Closing time for HCV protease

By Kai-Jye Lou, Staff Writer

Astex Pharmaceuticals Inc. has used its fragment-based drug discovery platform to identify a new allosteric binding site on the full-length HCV NS3/4A protein and thinks compounds that bind the site can inhibit the protein's helicase activity in addition to its proteolytic activity.¹ The result could be molecules with better efficacy than existing HCV drugs that target the protease active site.

The full-length HCV NS3/4A protein consists of a protease domain

linked to a helicase domain. According to Astex president, director and cofounder Harren Jhoti, R&D efforts to target the NS3/4A complex have previously focused on compounds that target the active site of the protein's protease domain due to screening limitations—it is more technically challenging to express and crystallize the full-length protein than individual domains for screening purposes.

Celia Schiffer, a professor of biochemistry and molecular pharmacology and director of the Center for AIDS Research at the **University of Massachusetts Medical School**, noted that another reason the HCV field has focused on developing compounds against the individual domains of the NS3/4A protein stems from the view that the protein's helicase and protease functions are essentially independent of one another.

Despite not working with the full protein, efforts to inhibit the HCV NS3/4A protease active site have borne fruit—notably the approvals of Victrelis boceprevir from **Merck & Co. Inc.** and Incivek telaprevir from **Vertex Pharmaceuticals Inc.** in May of last year.

There are at least 10 HCV NS3/4A protease inhibitors in various stages of clinical development, including three in Phase III trials.

Figure 1. Targeting the full-length HCV NS3/4A protein complex.

Researchers at **Astex Pharmaceuticals Inc.** have identified a new binding pocket on the full-length HCV NS3/4A protein complex that could be targeted to treat HCV infection. The full-length protein consists of a helicase domain linked to a protease domain.

[I] The complex switches between an active, open conformation and an inactive, closed conformation. In the closed conformation, the C-terminus of the helicase domain occupies the protease active site.

[II] Direct inhibition of the protease active site is the primary strategy drug developers have pursued to inhibit the complex. There are already at least 2 such drugs marketed to treat HCV infection and at least another 10 in clinical trials. Targeting this site inhibits the complex's proteolytic activity [II(a)].

The newly identified allosteric site is present when the full-length NS3/4A protein complex is in its closed conformation. Compounds that target the site stabilize the complex in this inactive conformation [II(b)]. Unlike inhibitors against the protease active site, compounds that target the allosteric site also have the potential to inhibit the complex's helicase activity in addition to its proteolytic activity.

I HCV NS3/4A protease complex conformational states

II Intervention strategies

Marketed and late-stage HCV NS3/NS4A active protease inhibitors:

Incivek telaprevir marketed by **Vertex Pharmaceuticals Inc.** (NASDAQ:VRTX), **Johnson & Johnson** (NYSE:JNJ) and **Mitsubishi Tanabe Pharma Corp.** (Tokyo:4508; Osaka:4508); Victrelis boceprevir marketed by **Merck & Co. Inc.** (NYSE:MRK); faldaprevir in Phase III by **Boehringer Ingelheim GmbH**; simeprevir in Phase III by **Medivir AB** (SSE:MVIRB) and Johnson & Johnson; vaniprevir in Phase III by Merck.

HCV NS3/NS4A allosteric inhibitors: AT26893, a small molecule allosteric inhibitor of HCV NS3/NS4A, in preclinical development by **Astex Pharmaceuticals Inc.** (NASDAQ:ASTX).

Jhota said compounds that target the same site on the HCV protein are likely to be affected by the same resistance mutations. Thus, rather than joining an already crowded space by pursuing compounds against the protease active site, the Astex team sought to identify alternative sites on the NS3/4A protein that could be targeted to inhibit its function.

The group generated crystals of the full-length NS3/4A protein and used X-ray-based crystallographic screening against a library of chemical fragments to identify compounds that bound to the target.

The crystal structures of the full-length protein in complex with screening hits revealed a new binding pocket at the interface of the protein's helicase and protease domains. The fragment screening hits had weak inhibitory activity but showed ligand efficiencies that suggested they could be optimized into potent compounds.

Ligand efficiency is a measure of how well a small molecule binds to a protein, regardless of its size. In fragment-based screening, it is used as a criterion to select chemical fragments that could be good candidates for further optimization.²

Structure-based optimization of a screening hit yielded a chemical probe that inhibited the full length NS3/4A protein with an IC_{50} of <10 nM. In cell-based replicon assays, the optimized probe showed antiviral activity with an EC_{50} of 8.3 nM.

Data from a series of *in vitro* biophysical assays suggested compounds binding to the newly identified pocket allosterically inhibit the function of the NS3/4A protein by stabilizing it in an inactive conformation (see Figure 1, "Targeting the full-length HCV NS3/4A protein complex").

Results were published in *Nature Chemical Biology*.

"They have discovered potent HCV NS3/4A inhibitors that simultaneously interact with both the protease and the helicase domains," said Schiffer, who also is co-director of the Institute for Drug Resistance at the University of Massachusetts Medical Center. "I think having small, reasonably bioavailable inhibitors that interact with conserved regions of both domains could be a very useful strategy for the various genotypes of HCV and may be a strategy for other flaviviruses as well."

However, Schiffer wanted to see additional experimental data to prove that the inhibitors are acting via the described allosteric mechanism.

"It would also be very interesting to know if these inhibitors impact the helicase activity at all," she added.

Known target, new strategy

Astex has selected a lead preclinical candidate, AT26893, which exploits the allosteric inhibitory mechanism against the full-length HCV NS3/4A protein. The company hopes to start Phase I testing in mid-2013.

"The goal of our HCV program is to find and develop molecules that will have superior physicochemical properties compared to protease active site inhibitors," said Jhota, corresponding author on the paper. "What we were trying to do in this study is determine whether our fragment-based screening approach could discover compounds that bind to a site on the NS3/4A protein that's different from the one protease active site inhibitors bind to."

"Because we are going after a different binding site on the protein, we get to work in a different chemical space that's also relatively unconstrained by existing IP."

—Harren Jhota,
Astex Pharmaceuticals Inc.

It is too early to know how Astex's allosteric inhibitor will compare with marketed and clinical-stage NS3/4A protease inhibitors. Nevertheless, Jhota said the company's molecule could have improved efficacy, better safety and lower risk of cross-resistance based on the compound's hypothesized mechanism and some unpublished preliminary preclinical data.

"Our compound appears to stabilize the NS3/4A protein in its closed, inactive conformation and thus could have the ability to inhibit both the helicase and protease enzymatic functions of the protein," he told *SciBX*. "Because of this, there is a possibility that the antiviral effect of our compound could be more pronounced than current protease active site inhibitors, which only inhibit the protein's protease function. However, we still need to confirm these potential advantages in experimental assays."

Moreover, telaprevir and boceprevir, as well as many protease inhibitors marketed to treat HIV infection, are known to inhibit cytochrome P450 3A4 (CYP3A4).^{3,4} The enzyme is involved in the metabolism of many known drugs, including the protease inhibitors themselves.

Because Astex's AT26893 comes from a different chemical class, Jhota thinks it should avoid many of the bioavailability, toxicity and drug-interaction issues caused by off-target inhibition of cytochrome P450 (p450) isoenzymes. He added that this could translate into more consistent efficacy and tolerability profiles between patients.

Finally, Jhota said the resistance profile of the company's allosteric inhibitor should be very different from that of active site inhibitors.

In addition to the potential therapeutic advantages, Astex expects that avoiding compounds that bind to the protease active site of the NS3/4A protein will give the company greater operating freedom in designing and optimizing lead candidates from screening hits.

"Many companies already have compounds that target the NS3/4A protease active site, and both the chemical space and IP space have become very crowded," Jhota told *SciBX*. "Because we are going after a different binding site on the protein, we get to work in a different chemical space that's also relatively unconstrained by existing IP."

He said the paper also demonstrates that the company's fragment-based platform could be used to discover leads that modulate the activity of disease-relevant proteins via allosteric sites. Astex plans to publish data soon related to pyruvate kinase M2 isozyme (PKM2) and also plans to report on efforts to target a protein-protein interaction involving X-linked inhibitor of apoptosis (XIAP). Both proteins are linked to cancer.

Astex has filed composition-of-matter patents covering its allosteric inhibitors of the full-length NS3/4A protein. The compounds are available for licensing.

Jhota noted that the company also is interested in forming collaborations and/or partnerships to help advance its HCV program.

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COMPANIES AND INSTITUTIONS MENTIONED

Astex Pharmaceuticals Inc. (NASDAQ:ASTX), Dublin, Calif.
Merck & Co. Inc. (NYSE:MRK), Whitehouse Station, N.J.
University of Massachusetts Medical School, Worcester, Mass.
Vertex Pharmaceuticals Inc. (NASDAQ:VRTX), Cambridge, Mass.