Going nuclear with influenza

By Lauren Martz, Staff Writer

Researchers from **The University of Hong Kong** have identified a new antiviral target—influenza A nucleoprotein—that could be effective against a range of influenza strains by blocking viral replication.[1] The team has generated a small molecule inhibitor of the target, although its efficacy probably needs to be optimized to be on par with that of marketed neuraminidase inhibitors, and its resistance profile also needs to be better understood.

There are two marketed classes of antivirals to treat influenza—neuraminidase (NEU1; SIAL1) inhibitors and the older, generic adamantanes, which target the influenza ion channel protein M2. Marketed neuraminidase inhibitors include Tamiflu oseltamivir from **Gilead Sciences Inc.** and **Roche** and Relenza zanamivir from **GlaxoSmithKline plc** and **Biota Holdings Ltd.**

However, all of the seasonal influenza A H3N2 strains are resistant to adamantanes,[2] and the majority of the 2009 H1N1 swine flu viruses are resistant to neuraminidase inhibitors, specifically Tamiflu.[3]

Thus, Richard Kao and colleagues at the University of Hong Kong have been searching for new druggable influenza targets. In a paper published in *Nature Biotechnology*, Kao's team described the use of a forward chemical genetics approach to screen a library of 50,240 compounds for activity against influenza *in vitro*.

The initial screen found 950 hits that protected against infection. A secondary screen for potency narrowed the hits to 39, 5 of which were found to prevent nuclear accumulation of influenza A nucleoprotein (NP).

NP is the most highly expressed viral protein throughout the course of infection. It accumulates in the nucleus during early infection, where it contributes to viral RNA transcription, replication and intercellular trafficking. NP later relocates to the cytoplasm, where it promotes viral assembly and maturation.[4]

With a good target in hand, Kao's team then generated an analog of the most potent and soluble hit from its screen. The analog, dubbed nucleozin, inhibited infection of cells by multiple influenza strains, including A/WSN/33, H3N2 and avian H5N1.

The analog also suppressed viral growth and production in a multicycle growth assay better than no treatment and with results comparable to those for zanamivir. In human alveolar basal epithelial cells infected with influenza A/WSN/33, fluorescence microscopy showed that nucleozin prevented accumulation of NP in the nucleus and caused the formation of NP aggregates in the cytoplasm compared with no treatment. Keeping NP out of the nucleus should prevent viral replication.

In mice, nucleozin increased survival following H5N1 infection. Half of the treated animals were alive at 21 days postinfection compared with none of the untreated mice.

Compared with no treatment, zanamivir and nucleozin both decreased viral loads in the lungs of mice six days after infection, but "unlike neuraminidase inhibitors that prevent the virus from releasing from the infected cells, nucleozin targets the viral nucleoprotein and stops viral replication," said Kao, assistant professor of microbiology at the University of Hong Kong.

Although 100% of zanamivir-treated animals survived in a parallel experiment, having drugs against a previously unknown target is important because of viral resistance.

"Many circulating viruses have already developed resistance against existing drugs. Therefore, having a new drug that utilizes an entirely different molecular pathway as described in this paper would be clearly valuable," said Christian Mandl, global head of virology for **Novartis AG**'s Novartis Vaccines & Diagnostics Inc. subsidiary.

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> ***—Christian Mandl, Novartis AG***

Robert Krug, chair of the Department of Molecular Genetics and Microbiology at the Institute for Cellular and Molecular Biology at **The University of Texas at Austin**, said the benefit of targeting NP over influenza surface proteins such as neuraminidase "is that there is less variability and genetic change. In influenza, there is a lot of resistance, and you can't rely on a single drug against a single target."

Indeed, Martin Schwemmle, professor in the Department of Virology at the **University of Freiburg**, thinks the real benefit of hitting NP is the potential for combination therapy against the virus. He suggested that nucleozin plus a neuraminidase inhibitor could increase efficacy and decrease resistance compared with either agent alone.

Resistance movement

One red flag raised in the *Nature Biotechnology* article is that the molecule had little effect on last year's H1N1 virus, which naturally expresses a version of NP to which nucleozin is unable to bind. The question, therefore, is whether swine flu is the exception or the rule.

"As with the existing drugs, it is likely that flu virus will be able to develop resistance against this new antiviral substance. In fact, the publication already shows examples of this happening," said Mandl. "How often this would happen under treatment conditions is totally unclear and needs to be addressed. So far, the activity in animals is not as good as that of Tamiflu; therefore, there is still a lot of optimization to be done."

Mandl told *SciBX* it will be necessary to outline resistance patterns among known strains of the flu virus and to optimize nucleozin's pharmacokinetics and toxicity profiles in animal models. "It may be possible to synthesize related substances that overcome this resistance to H1N1," he noted.

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Krug thinks the best way to improve the inhibitor "is to get the structure of the chemical with the protein it is targeting using X-ray crystallography. The researchers in the paper have used computer docking methods, which produce predictions that are not always reliable."

He added: "The two major problems with developing antivirals are finding compounds that don't allow the virus to escape easily and develop resistance and developing compounds without toxicities. What they face here is a compound that binds to a region of the protein that can readily change without compromising virus replication. Therefore, unfortunately the results don't reflect what they might have hoped for."

Kao said his team is in the process of lead optimization and that "the real drug to be tested will have a broader spectrum against all strains of clinically significant influenza viruses" than nucleozin.

He added that his next steps include testing the compound in nonhuman primates and then in clinical trials. He said the university has filed a patent application covering the results and that the IP has not been licensed.

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COMPANIES AND INSTITUTIONS MENTIONED

Biota Holdings Ltd. (ASX:BTA), Notting Hill, Victoria, Australia
Gilead Sciences Inc. (NASDAQ:GILD), Foster City, Calif.
GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K.
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