Overcoming amantadine resistance

By Lauren Martz, Staff Writer

A group of U.S. researchers has developed inhibitors of the influenza A virus matrix protein 2 mutant that is responsible for resistance to amantadine.¹ The first-generation inhibitor was widely used for years to treat flu, but in 2006 the **Centers for Disease Control and Prevention** recommended against its use due to resistance. **InfluMedix Inc.** has licensed the compounds and hopes to bring a new influenza antiviral to the market within five years.

Most mutations of the influenza A virus matrix protein 2 (M2) channel render the virus less transmissible than wild-type M2. As a result, only a handful of mutations are found in circulating influenza A viruses.

For example, virtually every amantadine-resistant transmissible influenza virus expresses the same M2 mutation—S31N—and more than 95% of circulating influenza A viruses express that mutation.

The problem is that the S31N mutation has a drug-binding site that is more difficult to target than the wild-type protein because the site in mutant M2 is smaller and more polar. To date, there have been no effective inhibitors of S31N M2.

Influenza virus instead is treated with neuraminidase (NEU1; SIAL1) inhibitors such as oral Tamiflu oseltamivir from **Roche** and **Gilead Sciences Inc.** or inhaled Relenza zanamivir from **GlaxoSmithKline plc** and **Biota Pharmaceuticals Inc.** The drugs are effective against influenza A viruses with the S31N mutation, but resistance to neuraminidase inhibitors is growing.

William DeGrado and colleagues therefore set out to find new therapeutic strategies to treat influenza A viruses with acquired resistance to amantadine and other drugs.

In 2008, DeGrado and colleagues first determined the high-resolution crystal structure of the wild-type M2 channel.² However, the mutant channel was harder to crystallize because its structure is more dynamic.

DeGrado is professor of pharmaceutical chemistry at the **University of California, San Francisco** and investigator at the university's Cardiovascular Research Institute. The team also included researchers from **Northwestern University**, **Temple University** and **InfluMedix**.

The group used *Xenopus laevis* oocytes to test inhibitors of the mutant channel and showed that analogs of amantadine with a CH₂-heteroaryl group conjugated to the drug's amine group blocked S31N M2 proton transport.

The group further modified the compounds to isoxazole-, isoxazoline- and oxadiazole-containing derivatives, some of which had IC₅₀ values <16 μM against S31N. This suggests the new inhibitors could be more potent against mutant M2 than amantadine is against the wild-type protein.

In an assay using influenza A virus expressing the S31N mutant, some of the most potent compounds completely inhibited the ability of the virus to infect and form colonies on a layer of host cells at 10 μM. A similar dose of amantadine showed almost no efficacy.

Finally, the team found that the new inhibitors locked the proton channel's conformation. This allowed the group to capture a high-resolution structure of S31N in complex with an inhibitor, which could aid the design of inhibitors with improved potency.

Antiviral demand

InfluMedix has licensed the compounds and IP related to the proton channel structure. CEO Lidia Cristian said that next steps include evaluating the compounds in animal models of influenza infection to establish safety and efficacy.

"Currently there are only four antiviral drugs approved for use against influenza infections in humans: two targeting the M2 proton channel and two targeting neuraminidase. The emergence of drug resistance to both classes of flu drugs poses a major problem," she said. "Tamiflu resistance is on the rise, and there are concerning reports of the emergence of flu strains with dual resistance to both M2 and neuraminidase classes of drugs."

Rimantadine, the other first-generation M2 inhibitor, is a related derivative of amantadine with similar problems of drug resistance.

Makoto Yamashita, senior chief researcher at **Daichi Sankyo Co. Ltd.**, noted that "we practically only clinically use neuraminidase inhibitors, and there does exist a necessity for agents that have a different mode of action."

Daichi's Inavir laninamivir is a second-generation neuraminidase inhibitor approved in Japan to treat influenza A and B. The company has Japanese rights to the compound from **Biota**.

"These S31N variants are fully susceptible to the neuraminidase inhibitors, but the opportunity to potentially resurrect the older amantadine class of drug is a worthy goal," said Simon Tucker, VP of research at **Biota**.

According to the CDC reports for the 2012–2013 influenza season, about 78% of flu patients in the U.S. are infected with the A strain and 22% are infected with the B strain. The new S31N M2 inhibitors have the potential to treat most influenza A subtypes because almost all of the circulating strains carry the mutation, but the influenza B virus strains would not be amenable to treatment because they do not express M2.

Anil Diwan, president and chairman of **NanoViricides Inc.**, said any approach that goes directly after viral targets will likely be hampered by resistance issues.

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—Anil Diwan, NanoViricides Inc.

“A major disadvantage of small chemical antiviral strategies is that mutant, resistant viruses can be generated rapidly. Viruses are intelligent nanomachines that reprogram themselves,” said Diwan.

NanoViricides's FluCide is a nanoviricide designed to mimic influenza-targeted cells, bind the virus and encapsulate it. The company has both i.v. and oral FluCide candidates in preclinical development.

Cristian noted that “the potential caveat, as with any newly developed antiviral, is the emergence of resistance. However, M2 is a remarkably conserved viral protein that tolerates very few mutations that are present within transmissible viruses and are of clinical relevance.”

She said that the first class of M2 inhibitors was used for more than 30 years before resistance became widespread and that Influmedix does not expect resistance to the new M2 inhibitors to develop quickly.

The **University of Pennsylvania**, where DeGrado conducted earlier work on this project prior to moving to UCSF, and Influmedix have filed a patent application covering the compounds. Influmedix has licensed the IP, and licensing opportunities are available through the company.

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COMPANIES AND INSTITUTIONS MENTIONED

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Centers for Disease Control and Prevention, Atlanta, Ga.
Daiichi Sankyo Co. Ltd. (Tokyo:4568; Osaka:4568), Tokyo, Japan
Gilead Sciences Inc. (NASDAQ:GILD), Foster City, Calif.
GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K.
Influmedix Inc., Radnor, Pa.
NanoViricides Inc. (OTCBB:NNVC), West Haven, Conn.
Northwestern University, Evanston, Ill.
Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland
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