Unfavorable outcomes in the search for antiviral drugs for COVID-19 treatment.

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The COVID-19 pandemic was declared by the World Health Organization (WHO) in early 2020, and it's still devastating. As of February 17, 2023, there have been 756,581,850 confirmed cases of COVID-19 globally, including 6,844,267 deaths, reported to WHO (1). Although there are some approved drugs with promising effects in the management of COVID-19 induced by SARS-COV-2, antiviral agents against the virus are still missing. Drug repurposing is a worthwhile strategy (2), as has been proven already for immunobiological drugs and steroids (3,4), for instance.

However, the ideal scenario would be access to antiviral drugs that could impair SARS-COV-2 reproduction or proliferation inside the human body, acting on the virus protein synthesis mechanism. Unfortunately, we seem to be far from this scenario, mainly because resistant virus strains rapidly emerge against new drugs. Moreover, the available vaccines do not completely resolve virus evasion from the generated antibodies,even after three or four jabs (5,6).

Antiviral agents such as Paxlovid®, Molnupiravir, Remdesivir, Azvudine, and Ensitrelvir have been therapeutically employed or tested against SARS-COV-2 as approved or experimental drugs for COVID-19 in many countries. Although some of them were hailed as "game changers", disappointing outcomes have been reported. For example, Paxlovid® is composed of the protease inhibitors nirmatrelvir (PF-07321332; a peptidomimetic designed specifically for the SARS-COV-2-3CL protease, or main protease (Mpro)) and ritonavir (used in HIV therapy and a potent CYP450 3A4 inhibitor). Paxlovid is a curious case of a drug in that clinical trials showed overwhelming efficacy, such as a reduction of nearly 90% in the risk of severe COVID-19 (7).

Therefore, one immediately asks why millions of people are still dying from COVID-19 all over the world if the pharmacology efficacy of Paxlovid is so high? Perhaps the explanation may be "rebound cases" related to the drug (8). This perplexing trend was observed in patients who were taking the drug and the symptoms from the disease and the virus disappeared initially, though unexpectedly returned a few days later. These intriguing results have no explanation and researchers are still trying to elucidate the Paxlovid® mystery.

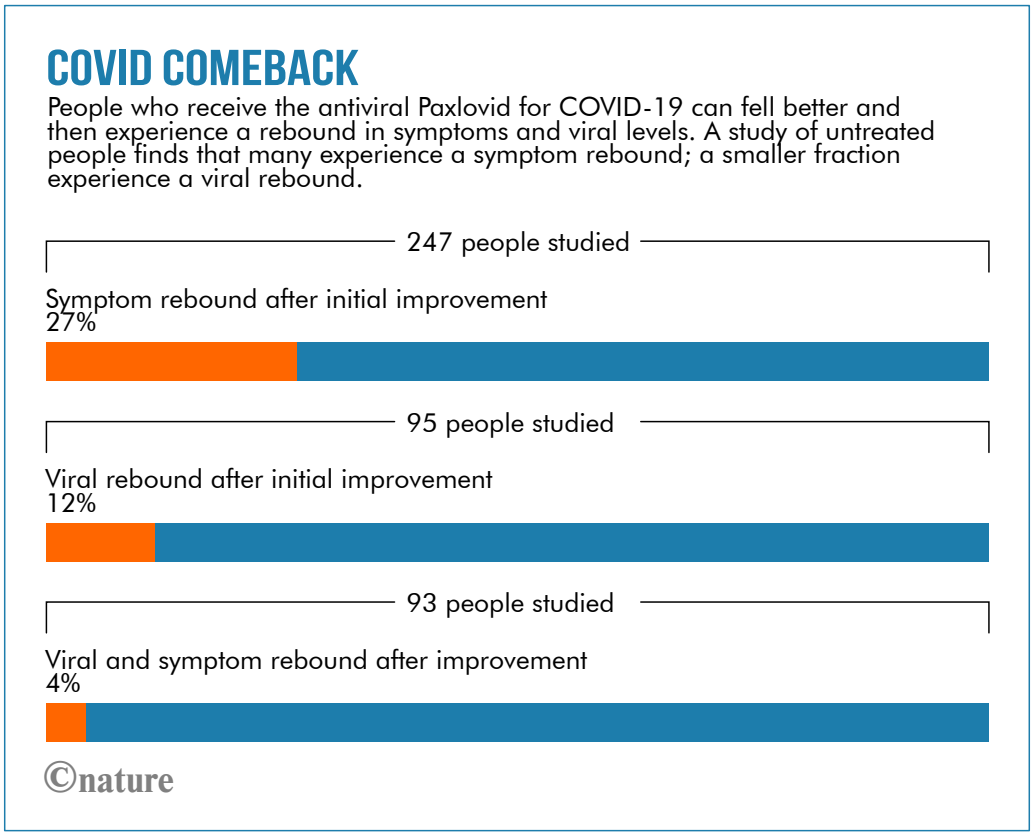

Figure 1. *Viral and Symptom Rebound in Untreated COVID-19 Infection. Figure extracted from Rink Deo et al, 2022 (9).*

Aside from this inexplicable chaotic therapeutic event, reports on the difficulty of getting Paxlovid have been raised. The high prices of the medicine, unequal distribution, and even the lack of knowledge on the drug account for them. For example, researchers have pointed out that physicians have prescribed the drug in only about 0.5% of new COVID-19 cases in the United Kingdom, and about 13% in the United States (10).

In this sense, the report from Dr. Christina Mangurian (11) on her saga to obtain Paxlovid® for treating her parents was impressive. She has had to overcome plenty of barriers from the US health system, as well as other physicians, to get the medicine and finally see her mother and father healed from SARS-COV-2 infection. She concluded that getting Paxlovid® to treat people (her parents, in this case) shouldn't be so hard. Despite these issues, the Paxlovid® blockbuster brought $18.9 billion in revenue for Pfizer in 2022. The big pharmaceutical group is expecting a decline of 33% in revenue in 2023, however, as the world emerges from the pandemic (12).

The antiviral drug Molnupiravir, was developed as a prodrug from the ribonucleoside analog β-DN4-hydroxycytidine (NHC) and thus, a direct-acting antiviral for several infections.Therefore, Molnupiravir is thought to be able to introduce mutations to the viral genome and help to clear virus-related infection (13).

Although the design of Molnupiravir for acting against the SARS-COV-2 virus seemed impeccable, unfortunately, clinical outcomes after its approval and therapeutic use were not accompanied by a significant reduction of the disease worldwide. Before getting emergency approval from the US Food and Drug Administration and from the Healthcare Products Regulatory Agency of the UK, Molnupiravir orally administered for 5 consecutive days in diagnosed symptomatic patients showed only a 30% reduction in deaths and hospitalizations (14). By contrast, some scientists have pointed out that Molnupiravir use cannot clear infections by SARS-COV-2 at all, thereby allowing some patients to continue to transmit the virus.

Actually, the genome sequence of SARS-COV-2 strains by Sanderson *et al.* (15) has revealed a peculiar result: a significant number of lineage samples has presented many more mutations than their nearest relatives. Astonishingly, most of the genetic changes were of the type induced by Molnupiravir. A close, consistent correlation between the use of the antiviral and the induced mutations was attained in a study that included

more than 13 million SARS-COV-2 sequences bearing Molnupiravir fingerprints.

Authors have concluded that Molnupiravir treatment has possibly generated the evolution of viral lineages carrying numerous mutations with great ability to spread to other individuals. The concern of generating more aggressive viruses had been expressed by some scientists in the search for new antiviral agents against the SARS-COV-2 virus (16), and the "Molnupiravir case" shall be taken as an example for continuing research on the new molecular template candidates for COVID-19 treatment.

Remdesivir (brand-named Veklury®) is a viral RNA-directed RNA polymerase (RdRp) inhibitor and approved in several countries for mild to moderate COVID-19 treatment in high-risk patients who are 12 years of age or older and weighing more than 40 kg. The treatment consists of an attacking dose of 200 mg on the first day, followed by a 100 mg dose for the subsequent days (17,18).

For hospitalized patients, a 5-day course of treatment should be given, but recently, a 3-day treatment for non-hospitalized patients with 7 days of symptoms showed a reduction in the risk of hospitalization and death by 87% (18). However, treatment of hospitalized patients was not proven to be so effective in preventing mechanical ventilation and death, only to enhance secondary outcomes such as hospitalization, adverse effects, and days of symptoms (17,19). Nevertheless, Velklury® sales are predicted to stay between $1.59–2.13 billion from 2023 through 2026 (20), even with the significant reduction of COVID-19 in the world.

In this sense, Chinese researchers have recently launched the first oral antiviral agent approved in China (21). Azvudine (FNC, 2′-deoxy-2′-β-fluoro-4′-azidocytidine) targets reverse transcriptase and the HIV-1 accessory protein (VIF) simultaneously, being the first-in-class dual inhibitor. Considering this basic premise, as an RNA-dependent RNA polymerase (RdRp) inhibitor, authors sought to investigate for its therapeutic potential against SARS-COV-2.

The published experimental results showed that Azvudine and the active phosphorylated triphosphate were mainly concentrated in the thymus and peripheral blood mononuclear cells, showing an immune-targeting feature. Azvudine inhibited SARS-COV-2 with an EC50 value ranging from 1.2 to 4.3 μM; in SARS-COV-2-infected rhesus macaque models, Azvudine reduced viral load, improved lymphocyte profiles, alleviated inflammation and organ damage, recuperated the thymus, and lessened ground-glass opacities. Single-cell sequencing studies demonstrated that Azvudine promoted thymus function. The authors concluded that the attained results indicate that Azvudine may cure COVID-19 patients through the thymus-homing feature and immunity promotion (21).

Concerning humans, after preliminary results in minor clinical trials, a phase 3 multicenter, randomized, double-blind, placebo-controlled study with 280 patients was launched. Azvudine significantly shortened symptomatic time and improved clinical symptoms (40.43%) in patients with moderate SARS-COV-2 infection, compared with those given a placebo (10.87%; n = 138, $p < 0.001$). Moreover, Azvudine inhibited SARS-COV-2 with a virus clearance time of about 5 days and was also effective against virus variants (Alpha, Beta, Delta, and Omicron). The drug was well-tolerated and did not increase the risk of subjects nor the incidence of adverse events when compared with the placebo group (21).

We feel that such results are quite promising and, most important, Azvudine has been therapeutically employed in China to combat the pandemic. Therefore, we are eager to realize the outcomes of its arrival in the pharmacology arsenal to combat the SARS-COV-2 virus there.

Recently, scientists from Japan have developed and tested a novel oral SARS-COV-2-3CL protease inhibitor, Ensitrelvir fumaric acid. The molecule has shown antiviral efficacy in both *in vitro* and *in vivo* animal studies and its pharmacokinetic profile has been assessed in a phase 1 study. The results from a multicenter, randomized, double-blind,placebo-controlled, phase 2/3 study to assess the efficacy, safety, and pharmacokinetics of the 5-day oral administration of Ensitrelvir were recently published (22).

The results obtained allowed the authors to favorably conclude rapid clearance of SARS-COV-2 with 5-day oral administration of Ensitrelvir. It was also well-tolerated in patients with mild-to-moderate COVID-19 or asymptomatic SARS-COV-2 infection. Nevertheless, some limitations of the study were pointed out. For instance, that most of the patients were infected by the Delta variant of the virus; therefore, the possible efficacy against the Omicron strain of SARS-COV-2 was only tested for *in vitro* experiments (22).

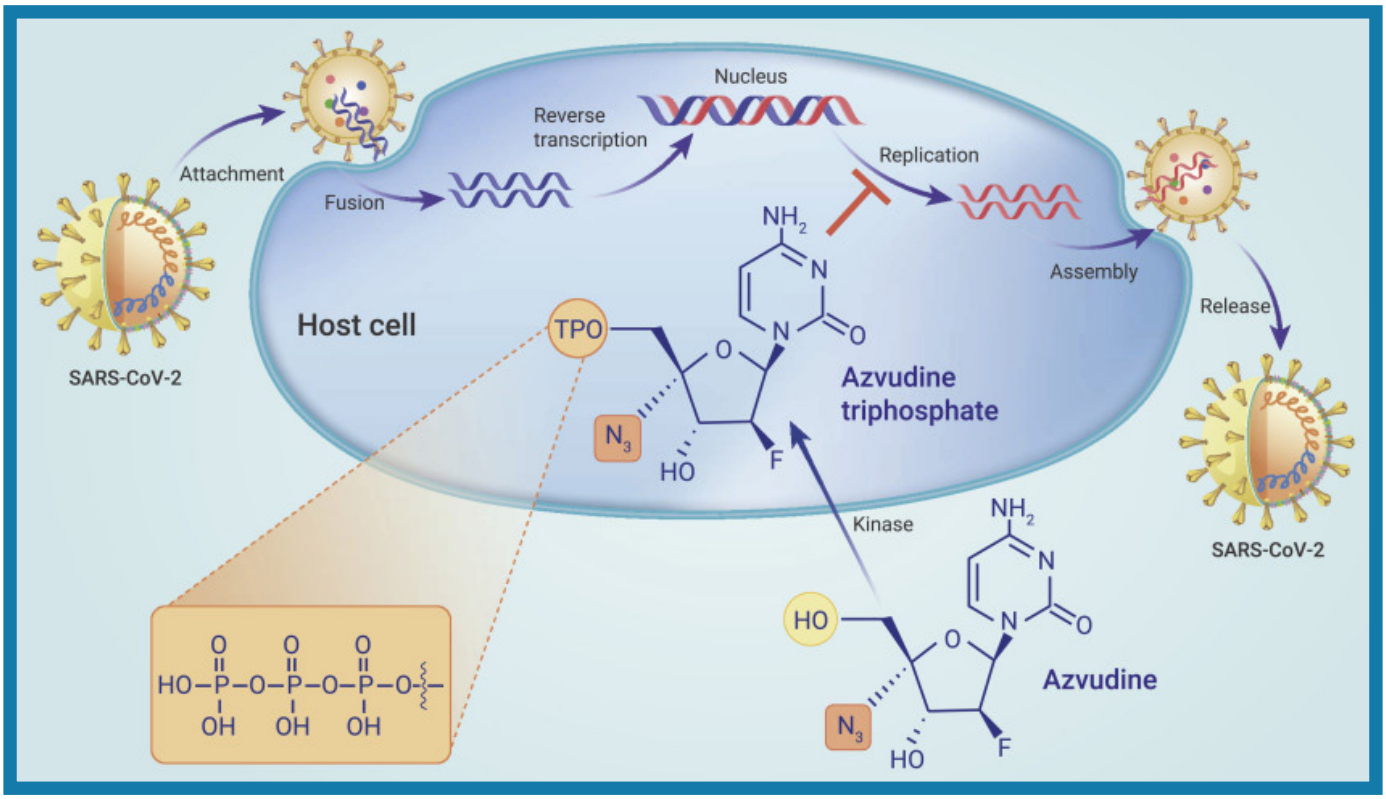

Figure 2. *Mechanism of Azvudine against SARS-CoV-2. Extracted from Bin Yu and Junbiao Chang (2022) (21).*

We feel that is of sensible concern since Omicron is the variant that shows myriad mutations and an increased risk of reinfections. It expands more easily than the original virus and the Delta variant, contaminating those who had been doubly or triply vaccinated (23).Thus, Ensitrelvir remains to be investigated for therapeutic use in COVID-19 infection.

Furthermore, considering the emergence of new variants from the SARS-COV-2, scientists in New York state in October identified the so-called XBB.1.5 variant. In fact, it is a subvariant that stems from a broader branch of the Omicron family tree known as "XBB," which emerged as a result of two earlier versions of omicron —BA.2.10.1 and BA.2.75 — according to the World Health Organization. These closely related Omicron subvariants had the opportunity to swap genes when they infected the same person at the same time (24). Indeed, researchers have pointed out that XBB.1.5, is a master of immune evasion because it carries numerous spike protein (S protein) mutations that blunt the potency of antibodies raised by vaccination and previous infections (10).

Considering all the panorama discussed in the previous paragraphs, it is imperative to continue the efforts to uncover effective antiviral agents, especially in new SARS-COV-2 variant or subvariant scenarios that persist in threatening life on the earth.

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PÍLDORA DE INFORMACIÓN

ESTUDIO CASO-CONTROL

Este tipo de estudios epidemiológicos persiguen la identificación de factores de riesgo para sufrir una determinada enfermedad. Por ejemplo, un estudio caso-control fue el que llegó a la conclusión de que los sujetos que tomaban aspirina con frecuencia tenían menos riesgo de padecer cáncer de colon. Estos estudios retrospectivos se realizan con la ayuda de grandes bases de datos que acumulan millones de historias clínicas de centros de salud, caso de la BIFAP de la Agencia Española de Medicamentos y Productos Sanitarios, o la base de datos de la Fundación Jordi Gol i Gurina de Cataluña.