir. Mutations accumulate over cycles, leading to 'error catastrophe' and viral death.

"It's actually quite interesting that we're still learning new ways to inhibit virus replication through the use of nucleoside analogues," says Daria Hazuda, vice president of infectious diseases and vaccine discovery at Merck.

Molnupiravir itself originates from George Painter's laboratory and Drug Innovations at Emory University. In 2013, Painter was looking for a drug to treat Venezuelan equine encephalitis virus (VEEV), a mosquito-borne pathogen that is on the rise in the southern USA. By targeting RdRp, Painter hoped to develop a broad-acting antiviral for RNA-encoded viruses. He chose to explore nucleoside analogues because they are generally potent, have high barriers to resistance, and can often be made orally available.

As the pandemic was kicking off, Painter and colleagues reported that molnupiravir had broad antiviral activity against SARS-CoV-2, MERS-CoV, SARS-CoV-1 and influenza in preclinical models of disease. Emory licensed the therapy to Ridgeback Biotherapeutics in March, and Merck secured exclusive worldwide rights to develop and commercialize the drug just two months later.

In keeping with the broad-spectrum potential of RdRp inhibitors, molnupiravir didn't need any COVID-19-specific refinements before it entered phase I trials in April 2020. Merck began the phase II/III MOVe-OUT trial in October 2020, and in October 2021 submitted interim results from this trial to the FDA for EUA. Merck, too, is testing whether its drug can be used prophylactically.

> It's actually quite interesting that we're still learning new ways to inhibit virus replication through the use of nucleoside analogues

Top-line results from molnupiravir in 1,433 patients show the drug reduced hospitalization or death by 30% when it was administered within 5 days of symptom onset. Rosier interim results, showing a 48% reduction, analysed fewer people; molnupiravir didn't have an edge in the second half of the trial.

In the FDA's advisory committee meeting on the drug, experts voted narrowly in favour of authorization. But 10 panellists felt that the drug's benefits did not justify its risks.

Because molnupiravir induces errors in viral RNA, one key concern is that it could theoretically speed up the evolution of this viral foe. This risk is greatest in immunocompromised patients, who may incubate and shed virus for longer, and in patients who take lower doses or shorter drug courses than prescribed. Merck's counterargument — that the virus was undetectable at the end of treatment in the clinic, suggesting that mutant viruses that arise are quickly cleared — did not convince all the committee members.

Another issue is that molnupiravir might be incorporated into human DNA, causing mutations in rapidly dividing human tissues including fetuses. These mutagenic concerns are not new: Pharmasset reportedly abandoned development of structurally related compounds in 2003 due to their mutagenic potential.

Molnupiravir causes mutations in the Ames test, which assesses mutagenic potential in bacterial cells. One follow-up study in mice produced inconclusive results, and another showed that the drug was not mutagenic in whole animals, Merck showed. Some committee members questioned Merck's models and controls.

Molnupiravir also induced fetal mutations in rats. "The FDA should not approve it for pregnant women except in exceptional circumstances," summarized David Eastmond, a toxicologist at the University of California and an FDA advisory committee member.

Other RdRp inhibitors, mostly repurposed drugs, are in clinical development (TABLE 1).

The early antiviral

If authorized, Pfizer and Merck's drugs could dramatically help alter the course of the pandemic.

Both drugs would likely be rolled out in outpatient settings, in both vaccinated and unvaccinated non-hospitalized patients. The USA has already committed to buying 10 million courses of Pfizer's drug and 1.7 million courses of Merck's — paying around US$530 to $700 per course, respectively — pending approval.

In sicker, hospitalized patients, however, these drugs might be less effective. Merck halted a clinical trial in hospitalized patients after an interim analysis. "The earlier you treat the better," says Hazuda. "Once somebody has an illness that requires hospitalization, all the evidence would suggest that most of that disease is no longer being driven by viral replication," she explains. At that point inflammatory responses, like those dampened by the anti-IL-6 receptor antibody tocilizumab, dominate. In Horby's RECOVERY trial, tocilizumab reduced death and the time those who recovered spent in hospital.

But Horby was still surprised when Merck halted its pivotal trial of molnupiravir in hospitalized patients after treating only around 300 patients. "That's a pretty small study to ditch clinical development in hospitalized patients, given that we have shown proof of principle for antivirals in hospitalized patients," he says.

Remdesivir, authorized only for inpatient use, may meanwhile be more effective if administered to outpatients. A trial of the intravenous drug in 562 non-hospitalized patients showed that treatment reduced hospitalization or death by 87% when given within 4 days of a COVID-19 diagnosis and within 7 days of symptom onset. In the hospital, by contrast, it reduces the time to recovery but does not offer a statistically significant effect on survival.

Gilead and others are working on oral formulations of remdesivir and remdesivir derivatives, which could address the practical challenges of using this drug in the community.

The need for early treatment may partly also explain the lacklustre impact of Roche's oseltamivir for the treatment of flu. Flu patients "feel bad for a couple of days, only then go to the doctor and get a prescription for Tamiflu [oseltamivir]," says Hilgenfeld, but "by then it's already too late."

Early intervention — in the first 3–7 days — depends on access to early testing. The availability of COVID-19 testing thus provides an important lever for the up-and-coming antivirals.

Shoulders for the future

Just as drug candidates that were developed for the original SARS were key starting points for the development of COVID-19 antivirals, molnupiravir and PF-07231332 could be critical to the development of antivirals for future outbreaks.

Horby recalls looking for phase III-ready antivirals to test at the beginning of the pandemic. "We had remdesivir and a bunch of repurposed, very weak, antivirals for which we had little confidence of success," he says. Novel antivirals are "definitely needed across the whole spectrum of virus families that are potential threats," he says. When something new emerges, the community needs to be well placed to properly evaluate these in big trials.

Hazuda expects molnupiravir to be a broad-spectrum contender. It could have "an important role to play in ending this pandemic, and potentially in future zoonotic transmissions from other coronaviruses," she says. Three coronaviruses — SARS-CoV-1, MERS-CoV and SARS-CoV-2 — have spilled over in the past 20 years.

> We need more investment

Mpro inhibitors tend to be narrower spectrum because there are more structural differences between the proteases of different viruses. However, the protease inhibitors from Pfizer, Hilgenfeld and others are built on the bones of older antiviral candidates.

Research programmes that study the structures of key viral proteins and the attributes of molecules that bind them should also give future antivirals a head start. Hilgenfeld thinks that these platforms should focus on well-conserved targets and processes, including RdRp and the main proteases. Various programmes — including the EU's €2.5 million Swift COronavirus therapeutics REsponse project, the IMI's €75 million Corona Accelerated R&D in Europe and the USA's $3 billion Antiviral Program for Pandemics — are doing precisely that.

"We need more investment," says Hilgenfeld.

The key to antiviral success will be to keep the funds flowing, adds Horby. The 2002–2004 SARS outbreak "kept people interested for a year or two and then interest rapidly dropped off," recalls Horby. "One hopes that won't happen again."