Pioneering a Global Cure for Chronic Hepatitis C Virus Infection

Silvia Vilarinho1 and Richard P. Lifton1,*
1Departments of Genetics and Internal Medicine, Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT 06510, USA
*Correspondence: richard.lifton@yale.edu
http://dx.doi.org/10.1016/j.cell.2016.08.038

This year’s Lasker~DeBakey Clinical Medical Research Award honors Ralf Bartenschlager, Charles Rice, and Michael Sofia, pioneers in the development of curative and safe therapies for the 170 million people with hepatitis C virus infection.

The liver plays a major role in energy homoeostasis, produces essential plasma proteins, and mediates detoxification and excretion of metabolic products. As a result, loss of liver function is lethal within days. Despite a remarkable capacity for regeneration, continuing insult leads to chronic disease, resulting in replacement of hepatocytes by fibrotic tissue. This results in high venous pressure, shunting blood through esophageal veins and resulting in varices that are prone to catastrophic rupture, and in fluid accumulation in the abdominal cavity (ascites) which frequently becomes infected. Additionally, loss of metabolite detoxification results in encephalopathy.

Causes and Impact of End-Stage Liver Disease

The major causes of end-stage liver disease (ESLD) are viral infection with hepatitis B or C and excessive alcohol consumption. ESLD accounts for 700,000 deaths per year world-wide (Xu et al., 2016) and, in the United States alone, results in $4 billion in health care expenditures and $11 billion in lost productivity and quality of life. Liver transplantation can restore hepatic function in suitable patients: the 2012 Lasker Clinical Award recognized Thomas Starzl and Roy Calne for their development of liver transplantation.

In the 1970s, the viruses causing hepatitis A and hepatitis B were identified. The development of serologic tests for hepatitis B led to its elimination from transfusion products, and highly efficacious vaccines were developed that prevent infection. Baruch Blumberg received the Nobel Prize in 1976 for his work on hepatitis B, and Maurice Hilleman received the 1983 Lasker Public Service Award for development of the hepatitis B vaccine.

Discovery of HCV and Its Prevalence

Serologic testing showed that hepatitis A and B viruses together explained only ~50% of viral hepatitis because plasma from patients with non-A, non-B hepatitis, after passage through a fine filter, could transmit hepatitis to chimpanzees. This led to the identification, in 1989, of hepatitis C virus (HCV), an enveloped plus strand RNA virus constituting a distinct genus of Flaviviridae. HCV encodes a single open reading frame of ~3,000 amino acids: this primary translation product is processed to produce ten mature proteins, including amino-terminal structural proteins and, distally, proteins required for pro-protein processing and replication (Figure 1).

The discovery of HCV allowed development of screening tests for chronic infection, resulting in elimination of HCV from transfusion products and recognition that 170 million people worldwide, including 3.2 million in the US, have chronic HCV infection, resulting in 350,000 deaths per year. HCV is predominantly transmitted via intravenous drug use and re-use of needles in medical care. Following acute infection, which is typically asymptomatic, 75%–85% of subjects develop chronic infection, and 20%–30% of these progress to cirrhosis within 20–30 years. Once cirrhosis is established, each year 1%–5% develop hepatic decompensation (ascites, variceal hemorrhage, and/or hepatic encephalopathy) and/or hepatocellular carcinoma (Hajarizadeh et al., 2013) (Figure 1). In the US, HCV is the most common cause of liver transplantation and liver cancer, and HCV causes more deaths annually (20,000) than HIV-1 (12,000). For their discovery of HCV, in 2000, Michael Houghton at Chiron and Harvey Alter at NIH received the Lasker Clinical Award.

Challenges to HCV Prevention and Treatment

Efforts to develop an effective HCV vaccine have not as yet been successful, in part, because HCV is highly heterogeneous, comprising six major genotypes (1 to 6), with numerous subtypes (e.g., 1a, 1b, etc.) and quasispecies. Genotype 1 is the most prevalent, but non-genotype 1 HCV collectively causes more than half of chronic HCV. The scope of the vaccine challenge is underscored by the fact that individuals who have spontaneously cleared HCV infection can be reinfected. These observations, along with the high burden of currently infected subjects who will progress to liver failure, have motivated efforts to develop efficacious therapeutics. Initial therapies were not specifically targeted to HCV. In 1991, the Food and Drug Administration (FDA) approved use of α-interferon, which produced sustained virologic response (SVR; no detectable virus 12 weeks after therapy, evidence of cure) in only 5%–10% of patients. In 2001, the addition of ribavirin, a guanosine analog with activity against many RNA and DNA viruses, increased SVR to 40% following 48 weeks of treatment. Unfortunately, this treatment regimen was poorly tolerated due to significant side effects, such as flu-like symptoms, depression, and bone marrow
suppression, requiring dose reduction or drug discontinuation. Moreover, treatment needed to be tailored to specific viral and host genotypes. Despite these limitations, patients cured of infection showed markedly reduced progression of liver failure, need for transplantation, and death (Morgan et al., 2010), providing strong incentive to develop improved therapeutics.

**Development of Subgenomic HCV Replicons**

Efforts to develop antivirals specifically targeting HCV gene products were greatly hindered by the inability to propagate virus or replicate the HCV genome in cell culture, requiring low-throughput testing in chimps or humans. The breakthrough overcoming this limitation came from two of this year’s Lasker Awardees, Charles Rice at Rockefeller University and Ralf Bartenschlager at University of Heidelberg. Considering the lack of infectivity of presumed full-length HCV genomes, Rice noted that the reported 3′ end of the viral genome was unusually short, and lacked structural features involved in replication of related viruses. His group purified viral RNA from infected patients, ligated oligonucleotides to the 3′ end, and used a complementary primer for cDNA synthesis, followed by PCR to amplify the complete viral 3′ end. The results demonstrated a previously missing 98-base extension of the HCV 3′ terminus, including a hairpin in the last 50 bases (Kolykhalov et al., 1996). Moreover, this sequence was highly conserved among different HCV genotypes, supporting its functional importance. Similar results were obtained independently by Kunitada Shimotohno of the Chiba Institute of Technology (Tanaka et al., 1996). Rice and colleagues then showed that now full-length HCV transcripts, when injected into the livers of chimpanzees, produced hepatitis, with seroconversion and production of virus containing RNA identical to the injected RNA (Kolykhalov et al., 1997). This provided formal proof that HCV alone can produce hepatitis and reinvigorated efforts to produce a robust cell culture model of HCV infection.

Building upon these efforts, Bartenschlager and colleagues (Lohmann et al., 1999), using methods previously applied to bovine diarrhea virus, produced subgenomic fragments of HCV that excluded genes encoding amino-terminal structural proteins but included a selectable marker linked to viral RNA-dependent RNA polymerase, viral proteases, and NS5A (a gene of then-unknown function). Introduction of these subgenomic constructs into a hepatoma cell line demonstrated their replication, albeit in only ~1/10^6 cells, suggesting that host cell factors inhibited replication.

A subsequent study from the Rice lab produced similar sub-genomic replicons from a different HCV genotype (Blight et al., 2000). In a clever extension of this experiment, they isolated clones of cells expressing the selectable marker and sequenced the replicated viral RNA. They found 9 independent clones with mutations that clustered within a 30 codon segment of the viral NS5A gene and a 47 amino acid deletion in a downstream segment of NS5A required for interferon’s ability to inhibit viral replication. They inferred that NS5A plays a role in viral replication and that host gene products inhibit replication via effects on NS5A. Importantly, by introducing NS5A mutations into subgenomic replicons, they dramatically increased the fraction of cells supporting viral RNA replication to >10%.

These subgenomic replicons were highly useful in enabling detailed characterization of aspects of HCV biology. Yet, more critically, they provided for the first time the essential substrate for cell-based screening for effective inhibitors of HCV replication, a critical asset for drug development.

**Development of Direct-Acting Antiviral Drugs and Combinations**

The first drugs specifically tailored to specific HCV gene products were inhibitors of
the viral protease (NS3-4A), boceprevir and telaprevir, which gained FDA approval in 2011. In combination with interferon and ribavirin for 24–48 weeks, these inhibitors increased SVR to 50%–80% for viral genotypes 2 and 3 but were less successful for genotype 1.

The next drug, sofosbuvir, was discovered by the third of this year’s Lasker awardees, Michael Sofia, then at Pharmasset. His team noted prior work demonstrating that a uridine analog showed no inhibition of HCV replication; however its 5’ triphosphate was a potent inhibitor. They devised a clever strategy, using a prodrug approach first developed by the late Chris McGuigan of Cardiff University. McGuigan discovered that nucleoside 5’ monophosphates, commonly highly impermeable to cells, can traverse membranes when charges on the phosphate are neutralized. Upon entering cells, certain derivatives are metabolized to the nucleoside monophosphate. Sofia and colleagues developed a phosphorimidate prodrg of the 5’-phosphate derivative of the β-D-20-deoxy-20-R-fluoro-20-β-C methyluridine nucleoside that entered hepatocytes, was metabolized to its monophosphate derivative, and then phosphorylated by the host cell to a potent triphosphate inhibitor. This compound was a sub-micromolar inhibitor of HCV replication in cultured cells, and produced high levels of the triphosphate derivative in liver when administered in vivo (Sofia et al., 2010). In early-phase clinical trials, sofosbuvir showed potent antiviral activity in combination with interferon and ribavirin.

These findings were not lost on Gilead Pharmaceuticals. Gilead was highly successful developing multidrug therapies for treatment of HIV-1 infection and was taking transformative therapies for HCV. They saw sofosbuvir as a potentially key drug for combination therapy and, in 2011, purchased Pharmasset for the breathtaking sum of $1 billion, a notably steep price for a molecule that had not successfully completed a phase III clinical trial. Subsequent pivotal trials showed that sofosbuvir in combination with interferon and ribavirin for patients with HCV genotypes 1 and 4 and with sofosbuvir plus ribavirin for genotypes 2 and 3 produced SVR in 80%–95% of patients in 12–24 week treatment regimens. These treatments received FDA approval in 2013 and represented a major advance, while leaving interferon and ribavirin in treatment regimens.

The next direct-acting anti-HCV drug came from Min Gao and colleagues at Bristol-Myers Squibb (Gao et al., 2010), who performed an elegant unbiased screen to identify novel inhibitors of HCV replication using subgenomic replicons. They identified daclatasvir, which showed unprecedented pM inhibition in the replicon system and also inhibited replication of a full-length HCV virus, isolated by Takaji Watanaka at National Institute of Infectious Diseases in Japan. By sequencing resistant viral replicons, they found that resistance mutations clustered within the N-terminal 100 amino acids of NS5A, implicating this as the drug target, a finding foreshadowed by Rice’s prior finding that NS5A mutations promoted viral RNA replication. They also demonstrated additive or synergistic effects of these NS5A inhibitors when combined with NS5B inhibitors, protease inhibitors or interferon/ribavirin, and showed a remarkable >3 log reduction in viral load following a single oral 10 mg dose in people with genotype 1 HCV infection. This discovery underscores the value of unbiased screening for the desired phenotype; the normal biochemical function of NS5A remains poorly understood.

Daclatasvir’s picomolar IC50 made it attractive for formulation with other highly active drugs. Gilead developed its own NS5A inhibitor, ledipasvir, formulated a fixed dose one pill per day combination of sofosbuvir/ledipasvir, and performed clinical trials with or without ribavirin. The combination of two highly efficacious drugs in a single tablet is particularly important for preventing the emergence of resistant virus. The results were spectacular: 96%–99% of previously untreated patients with and without cirrhosis achieved SVR after 12 weeks of treatment. Similarly, among previously treated patients who had not achieved SVR, 94% achieved SVR at 12 weeks with ledipasvir/sofosbuvir and 99% at 24 weeks (Afdhal et al., 2014). The addition of ribavirin conferred no significant benefit, allowing its elimination from the treatment regimen. Sofosbuvir/ledipasvir was approved by the FDA in October, 2014 and immediately changed the treatment of HCV. With extremely high cure rates, and outstanding safety, there was no longer a compelling clinical reason for patients to wait to be treated. Additional combination treatments free of interferon and ribavirin have obtained FDA approval for treatment of select HCV genotypes.

In 2015, Gilead reported a fixed-dose combination of sofosbuvir plus velpatasvir, another NS5A inhibitor, which had improved pan-genotypic effect. After 12 weeks of treatment, 99% of patients with all genotypes except genotype 3 achieved SVR (95% for genotype 3) including untreated and previously treated patients with and without cirrhosis (Curry et al., 2015; Foster et al., 2015; Feld et al., 2015). The combination was extremely safe, with no recurrent serious adverse events. This combination was approved by the FDA in June, 2016.

Lastly, the recurrence and accelerated progression of HCV infection in transplanted livers has been the main cause of graft loss, with 20% of infected transplants progressing to cirrhosis within five years. Sofosbuvir/ledipasvir plus ribavirin for 12 weeks has proved highly effective in eradicating post-transplant infection.

The Goal and Challenge of Treating All Infected People

The development of highly efficacious and non-toxic therapy that cures virtually all patients with HCV infection is a triumph of biomedicine. This advance has come with some controversy. Initially priced at $94,000 for a 12-week course of sofosbuvir/ledipasvir, treating all in the US diagnosed with HCV would cost >$130 billion over 5 years, with only 20% of that amount offset by reduced cost of care of these patients. Nonetheless, the cost of a 1-year gain in quality-adjusted life years was estimated at $55,000 (Chhatwal et al., 2015), which compares favorably with other new therapeutics. While competition has already cut typical cost by half, this price still leaves treatment of all who would benefit far beyond reach. Companies have shown some willingness to provide drugs for free or at a steep discount and/or have allowed poorer countries to make the drugs for use in their own populations.

Given the high efficacy and safety, it would be ideal to cure all chronically infected individuals. This would both
eliminate their risk of progressing to advanced liver disease and cancer and would drastically reduce viral transmission and new infection. This is currently considered financially impractical, forcing the question of who to treat. The American Association for the Study of Liver Disease (AASLD) and the Infectious Disease Society of America (IDSA) initially recommended “highest priority” for treating patients with advanced fibrosis and cirrhosis and “high priority” for those with moderate liver fibrosis. Recently, as efficacy and safety data mount, AASLD and IDSA recommendations have been revised to recommend treatment for virtually all patients with chronic HCV infection. Reconciling this recommendation with the current financial burden of treatment poses a significant challenge.

Perspective
Thirty years ago, people died from non-A non-B viral hepatitis without even obtaining a definitive diagnosis. The discovery of HCV allowed its diagnosis and elimination from the blood supply. The exceptional work of this year’s Lasker Awardees, Bartenschlager, Rice, and Sofía along with many other contributors to drug development and clinical trials, has dramatically improved the future of millions of people with HCV infection. The simplicity, efficacy, and safety of current regimens now generally allows therapy to be administered by physicians without specialized training or the need for viral genotyping, with treatment of all infected people worldwide limited largely by the high cost of therapy. Rather than withholding treatment until people have advanced liver disease, it would seem wise to establish drug pricing that would incentivize health care systems to treat all infected subjects—a much larger cohort—thereby both eradicating infection and risk of ESLD while concomitantly reducing viral transmission and new infection.

These remarkable advances in the treatment of HCV infection underscore the critical role of a vibrant biomedical ecosystem that can effectively combine clinical observation, basic science, drug development, clinical trials, and health care delivery to dramatically change the course of a major public health problem and improve the lives of millions of people. Such a successful outcome relies on the strength of each component, along with effective integration across disciplines. When this occurs, the understanding of disease pathogenesis, followed by development of transformative therapeutics and their delivery to those in need, can have profound effects on human health.

REFERENCES