

Hepatitis C replicon system and drug development

2016 Lasker~DeBaakey Clinical Medical Research Award



Ralf F.W. Bartenschlager
Heidelberg University



Charles M. Rice
Rockefeller University



Michael J. Sofia
Arbutus Biopharma

For development of a system to study the replication of the virus that causes hepatitis C and for use of this system to revolutionize the treatment of this chronic, often lethal disease.

The 2016 Lasker~DeBaakey Clinical Medical Research Award honors three scientists who developed a system to study the replication of the virus that causes hepatitis C and used this system to revolutionize the treatment of this chronic, often lethal disease. For more than a decade, **Ralf F. W. Bartenschlager** (University of Heidelberg) and **Charles M. Rice** (Rockefeller University) attempted to coax the hepatitis C virus (HCV) to multiply inside lab-grown host cells. They

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triumphed. With similar perseverance and imagination, **Michael J. Sofia** (formerly at Pharmasset; now at Arbutus Biopharma) then exploited this system to test and invent candidate drugs, whose novel design allowed targeting of the liver, where HCV resides, and creation of a medicine with unprecedented potency and safety.

— **More Info**

HCV causes chronic liver infection in 130-170 million people worldwide and leads to more than 350,000 deaths each year. No vaccine is available and, without treatment, about 15-30 percent of infected individuals develop liver failure or cancer. Until the work of Bartenschlager, Rice, and Sofia, therapy included toxic drugs that many people can't tolerate and that often don't cure the disease.

Reaching consensus

To study a virus and develop drugs against it, scientists need a way to grow it in the lab. With the isolation of HCV in 1989, Michael Houghton ([Albert Lasker Clinical Medical Research Award, 2000](#)) opened a new avenue toward that end. Investigators could use standard recombinant DNA techniques to produce the virus's RNA. After putting it into cells, they expected that the host machinery would use this genetic code to construct infectious HCV.

The approach flopped.

As researchers tried to prod HCV to replicate in cells, they began wondering whether they had captured its entire sequence. Knowing that viral-preparation methods could introduce errors, Rice (then at

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Alexander Kolykhalov deployed a method for defining the complete HCV RNA that would avoid these technical pitfalls.

In 1996, their strategy revealed an unanticipated structure at the end of the virus. This finding, also reported by Kunitada Shimotohno (then at the National Cancer Center Research Institute, Tokyo), implied that all of the constructed genomes had failed to replicate in the lab because they lacked this crucial feature.

With high hopes, Rice made a series of HCV sequences that included the bona fide end and injected them into chimpanzees. Unfortunately, these RNAs produced no hepatitis or other signs of infection.

Undaunted, he performed further analysis and found that the RNAs had acquired sequence changes—either while being amplified in the lab or before that, when the virus was replicating inside an infected human. These genetic errors, he reasoned, weakened HCV's ability to propagate. He created a "consensus" genome—one that, at every position, held the most common RNA letter rather than a deleterious one.

The resulting HCV RNA infected chimps and gave them hepatitis, Rice and Jens Bukh (National Institutes of Health) independently reported in 1997. Researchers had designed and produced a functional virus in the lab. Surely the consensus RNAs would generate HCV in lab-grown cells and thus allow investigators to dissect its detailed biology.

Replicating success

In the meantime, Bartenschlager (then at the University of Mainz) had made a different consensus

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cells, but never detected replication. Rice's consensus RNA also flunked this test.

Bartenschlager had an idea. He knew that relatives of HCV could lose a chunk of their genomes—the region that encodes viral-packaging proteins—yet still multiply inside host cells. He wondered whether he could replace these dispensable sequences with a genetic marker that would expose cells that contain replicating HCV.

In the late 1990's, he and postdoctoral fellow Volker Lohmann gutted their consensus RNA and inserted a gene whose product confers resistance to a lethal drug. If the HCV RNA-copying machinery did its job, it would amplify not only viral RNA, but also that of the drug-resistance gene. Consequently, host cells that carried this “replicon” would withstand the otherwise deadly toxin.

The scheme worked. The ability of the replicons to bestow a survival advantage upon host liver cells had thus allowed researchers to detect HCV replication in the lab. Still, as they clinked in celebration, their brows wrinkled. Surviving cells contained thousands of RNAs, but only about one in a million cells that received the input RNA survived.

The replicons that flourished, it turned out, had picked up adaptive sequence changes inside cells, Rice and Bartenschlager independently showed. After the investigators engineered these replication-enhancing mutations back into the starting RNA, production of infected cells jumped 500- to 10,000-fold.

For the first time, researchers had generated efficient HCV replication in the lab. Because the replicons did not produce infectious virus particles, the system could

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Furthermore, the mini-genomes encoded proteins critical for HCV multiplication, important drug targets.

Unmasking a champion

The pharmaceutical industry now had a manageable way to test whether candidate agents thwart HCV inside living liver cells. New medicines were sorely needed. Standard regimens required weekly injections of interferon, which delivers severe side effects. Furthermore, treatment took 24-72 weeks and frequently failed.

In their quest to improve therapy, scientists at Pharmasset, a small biotech company founded by Raymond Schinazi, focused on HCV's RNA-copying enzyme. Its active site was similar among disparate types of HCV and the enzyme has no human counterpart, so perhaps the team could develop an inhibitor that would combat multiple HCV genotypes without disrupting host physiology.

The investigators set their sights on chemicals that resemble normal RNA building blocks, but differ in a crucial way. These so-called nucleoside analogs attach to a growing RNA chain, but when the copying enzyme tries to add the next subunit, it can't. The analogs' extra chemical groups interfere, and RNA elongation stops.

In 2005, Jeremy L. Clark, a member of the Pharmasset team, identified a nucleoside analog that blocks HCV replication in the replicon system. In people, its safety profile looked promising, but most of it broke down into an inactive form.

By modifying its chemical properties, the Pharmasset scientists, now under the direction of Michael Sofia,

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combined with a different class of drug that inhibits a different HCV enzyme, but large and frequent doses of the analog were required.

Aiming to boost its potency, Sofia hit on an unconventional idea. The team's studies had revealed that the original analog might not be a dead-end after all. Through a series of reactions, enzymes in the body convert a small proportion of it into a different compound that foils HCV replication and persists intact in liver cells.

To harness the potential of this powerful and stable inhibitor, Sofia had to overcome a substantial challenge. He needed to supply not the original analog, which is transformed inefficiently into the desired chemical, but a specific molecular relative. This relative carries a phosphate chemical group, whose negative charge renders it unable to traverse oily cell membranes.

Sofia and his colleagues aimed to mask the phosphate so the compound would slip into cells. In his dream scenario, liver enzymes would then disrobe the agent and, with its charged portion revealed, it would be stuck. Conversion enzymes would set upon it and turn it into the active drug. His vision also promised to minimize harmful effects that might result from delivery to other parts of the body. Only liver cells possess the natural metabolic capabilities that trap the compound inside.

With intense tweaking and evaluation, Sofia designed a chemical candidate that performed well in the replicon system and passed other tests. Early clinical trials showed extremely promising results, and Gilead Sciences acquired Pharmasset in early 2012. In January 2013, the Pharmasset group, Gilead and

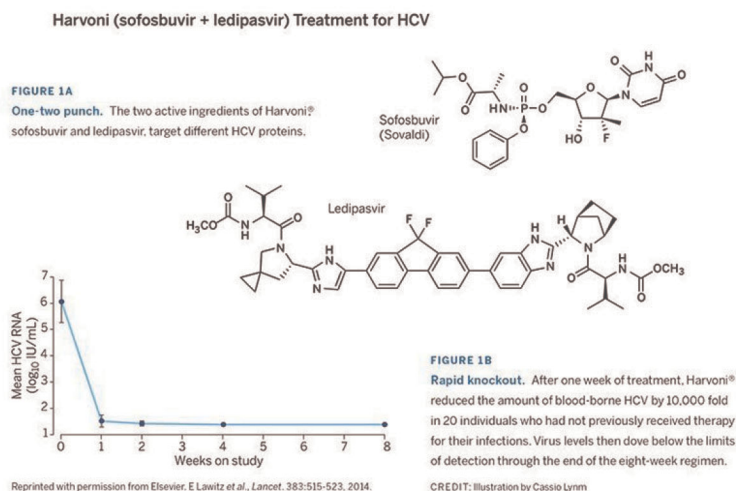
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dramatic findings. In combination with ribavirin, a toxic but non-interferon-based antiviral agent, the new drug, sofosbuvir, eradicated HCV long term. In people infected with some viral genotypes, no virus could be detected 24 weeks after a 12-week treatment period ended. The absence of interferon in this regimen launched a new era in curative HCV therapy.

On December 6, 2013, a sofosbuvir (Sovaldi®) regimen was approved by the US Federal Drug Administration (FDA) for some HCV genotypes. Finally, people with chronic HCV infections had an interferon-free therapy. Subsequently, sofosbuvir proved effective in diverse patient populations and across viral genotypes.

In the meantime, the replicon assay was fueling other scientists' discovery of new drugs. A Bristol-Myers Squibb group led by Min Gao identified a compound that targets an HCV protein of unknown function. This achievement showcased another strength of the replicon system—its ability to let the cell reveal what is important for replication, regardless whether scientists understand the underpinnings.



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Gilead rapidly developed a derivative of the compound Gao and colleagues had pioneered. The combination of Gilead's new agent, ledipasvir, plus sofosbuvir (Figure 1A) rapidly quashes the virus. This regimen boasts cure rates of 94-99% in only 8-12 weeks of therapy (Figure 1B), even among difficult-to-treat patients. The ledipasvir/sofosbuvir combination, Harvoni®, is now FDA-approved for numerous types of HCV infection, including the most common form in the US and Europe. It is the first HCV treatment that avoids both interferon and ribavirin. Four other such medications have since been approved. Sofosbuvir serves as the backbone of two, and the other two are based on different compounds.

Bartenschlager, Rice, and Sofia surmounted numerous hurdles as they devised innovative solutions to the biological and chemical obstacles that confronted them. Their victories culminated in a safe, effective, oral therapy for HCV that set a new standard and transformed the treatment of a devastating illness.

by Evelyn Strauss

Key Publications of Ralf F.W. Bartenschlager

Lohmann, V., Körner, F., Koch, J.O., Herian, U., Theilmann, L., and Bartenschlager, R. (1999). Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. *Science*. 285, 110-113.

Bartenschlager, R. and Lohmann, V. (2000). Replication of hepatitis C virus. *J. Gen. Virol.* 81, 1631-1648.

Krieger, N., Lohmann, V., and Bartenschlager, R. (2001). Enhancement of hepatitis C virus RNA replication by cell culture-adaptive mutations. *J. Virol.* 75, 4614-4624.

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Wakita, T., Pietschmann, T., Kato, T., Date, T., Miyamoto, M., Zhao, Z., Murthy, K., Habermann, A., Kräusslich, H.-G., Mizokami, M., Bartenschlager, R., and Liang, T.J. (2005). Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. *Nat. Med.* 11, 791-796.

Pietschmann, T., Kaul, A., Koutsoudakis, G., Shavinskaya, A., Kallis, S., Steinmann, E., Abid, K., Negro, F., Dreux, M., Cosset, F.L., and Bartenschlager, R. (2006). Construction and characterization of infectious intragenotypic and intergenotypic hepatitis C virus chimeras. *Proc. Natl. Acad. Sci. USA.* 103, 7408-7413.

Key Publications of Charles M. Rice

Grakoui, A., Lin, C., Wychowski, C., Feinstone, S., and Rice, C.M. (1993). Expression and identification of hepatitis C virus polyprotein cleavage products. *J. Virol.* 67, 1385-1395.

Kolykhalov, A.A., Feinstone, S.M., and Rice, C.M. (1996). Identification of a highly conserved sequence element at the 3' terminus of hepatitis C virus genome RNA. *J. Virol.* 70, 3363-3371.

Kolykhalov, A.A., Agapov, E.V., Blight, K.J., Mihalik, K., Feinstone, S.M., and Rice, C.M. (1997). Transmission of hepatitis C by intrahepatic inoculation with transcribed RNA. *Science.* 277, 570-574.

Blight, K.J., Kolykhalov, A.A., and Rice, C.M. (2000). Efficient initiation of HCV RNA replication in cell culture. *Science.* 290, 1972-1974.

Lindenbach, B.D., Evans, M.J., Syder, A.J., Wölk, B., Tellinghuisen, T.L., Liu, C.C., Maruyama, T., Hynes, R.O., Burton, D.B., McKee, J.A., and Rice, C.M. (2005).

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Complete replication of hepatitis C virus in cell culture. *Science*. 309, 623-626.

Key Publications of Michael J. Sofia

Sofia, M.J., Bao, D., Chang, W., Du, J., Nagarathnam, D., Rachakonda, S., Reddy, P.G., Ross, B.S., Wang, P., Zhang, H.R., Bansal, S., Espiritu, C., Keilman, M., Lam, A.M., Steuer, H.M., Niu, C., Otto, M.J., and Furman, P.A. (2010). Discovery of a β -D-2'-deoxy-2'- α -fluoro-2'- β -C-methyluridine nucleotide prodrug (PSI-7977) for the treatment of hepatitis C virus. *J. Med. Chem.* 53, 7202-7218.

Sofia, M.J., Furman, P.A., and Symonds, W.T. (2010). 2'-F-2'-C-methyl nucleosides and nucleotides for the treatment of hepatitis C virus: from discovery to the clinic. In: *Accounts in Drug Discovery: Case Studies in Medicinal Chemistry*. Chapter 11. Royal Society of Chemistry, London, pp. 238-266.

Sofia, M.J. (2013). Nucleotide prodrugs for the treatment of HCV infection. *Adv. Pharmacol.* 67, 39-73.

Sofia, M.J. (2014). Beyond sofosbuvir: what opportunity exists for a better nucleoside/nucleotide to treat hepatitis C? *Antiviral Res.* 107, 119-124.

Sofia, M.J. (2015). Sofosbuvir: a breakthrough curative therapy for the treatment of HCV infection. In: *Medicinal Chemistry Reviews*. Vol. 50. Med. Chem. Div., Am. Chem. Soc., Foster City, CA. pp. 397-416.

Editorial Comments on Therapy for Hepatitis C

Hoofnagle, J.H. and Sherker, A.H. (2014). Therapy for Hepatitis C – the costs of success. *New Engl. J. Med.*

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Liang, T.J. and Ghany, M.G. (2014). Therapy of Hepatitis C – back to the future. *New Engl. J. Med.* 370, 2043-2047.

Award presentation by Harold Varmus



2016 Clinical Award Overview - Harold Varmus

Lasker Foundation

09:01

keeping us in metabolic balance. But when damaged by alcohol, infiltrated by metastatic cancer, or felled by a viral infection, it can no longer be ignored.

Hepatitis C virus (or HCV) is one of the things that can bring the liver to our attention. The virus is growing persistently in the livers of an estimated 170 million people worldwide and over 3 million in the US. With time, HCV can destroy the liver's essential functions, since an infection is often a prelude to cirrhosis or liver cancer.

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Today we celebrate three people whose work has enabled medicine to eliminate infection with HCV. To eliminate, not just control, a chronic viral infection is unprecedented. And the story is unusual in other ways too.

First, why the “C”? In medical school, I learned about hepatitis A, a self-limited viral infection usually transmitted by food. And about hepatitis B, a chronic infection, usually contracted at birth or by transfusions or intravenous drug use, often causing cirrhosis or cancer.

Over the next twenty years, vaccines were developed to prevent these infections, and tests for hepatitis B virus made transfusions safer. But a big part of the hepatitis story was still missing. Then Harvey Alter, at the NIH Clinical Center, showed that another hepatitis virus, neither A nor B, must be in our blood supply: he could transmit it to non-human primates. But the usual methods did not help him to identify the so-called “non-A, non-B” hepatitis virus.

Where traditional virology failed, molecular biology rescued. In 1989, Michael Houghton and his colleagues at Chiron isolated a piece of nucleic acid from the blood of a patient infected with the non-A, non-B virus; they found that it was part of the genome of a novel virus, now called HCV, belonging to a known class of human pathogens. The fragment was enough to design a diagnostic test for HCV, protecting the millions who receive transfusions each year. In 2000, Alter and Houghton received a Lasker Clinical Prize for their work.

That fragment of the HCV genome could have been the toe-hold that led quickly to production of abundant virus and to the design of a vaccine and anti-viral

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and perplexing virus. Here is where today's heroes enter the story.

Charles Rice had been trained at CalTech, working on the class of viruses to which HCV belongs. So when that HCV fragment was announced, he saw its potential. By then, he had joined the faculty at Washington University in St. Louis, and his laboratory aimed to extend the fragment into a complete genome—a starting point for making infectious HCV in cultured cells. It should have worked easily, but it didn't. When he and others finally found the missing pieces, he learned that variations in HCV genomes impeded the production of virus in cultured liver cells. Finally he was able to puzzle together a genome that made virus, and the virus could infect a chimpanzee, a major achievement. But it still didn't multiply in cultured cells, so essential studies were stymied.

In a parallel universe, Ralf Bartenschlager trained in Heidelberg, working on the hepatitis B virus. After joining a pharmaceutical company in Germany, Ralf also saw the opportunities inherent in Chiron's piece of the HCV genome. Then he experienced many of the same frustrations. He and Charlie commiserated about them at international meetings for nearly a decade.

After returning to the university in Heidelberg, Ralf and one of his trainees, Volker Lohmann, conceived an interesting plan: they would try to multiply only a part of the viral genome in liver cells. The strategy precluded production of complete, infectious virus. But it allowed them to select partial genomes that multiplied efficiently. In turn, those efficient partial genomes allowed drug companies to seek compounds that inhibit viral functions.

It's remarkable that looking for anti-viral drugs was

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development of drugs that inhibit HIV, few anti-viral drugs existed. Treatment of viral infections, like HCV, if possible at all, usually depended on toxic drugs, like interferons, that bolstered host defenses. HIV taught us how to interfere specifically with enzymes essential for virus growth.

So ambitious chemists got to work on the partial genomes of HCV. Michael Sofia was among those. In 2005, Mike had just left a large pharmaceutical firm to work at Pharmasset, a much smaller company, which sought drugs against a few viruses, including HCV. One type of compound looked especially promising: it inhibited the viral enzyme that copied HCV RNA. But it didn't behave well in animals—rapidly decaying or failing to get into liver cells. With a series of chemical tricks, Mike and his team turned it into a drug that was concentrated and then activated in the liver. That drug, now called sofosbuvir, turned out to be highly effective against many strains of HCV; was impervious to drug-resistance; and became the common component of the several drug combinations approved by the FDA over the past three years. These combinations can eliminate HCV from as many as 99% of infected patients in several weeks of treatment, with minimal toxicity.

Some benefits to patients have been immediate: improved liver function and loss of infectiousness. Others are very likely: sharply reduced risks of cirrhosis or liver cancer.

These results are extraordinary. But thus far the number of HCV-infected people has declined only slightly, because most have not received the drugs. One reason is their cost. The public outcry about the costs has not escaped the notice of those of us on the Lasker Jury. We have also noted that routes to wider access



Clinical Award

Award

Presentation

**Acceptance
Remarks**

Award Video

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Our job today is to celebrate those who walked a winding scientific trail that led to miraculous drugs and the benefits of using those drugs. At the same time, let us resolve to find the means to provide them to all who can benefit. And let us hope that we have today explained why it is so important to do so.

Acceptance remarks

Ralf Bartenschlager



The 2016 Lasker Awards - Ralf Bartenschlager

Lasker Foundation

05:32

Cell Interview



Acceptance remarks, 2016 Lasker Awards Ceremony

Passion, most often requiring an adequate trigger, supporting people, and luck are the most important ingredients for a scientific career. I had the fortune to have all of them.

When I grew up with my grandparents in an

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future profession would be. This changed in high school where I met an excellent teacher who raised my interest in biology. Recognizing my new inspiration, my grandparents bought me a small microscope as a Christmas present that I used in my spare time to examine all sorts of biological samples, although often with disappointing results because of its poor resolution.

During those days I decided to study biology even though people were telling me at that time, the late 1970s, that the demand for biologists in Germany is very low; one biologist per year, so why should I be this person? Nevertheless, my passion for this topic was stronger than any concerns about my professional future, so after a 4-year interim time during which I was trained and practiced as a police officer, I began to study biology at Heidelberg University.

During my time as student, I developed a growing interest in molecular biology, which in Germany was at its infancy. Fortunately, there were several professors in Heidelberg, who did postdocs in the US and after their return to Germany started to build up labs that used most modern molecular biology methodologies. One of these labs was headed by Heinz Schaller. Heinz worked on hepatitis B virus, a topic that I found fascinating, but getting access to his lab was not an easy task. resistance. Fortunately, he accepted me as lab practical student to establish a method for cloning duck hepatitis B virus mutants. I assume I did my job right, because Heinz took me under his wing and really taught me the secrets of scientific working. I left his lab in 1991, and in 2002 I got the chance to return back to Heidelberg to set up a new

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research department made possible by a foundation set up by Heinz and his wife Chica.

Success in science also requires luck. My biggest luck was the privilege to host numerous incredibly talented students and postdocs in my lab. While all have contributed tremendously, there was one person who influenced my career most: Volker Lohmann, my very first PhD student. Recruiting Volker was one of the biggest strokes of luck in my scientific life as he was not only the key person to set up the first HCV cell culture system, but also an exceptional mind creating true team spirit and synergy within the research team.

Being a scientist is not just a job, it is a vocation—the pursuit of knowledge and the elation of discovery are the twin triggers of passion in science. But this vocation also brings long working hours, hectic days, and frequent travel. Without the continuous encouragement of my wife Judith, and without the enrichment that my children Marie, Nora, and Lorenz have provided to my life, I would not be here today. Thank you.

Passion, most often requiring an adequate trigger, supporting people, and luck are the most important ingredients for a scientific career. I had the fortune to have all of them.

When I grew up with my grandparents in an industrial suburb of Mannheim, my main interest was playing soccer and I had no clue what my future profession would be. This changed in high school where I met an excellent teacher who raised my interest in biology. Recognizing my new inspiration, my grandparents bought me a small microscope as a Christmas present that I used in

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Charles Rice

Michael Sofia

The 2016 Clinical Award video

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