Profile of Charles M. Rice, Ralf F. W. Bartenschlager, and Michael J. Sofia, 2016 Lasker–DeBakey Clinical Medical Research Awardees

Hepatitis C: From chronic to curable

Peter Palese[a,1]

Three researchers, Ralf F. W. Bartenschlager (University of Heidelberg), Charles M. Rice (The Rockefeller University), and Michael J. Sofia (Arbutus Biopharma, Inc.) (Fig. 1) share the 2016 Lasker–DeBakey Clinical Medical Research Award for their work, which has allowed for the development of a therapeutic drug against chronic hepatitis C. Finding a cure against this dreadful disease represents a major medical breakthrough, the importance of which cannot be overstated.

Hepatitis viruses, as the name implies, infect the liver and can be associated with a variety of illnesses. Hepatitis A viruses lead to a self-limited disease, which can be seriously debilitating for months. Hepatitis B viruses can cause an acute, short-term illness, but can also be associated with long-term chronic infection resulting in cirrhosis or liver cancer. Both hepatitis A and hepatitis B vaccines are highly efficacious and have been available for decades in the United States. Consequently, hepatitis A and hepatitis B are both preventable diseases.

What is different about hepatitis C? Critically, there is no vaccine available against this disease. Unfortunately, an estimated 170 million people are chronically infected with hepatitis C virus and ~350,000 lives are lost every year worldwide (1). These high numbers are comparable to the morbidity and mortality of other viral diseases, such as HIV and influenza. The WHO estimates that more than a million people die annually of AIDS-related illnesses and up to half a million people succumb to influenza globally every year (2, 3). The common trait shared by these top three viral diseases, hepatitis C, AIDS, and influenza, is extensive antigenic variation, which makes vaccine development and vaccination strategies a particular challenge (Fig. 2). Hepatitis C viruses come in many different flavors; therefore, an effective vaccine against hepatitis C has never been developed. Similarly, HIV strains undergo many antigenic changes, and effective vaccines against HIV (eliciting sterile immunity) are at best a lofty dream for the future. Even for influenza, an effective universal vaccine protecting against all seasonal and pandemic strains has not yet been achieved. Although anti-HIV drugs have changed the landscape for AIDS patients, there is no cure in sight against HIV infections. Similarly, antiviral drugs for influenza have been of limited value as well.

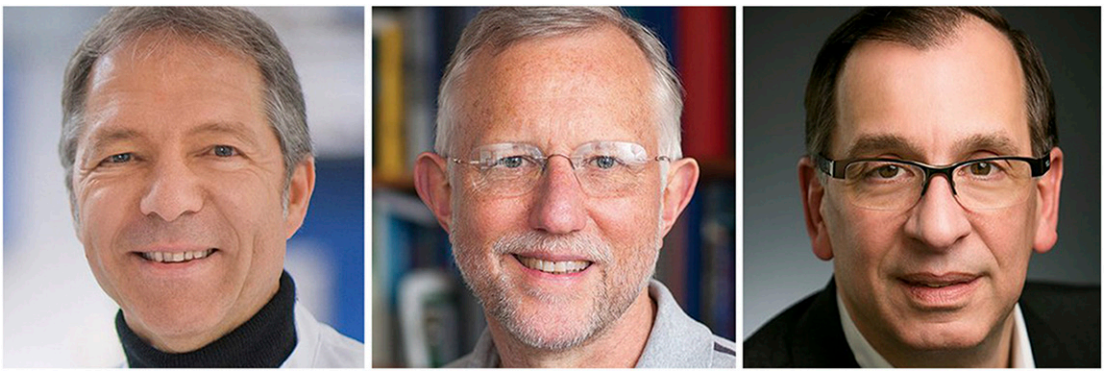

Fig. 1. Ralf F. W. Bartenschlager, Charles M. Rice, and Michael J. Sofia, pictured in order above, shared the 2016 Lasker–DeBakey Clinical Medical Research Award for their work on hepatitis C. Images courtesy of the Albert and Mary Lasker Foundation.

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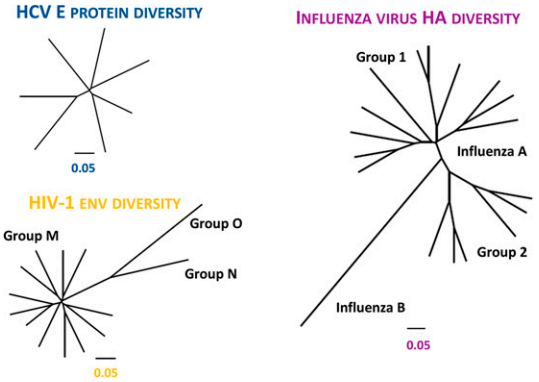

Fig. 2. Surface glycoprotein diversity of different viruses. The immunodominant surface proteins of hepatitis C, HIV, and influenza viruses dramatically vary antigenically. The three phylogenetic trees reveal large differences among the hepatitis C virus E proteins, the HIV envelope (Env) proteins, and the influenza virus hemagglutinin (HA) proteins. For each protein cluster, the scale bar shows a 5% amino acid difference. The variability of these proteins is one of the reasons that the development of effective vaccines against these viruses has not been successful. Figure provided by Florian Krammer.

Enter the 2016 Lasker–DeBakey Award Winners Developing a Curative Treatment for Hepatitis C

Charles "Charlie" M. Rice completed his graduate studies in the laboratory of Jim and Ellen Strauss at the California Institute of Technology, where he worked with Sindbis virus to determine the first genome sequences for this RNA-containing virus. By 1987, Charlie had succeeded in generating the first infectious clone for Sindbis virus, which was a notable achievement at the time (4). Importantly, this milestone would pave the way for overcoming a key obstacle in the hepatitis C virus field. Harvey Alter, at the NIH, had found evidence for a non-A and non-B hepatitis, and Michael Houghton, at the time at Chiron Corporation, discovered the RNA-containing virus associated with this disease. Building on this finding, Charlie set out to map all of the proteins of the hepatitis C virus and to thoroughly study the RNA of the virus (Fig. 3). Charlie and Kunitada Shimotohno in Japan demonstrated that the 3′ terminus of the virus's RNA (as published at the time) was incomplete (5). Having the correct sequence, Charlie's group was then able to generate an infectious molecular clone, which allowed his laboratory to complete early molecular studies on hepatitis C virus (6). Another of Charlie's wonderful scientific achievements was the work on the structure and function of the hepatitis C virus NS5A protein. This phosphoprotein has now become a target for several Food and Drug Administration (FDA)-approved drugs. Charlie's discoveries and, equally important, the many tools he developed, have been essential for identifying potent drugs against hepatitis C viruses (7) (Fig. 3).

In Germany, Ralf Bartenschlager came to viruses via a more circuitous route. His educational path was rather unusual. Having first been trained as a police officer, Ralf became fascinated with one aspect of police work, the forensic laboratory. From the Police Academy, Ralf went on to study at the University of Heidelberg and joined the laboratory of the late Heinz Schaller, an established hepatitis B virus researcher. The rest is history. After completing his graduate studies, Ralf joined Hoffmann-La Roche in Switzerland. There, he presciently started a program on hepatitis C viruses. Ralf left the "dark side" of industry after a short period, and with Volker Lohmann went on to start an academic laboratory at the University of Mainz. Together, Ralf and Lohmann succeeded in building a subgenomic replicon system of the hepatitis C virus in tissue culture, which represented a major breakthrough (8). Such a self-replicating RNA system became the basis for much of what Ralf's laboratory achieved in the following years. Even though such a system does not necessarily produce infectious viruses, it is indispensable as a first step for studying the life cycle of the virus, functions of the viral proteins, as well as the interactions with the host cell. Working in collaboration with Takaji Wakita, improvements to this system subsequently led to the successful replication of hepatitis C viruses in tissue culture (9–11). The virus produced from this system not only replicated in tissue culture, but also in chimpanzees and humanized mice. This was the Holy Grail for hepatitis C virus research.

Research Culminating in a Cure

Before the availability of NS5A, NS5B, and protease inhibitors against hepatitis C viruses (Fig. 3), treatments involving the administration of IFN and ribavirin were 6- to 12-months long and were associated with severe side effects. Noncompliance by patients was a serious problem and the overall cure rate was seldom better than 50%. As the replicon system became available and molecular biology revealed the Achilles heels of the virus, a path toward a cure suddenly became visible. Novel chemical entities could be tested in vitro and

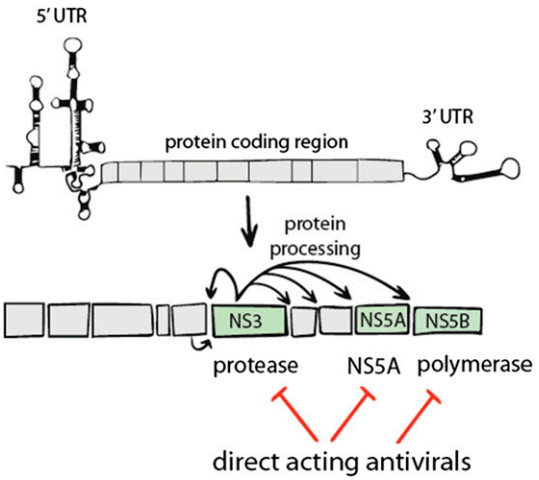

Fig. 3. Hepatitis C virus (HCV) genome structure. The plus strand RNA virus with a genome of fewer than 10,000 nucleotides codes for structural and nonstructural genes (5′ UTR: 5′ untranslated region; 3′ UTR: 3′ untranslated region). The expressed polyprotein is processed into a series of viral proteins by viral and cellular proteases. FDA-approved direct-acting antivirals have been identified targeting the NS3 protease and the NS5A and the NS5B proteins (green). Cartoon kindly provided by William Schindler and Charles Rice.

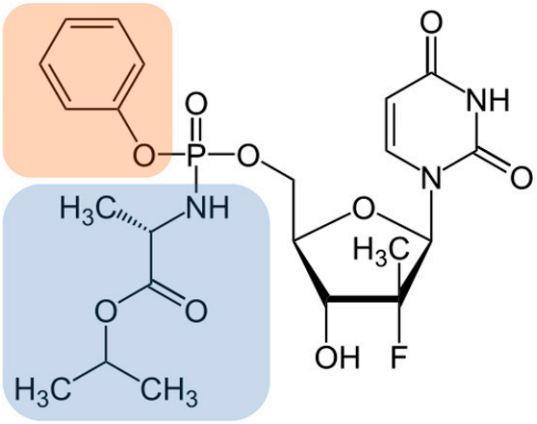

Fig. 4. Structure of sofosbuvir. This prodrug of a potent RNA-dependent RNA polymerase inhibitor of the hepatitis C virus has a 2′-α-fluoro-2′-β-C-methyluridine core structure. It was found that the monophosphate of this core structure was readily metabolized to the triphosphate within cells and had potent antiviral activity. However, it was also found that the monophosphate had a low bioavailability. Interesting new chemistry allowed masking the charged phosphate by synthesizing a phosphoramidate (blue) prodrug in which the second OH group of the phosphate was also blocked (pink). This prodrug enters liver cells and gets metabolized into the highly active triphosphate derivative. The final prodrug is actually an isomer which required the design of a previously unknown chemical synthesis step.

sophisticated chemistry was applied to generate hundreds of derivatives.

The third recipient of the Award, Michael Sofia, received his undergraduate degree in chemistry from Cornell University and his doctorate in organic chemistry from the University of Illinois Urbana–Champaign. Following two decades as a research scientist at the Squibb Institute for Medical Research, Eli Lilly and Company, Bristol-Myers Squibb, and several smaller companies, Michael Sofia joined Pharmasset in 2005. The company had previously identified a nucleoside with antiviral activity through the hepatitis replicon assay. This molecule (PSI-6130) targeted the NS5B RNA polymerase (12) and was then derivatized to become more bio-available and to be taken up easily by liver cells. Elegant chemistry by Michael Sofia's group was applied to mask the monophosphate of the uridine derivative, which allowed the compound to enter liver cells. In the liver, the masking group is removed by tissue-specific enzymes to allow the addition of two new phosphates to generate the derivatized uridine triphosphate, which serves as a chain terminator for the RNA-dependent RNA polymerase of the virus. Therefore, the drug not only works in a tissue-specific way, but also later was found to prevent the emergence of large numbers of viral escape mutants. The latter finding is unexpected and adds to the success story of this drug. In clinical trials, it was shown that this compound, PSI-7851, was safe and that it could be given only once a day. After further optimization studies, the isomer PSI-7977 (sofosbuvir) (Fig. 4), proved to be exceptionally potent in humans (13). First, the isomer was given together with IFN but soon it was found to be equally effective in combination with only ribavirin. FDA approval for sofosbuvir, in combination with ribavirin or in combination with pegylated IFN and ribavirin, came in 2013. Later, sofosbuvir was tested with the NS5A inhibitor ledipasvir and found to be extraordinary in curing people infected with the virus. FDA approval for this combination came in 2014. With this treatment option, and several other nonsofosbuvir-based medications that are now available, a cure rate approaching 100% following a mere three-month treatment course is possible.

What Made a Cure of Hepatitis C Possible?

Few, if any, developments in medical research have been so successful in the last decade as the curative antihepatitis C medication. Major advances in molecular biology and a greatly enhanced understanding of the virology of hepatitis C viruses made it possible to develop replicon systems in tissue culture. For many years, the virus successfully resisted attempts to be grown in the laboratory, and the only animal species in which the virus grew were nonhuman primates. The tissue-culture replicon system allowed the screening of small molecular-weight compounds with antiviral activity and—once a lead compound was identified—it allowed meaningful structure activity relationship studies. The chemistry necessary to make the compounds bio-available and effective was by no means trivial. Thus, the confluence of exceptional molecular biology/virology and cutting-edge chemistry at the right time were key to success. These efforts were generously funded by the NIH, German agencies, and philanthropy (both in the United States and in Germany). One piece of the puzzle that frequently goes unmentioned is the entrepreneurial spirit that provided the vision and imaginative breeding ground allowing the work on nucleoside derivatives to go forward. A small company, Pharmasset, founded by two Emory University scientists, Raymond Schinazi and Dennis Liotta, focused on nucleoside antivirals, whereas larger companies pursued other avenues. Without the entrepreneurial drive and the open business climate in this country, it is unlikely that sofosbuvir could have been developed so effectively and in such a short timeframe. One can only hope that such a winning combination of wonderful basic science, remarkable chemistry, and entrepreneurial spirit will be equally successful against many other unconquered diseases.

There is plenty left to do!

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