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Blocking and watching HCV

By Tracey Baas, Associate Editor

Papers from **Stanford University** researchers and a **Massachusetts Institute of Technology–The Rockefeller University** team provide a new target for HCV and a new way to study the virus in real time, respectively. The Stanford group has already identified two small molecule inhibitors of the target—the 4BAH2 motif of non-structural protein 4B—and exclusively licensed them to **Eiger Biopharmaceuticals Inc.**[1]

Standard care for chronic HCV is combined therapy with pegylated interferon-α and ribavirin. However, the treatment is only effective in just over 50% of patients and side effects include flu-like illness, fever, fatigue, hematological disease, alopecia and depression.[2,3] The response rate is highest in patients infected with HCV genotypes 2 or 3 and lowest in patients infected with genotypes 1 or 4.[2]

The highest-profile targets are HCV protease and polymerase (*see* **Figure 1, "Targeting HCV"**), and several companies have HCV protease or polymerase inhibitors in clinical development (*see* **Table 1, "HCV therapeutic pipeline"**).

A less hotly pursued target is non-structural protein 4B (NS4B). For years the target's function was unknown, although recent reports have implicated NS4B in various roles of the HCV life cycle, including the formation of a distinct intracellular membranous web in which viral replication is thought to take place.[4]

The Stanford team, led by Jeffrey Glenn, determined that 4BAH2, the second amphipathic helix in NS4B, mediates HCV replication,

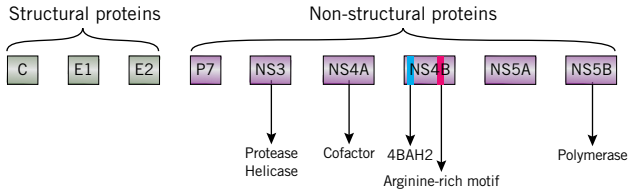

Figure 1. Targeting HCV. New HCV target 4BAH2 is an amphipathic helix (blue stripe) found within the virus' non-structural protein 4B (NS4B). This helix is involved in NS4B oligomerization and membrane association, resulting in the formation of an intracellular membrane structure that facilitates viral replication. Researchers previously identified an arginine-rich motif (red stripe) in NS4B as a target. This motif is involved in binding negative viral RNA.

Beyond NS4B, HCV is made up of nine other proteins, of which three (C, E1 and E2) are used to form the structure of the virus and six are non-structural proteins, like NS4B. Most HCV therapeutics target NS3 protease, NS4A, which is a cofactor for NS3, and the polymerase NS5B.

Table 1. HCV therapeutic pipeline. Selected compounds in clinical development targeting HCV non-structural protein 3 (NS3), NS4A, NS5A and NS5B.
Source: BCIQ: BioCentury Online Intelligence

Company	Compound	Target	Status
Merck & Co. Inc. (NYSE:MRK)	Boceprevir	NS3	Phase III
Vertex Pharmaceuticals Inc. (NASDAQ:VRTX)/**Mitsubishi Tanabe Pharma Corp.** (Tokyo:4508; Osaka:4508)/**Johnson & Johnson** (NYSE:JNJ)	Telaprevir (VX-950)	NS3/NS4A	Phase III
InterMune Inc. (NASDAQ:ITMN)/**Array BioPharma Inc.** (NASDAQ:ARRY)/**Roche** (SIX:ROG; OTCQX:RHHBY)	ITMN-191 (RG7227)	NS3/NS4A	Phase IIb
Medivir AB (SSE:MVIR B)/Johnson & Johnson	TMC435	NS3/NS4A	Phase IIb
Anadys Pharmaceuticals Inc. (NASDAQ:ANDS)	ANA598	NS5B	Phase II
AstraZeneca plc (LSE:AZN; NYSE:AZN)	AZD7295	NS5A	Phase II
Boehringer Ingelheim GmbH	BI 201335	NS3/NS4A	Phase II
Bristol-Myers Squibb Co. (NYSE:BMY)	BMS-790052	NS5A	Phase II
Gilead Sciences Inc. (NASDAQ:GILD)	GS 9190	NS5B	Phase II
Idenix Pharmaceuticals Inc. (NASDAQ:IDIX)	IDX184	NS5B	Phase II
Merck	MK-7009	NS3/NS4A	Phase II
Pfizer Inc. (NYSE:PFE)	PF-868554	NS5B	Phase II
Roche	R7128	NS5B	Phase II
Achillion Pharmaceuticals Inc. (NASDAQ:ACHN)	ACH-1625	NS3	Phase Ib
Eiger Biopharmaceuticals Inc.	Clemizole	NS4B	Phase Ib
Idenix Pharmaceuticals	IDX375	NS5B	Phase I
Vertex Pharmaceuticals	VX-759	NS5B	Phase I
Vertex Pharmaceuticals	VX-222	NS5B	Phase I

NS4B oligomerization, NS4B membrane association and lipid vesicle aggregation. The researchers therefore hypothesized that inhibiting 4BAH2 should block the virus' life cycle.

Glenn's group designed a high throughput lipid vesicle aggregation assay to identify inhibitors of 4BAH2. They screened a small molecule library from the Stanford High Throughput Bioscience Center and found several hits.

The next question was whether the compounds that disrupted 4BAH2-mediated lipid vesicle aggregation would also inhibit other 4BAH2-mediated functions and thus impede the life cycle of the virus.

The group found that two of the small molecules, C4 and A2, showed dose-dependent inhibition of viral replication in an HCV replicon assay.

The work was published in *Science Translational Medicine*.

Typing class

Although the replicon assay used subtypes of HCV genotypes 1 and 2 to confirm the activity of C4 and A2, Glenn noted that 4BAH2 is conserved across all publicly available HCV genotypes and therefore should have broad activity against the infection.

Flossie Wong-Staal, professor emeritus of medicine at the **University of California, San Diego** and EVP and CSO of infectious disease company **ItherX Pharmaceuticals Inc.**, described the molecules as "potentially exciting," but added that she wants to see direct testing to show that they have specificity for the target and activity against all HCV genotypes.

> **"Time and cost savings can now be reallocated to development candidates. I think that it will be very important in future HCV drug discovery."**
>
> ***—Flossie Wong-Staal, ItherX Pharmaceuticals Inc.***

Eiger holds an exclusive license to the assay and the use of small molecules to treat HCV. The license covers C4 and A2 as well as Eiger's lead compound, clemizole, which is an inhibitor of the NS4B RNA-binding motif.[5] Clemizole is in Phase Ib testing for HCV.

The biotech has developed derivatives of C4 and A2 and plans to start pharmacokinetic and toxicology studies within 12–18 months.

Glenn is founder of Eiger and consults for the company. He is an associate professor of gastroenterology and hepatology and director of the Center for Hepatitis and Liver Tissue Engineering at Stanford University School of Medicine.

According to Glenn, unpublished results from his laboratory suggest that clemizole plus the C4-based 4BAH2 inhibitors can have a synergistic effect. In contrast, he noted that combinations of HCV protease or polymerase inhibitors have generally shown additive effects.

Sliver of liver

Whereas Glenn used conventional *in vitro* replicon assays to evaluate the efficacy of the 4BAH2 inhibitors, a team from MIT and Rockefeller University has developed a tool that may overcome limitations of the current *in vitro* system. Their tool for real-time visualization of HCV infection and viral inhibition might allow researchers to more quickly determine the efficacy, toxicity and metabolism profile of therapeutics by giving researchers a more relevant *in vitro* liver tissue model.

Cell cultures used to propagate HCV replicons and infectious viral particles have inherent drawbacks. Compared with an infected human liver, the cell cultures show abnormal proliferation, deregulated gene expression, aberrant signaling and endocytic functions, and limited viability and liver-like phenotype.[6,7]

In 2008, Salman Khetani and Sangeeta Bhatia at MIT took a step toward circumventing the lack of a good *in vitro* liver tissue model. The group developed a microscale liver tissue culture of precisely arranged primary human hepatocytes embedded in supportive stromal cells.[6]

The architecture of the microscale liver tissue cultures was similar to that of human liver slices and retained full hepatic phenotypic functionality, such as albumin secretion, urea synthesis and cytochrome P450 activity. In addition, the cultures expressed liver-specific genes that are relevant for evaluating drug metabolism and toxicity.

Khetani was a postdoctoral associate at MIT when the manuscript was published. He is now research director at **Hepregen Corp.**, which is using the microliver technology, dubbed HepatoPaC, to provide toxicity and drug screening services to companies. Bhatia is a professor of health sciences and technology, and electrical engineering and computer science at MIT and is the chair of Hepregen's scientific advisory board.

Although the MIT team was focused on using its system to assess pharmaceuticals for hepatotoxicity, the stage was set for possible assessment of the HCV life cycle in a more relevant *in vitro* tissue culture system.

Now, a team led by Bhatia and Charles Rice, professor and head of the laboratory of virology and infectious disease at Rockefeller University, has developed microscale liver tissue cultures that express all known HCV entry factors. The cultures are capable of sustaining persistent HCV infection and show that infection is inhibited when treated with known antivirals.[7]

The group has also developed the HCV-dependent fluorescence relocalization (HDFR) reporter system, which it thinks circumvents the problem of detecting low levels of infection.[8]

Rather than relying on a standard fluorescence signal from an HCV reporter virus or immunostaining, the HDFR system uses a fluorescent recombinant host protein that HCV cleaves in an attempt to thwart the innate immune response. If HCV is present, the cleaved form of the host protein accumulates. Thus, the technique provides a real-time model for studying compounds that inhibit the virus.

The work on HCV microcultures was published in the *Proceedings of the National Academy of Sciences* and research on the HDFR system was published in *Nature Biotechnology*.

"This novel and unique assay system, in which cytoarchitectonic and functional characteristics of human liver tissue have been maintained in culture, can be used to address the critical need for rapid functional assessment of compounds," said Wong-Staal. "Time and cost savings can now be reallocated to development candidates. I think that it will be very important in future HCV drug discovery."

Wang-Staal believes Bhatia and Rice's microscale primary human hepatocyte cultures could be instrumental in dealing with the next steps in moving C4 and A2 toward the clinic. She said these include "the screening of compounds to develop an SAR [structure-activity relationship]

database in order to optimize compounds for metabolic stability, enzymatic clearance and hepatotoxicity in human target cells."

ItherX's ITX5061, an HCV entry inhibitor, is expected to start Phase Ib testing later this year.

Patent applications for the HCV-infected microscale liver tissue cultures and HDFR reporter system have been filed by MIT and Rockefeller. The licensing status was not disclosed.

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