IN THE PIPELINE

Paxlovid Resistance: Is It Just a Matter of Time Now?

11 JUL 2022 • BY DEREK LOWE • 6 MIN READ • COMMENTS

Pfizer's coronavirus protease inhibitor Paxlovid (nirmatrelvir and ritonavir) is being widely used now, and it's been clear since the beginning that resistant strains of the virus could appear against it. After all, that's what viruses do. With their vast numbers, fast generation time, and number of mutations, resistance to a given small molecule is generally just a matter of "when", not "if". The usual way around this problem is to try to use a drug cocktail, hitting the pathogen simultaneously with compounds that target more than one mechanism. That's the idea behind the two most successful small-molecule viral therapies we have, against HIV and Hepatitis C.

The exact equivalent for the coronavirus would be to use a protease inhibitor like Paxlovid along with a viral polymerase inhibitor. That's how Merck's molnupiravir works, but unfortunately clinical trials showed that it doesn't work all that well. Still, a combination of the two might be quite valuable, but I have yet to hear of any clinical activity whatsoever that is looking at this - nor have I yet heard of a good explanation for why there isn't any. At least it seems to work in mice! As it stands, it looks like various parts of the world are using one or using the other, which sounds like a good way to generate resistant strains in all directions, should one have some perverse desire to do that.

Here's a good article here at *Science* that looks at the situation as of about two weeks ago, and lays out the general story very well. And here's a new paper looking at the structure of the coronavirus main protease (MPro) with an eye to where such resistant variants might develop. The authors identify amino acids 45-51 in general, along with several other residues (M165, L167, P168, R188, and Q189) that can also affect the active binding site. (*For those outside the molecular biology field, that notation uses the single-letter amino acid abbreviations along with the numbered position in the protein sequence. For example, "L50F" tells you that the leucine amino acid (L) at position 50 of the protein has been replaced by a phenylalanine (F) amino acid*). That's not an exhaustive list by any means. The regions of the protein that interact directly with the small molecule are 40–44, 45–51, 140–146, 163–169, and 186–192, but there are many examples known of point mutations in some more distant part of a protein that affect the structure of an active site. Predicting those things from first principles is unfortunately generally not feasible. The authors note that mutations in their regions of concern are already known from wild-type sequences in human infection, so they're at least feasible. They might, in fact, already be contributing to a spectrum of Paxlovid activity among patients taking the drug. Here's a preprint looking at such a list of mutations that are already known from clinical sequencing efforts.

Now, any such mutations can be a tradeoff between the general fitness of an enzyme to do its job and its ability to evade the binding of the small molecule inhibitor. There are surely plenty of mutations that would keep Paxlovid's protease inhibitor component (nirmatrelvir) from binding at all, but would also keep many of the enzyme's substrates from binding, either, so those are dead ends, evolutionarily. But what you really don't want are changes that give you perfectly competent enzyme variants that also shrug off the small-molecule inhibitor, and we're not in a position to rule such things out yet. As you will see.

This new preprint is one of several trying to bring some experimental data in. Getting that directly on a human pathogen is a potentially dangerous gain-of-function experiment that needs to be done under containment conditions, but there are ways to get some answers in less risky ways. In this case, these authors (from Innsbruck) used VSV, an animal pathogen that rarely infects humans and does not cause severe illness when it does. It's been used over the years in many viral evolution studies, and has been investigated as a platform for human therapies (Merck had a failed coronavirus vaccine candidate that was going to use VSV, for example). This team engineered VSV to make it dependent on the coronavirus main protease, and then exposed this chimera virus to gradually increasing amounts of nirmatrelvir over time as it grew in hamster kidney cells.

Pfizer's own team had done similar experiments, but using mouse hepatitis virus (MHV), another member of the coronavirus family. This preprint argues that MHV was not a very good choice, since nirmatrelvir is not a very potent inhibitor against it right from the start - the authors believe that using the real SARS-2-CoV protease in an error-prone replication system like VSV is a better way to survey the mutational landscape. (Similar VSV chimeras have already been used to study potential mutation in the coronavirus Spike protein).

The authors found a range of mutations under these conditions, scattered throughout the whole coronavirus sequence. There were some in the actual binding-site residues that they studied closely, along with the most common mutations from elsewhere in the protein. These all seemed to have well-functioning protease enzyme after these changes, which included Y54C, L167F, Q192R, and F305L. Some of these line up with the predictions in the structure-based paper linked above, and some don't. A double mutant (Q192R/F305L) was unfortunately able to replicate to high VSV viral titer values even in the presence of 100 micromolar nirmatrelvir, indicating that it was pretty much completely resistant to Paxlovid. It remains to be seen if this situation is any different in wild-type coronavirus as opposed to a VSV chimera, but you don't like to see this sort of possibility.

We have some other data to consider. Here's a preprint from Belgium where the SARS-Cov-2 virus was passaged through Vero cell cultures in the presence of increased amounts of a new inhibitor of the coronavirus protease (ALG-097161), so these people were getting right to those gain-of-function experiments on the virus itself. The team noted that resistance was actually not very quick to develop, which is good to see. After some 20 to 30 days, they saw L50F, E166A, and L167F mutations appearing, eventually to the triple combination of all of them. They went on to evaluate those exact mutant enzymes directly, and found that all of them showed impaired activity compared to wild-type enzyme, all of them under 20% as active. The activity of both their inhibitor and nirmatrelvir were lower, by 30- to 70-fold, but the replication efficiency of the virus as a whole was decreased. This report is (comparatively) good news, but it still shows that mutations are ready to form out there.

On the other hand, here's a preprint from Denmark, where the authors also looked at SARS-CoV-2 in Vero cells. They found L50F and E166V mutations among others, with the combination of those two showing up to 80-fold resistance to nirmatrelvir along with high reproductive fitness as compared to wild-type. The E166V mutation by itself had worse fitness, but that was made up for by adding the L50F mutation on top of it. These authors noted that remdesivir efficacy was unaffected by these mutations, and suggest dual use of these drugs if we get into a bad resistance situation in the general population.

Putting these various papers together, we can say something about these various mutants and how they likely affect nirmatrelvir binding. L50F is believed to further change the conformation of the Q189 residue (see below), but the Danish group's calculations say that this by itself might actually *improve* nirmatrelvir binding through better interactions with R188 and T190, so that one by itself seems less likely to be trouble - only being worrisome when it's mixed in with other mutations, as noted above. The E166A and E166V mutations kill off a key hydrogen bond in the interaction with that site's normal glutamate residue. The L167F increases the size of the binding pocket and lowers the general interaction with the inhibitor (not as close a fit with hydrophobic residues). Q189 mutations disrupt another interaction with nirmatrelvir, this time through a network of water molecules in the binding site.

The package insert for Paxlovid notes a range of mutations that were already observed during the clinical trials as more common in Paxlovid-treated patients than in the control group (see the bottom of page 28 here). There are a number of them that are not on the lists above, but E166V is there. On the other hand, the L167F mutation that shows up in the above experiments is not on that human-data list, for whatever reason. The company also mentions that none of the mutations on their list were seen in Paxlovid patients who were also hospitalized, making their clinical significance hard to evaluate.

So where are we? I'd say that: (1) mutations in the coronavirus are definitely taking place in response to Paxlovid therapy, (2) that there are a number of regions in the protein sequence that could (in theory) lead to viral sequences that are still reproductively fit but are much less affected by Paxlovid, and (3) that a number of potential mutation sites identified by these

in vitro studies also have appeared in mutations isolated from wild-type coronavirus in humans, showing that they are feasible under real-world conditions. But what we don't know - yet - is how all the dots might connect. We do not know if there are reproductively fit mutations that are Paxlovid-resistant that are current spreading in the human population, for example. The evidence isn't there, or perhaps isn't quite there yet? But we absolutely should expect that this could happen. Could be happening now, could have already happened. We shall see.

ABOUT THE AUTHOR

Derek Lowe

Derek Lowe, an Arkansan by birth, got his BA from Hendrix College and his PhD in organic chemistry from Duke before spending time in Germany on a Humboldt Fellowship on his post-doc. He’s worked for several major pharmaceutical companies since 1989 on drug discovery projects against schizophrenia, Alzheimer’s, diabetes, osteoporosis and other diseases.

VIEW MORE

COMMENTS

sagafemina • a month ago

One does have to wonder, given the number of exquisitely vaccinated persons who are reportedly coming down with Rebound after Paxlovid, whether somehow vaccines are preventing variants that do NOT rebound, and allowing breakthrough infections in those that DO show a propensity for rebound.
If true, taking Paxlovid would NOT seem to be prudent in the Omicron era. It is known that rebound prolongs infectivity of others.
This virus continues to outsmart us.

Still to be determined is, beyond a few more days of symptoms and the nuisance of further isolation, whether prolonged experience of illness correlates at all with long haul Covid.

• Reply • Share ›

Blister • a month ago

Derek, a year ago you wrote Vaccines Will Not Produce Worse Variants. In light of what you are saying now about Paxlovid selecting for mutations have you changed your views on vaccine induced mutation selection?

• Reply • Share ›

Dereklowe Mod ➦ Blister • a month ago

Nope. The vaccines are producing Spike protein, which the natural infections does as well. There's no extra selection pressure. Paxlovid is a different beast - a small-molecule enzyme inhibitor which the virus can (and likely will) evolve resistance to if given enough chances.

• Reply • Share ›

JIA12_12 • 2 months ago

Hi Derek, when you wrote "mutations in the coronavirus are definitely taking place in response to Paxlovid therapy", I think you took a verbal shortcut. You presumably meant "Randomly occurring mutations in the coronavirus are definitely being selected for in response to Paxlovid therapy". Paxlovid itself is not a mutagen, per the animal and in vitro tox studies. I guess I sound like I'm quibbling here, but your blog is read widely by a lay audience, and I would hate for them to take away the wrong message from your phrasing. There are already enough conspiracy theories around coronavirus therapies. Thanks!

1 • Reply • Share ›