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Development of sofosbuvir for the treatment of hepatitis C virus infection

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The nucleotide analog NS5B polymerase inhibitor sofosbuvir was approved by the U.S. Food and Drug Administration (FDA) in December 2013 for the treatment of chronic hepatitis C virus (HCV) infection in combination with ribavirin or peginterferon and ribavirin. Sofosbuvir was developed to meet an urgent medical need for shorter, safer, simplified, more effective HCV treatment regimens and to reduce or eliminate the need for peginterferon. New treatment regimens were especially required for patient populations with limited treatment options, including patients who had failed prior HCV therapy, those with compensated and decompensated cirrhosis, and those who were either intolerant of or had contraindications to interferon. Sofosbuvir plus ribavirin for patients with genotype 2 or 3 HCV infection was the first approved all-oral treatment option. Sofosbuvir is also the backbone of the first regimen available for patients awaiting liver transplantation to prevent HCV recurrence, as well as the first oral interferon-free regimen for patients coinfected with HCV and HIV. This paper describes the development of sofosbuvir up to its original FDA approval.

Keywords: hepatitis C; sofosbuvir; direct acting antivirals; drug development

Background: hepatitis C virus

First identified in 1989 as the previously elusive causative agent of non-A, non-B hepatitis, hepatitis C virus (HCV) is a single-stranded RNA virus transmitted primarily through blood or blood product exposure. The incidence of acute infection in the United States declined dramatically following the introduction of universal HCV antibody screening of blood donors in 1992.[1] While spontaneous eradication of acute infection occurs in 10–50% of cases, most patients develop chronic HCV infection.[2]

Chronic HCV infection is a serious, progressive, and potentially life-threatening disease affecting an estimated 180 million people worldwide, including more than three million people in the United States.[3,4] Asymptomatic progression of liver disease can occur gradually over several decades;[2] many with cirrhosis today were infected with HCV decades ago.

Depending on several cofactors, 10–40% of patients with chronic HCV will develop cirrhosis of the liver and will be at risk for such complications as bleeding varices, ascites, hepatic encephalopathy, and hepatocellular carcinoma.[5] Chronic HCV infection results in approximately 10,000 deaths each year in the United States alone, and in 2007, HCV surpassed HIV as a cause of death.[5,6]

Belonging to the genus *Hepacivirus* in the family Flaviviridae, HCV is an enveloped virus with a positive-sense, single-stranded RNA genome.[7] The virus has significant genetic (RNA sequence) variability and is classified into at least six genotypes. The most common HCV genotype in North America and Europe is genotype 1, followed by genotypes 2 and 3.[8,9] Genotype 4 is most prevalent

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in the Middle East, while genotypes 5 and 6 are rare and found primarily in South Africa and Southeast Asia, respectively.[10]

Patients with HCV genotypes 1 and 4 have historically had lower response rates with interferon-based therapy than those with genotypes 2 or 3, while those with genotypes 5 and 6 have had intermediate response rates.[11–15] The virus' rapid replication—with an estimated 10^{12} virions produced per day in an untreated patient[16]—combined with the lack of a proofreading mechanism results in the development of many genetic variants, or quasispecies, within an infected individual,[17] which can result in rapid selection of resistant variants during antiviral treatment.

History of HCV therapy

Unlike HIV and other chronic viral diseases, HCV can be cured by finite treatment. The established surrogate end point for cure in clinical trials is sustained virologic response (SVR), which is defined as HCV RNA below the limit of quantification a fixed interval (12 or 24 weeks) after the end of treatment. Two distinct periods mark the history of HCV treatment: the interferon era, beginning in the mid-1980s, and the era of direct-acting antivirals (DAAs), launched 25 years later with the approval of first-generation DAAs for HCV.

The first effective HCV treatment was injected recombinant interferon-α monotherapy. The efficacy of interferon was improved incrementally by extending treatment from 24 to 48 weeks, by combining with oral ribavirin, and later by substituting convention interferon with long-acting pegylated interferon (which also reduced the frequency of administration from three times per week to once weekly) (Fig. 1). Peginterferon/ribavirin remained the standard of care for more than a decade.

The introduction of the first direct-acting antivirals—the protease inhibitors boceprevir and telaprevir in 2011—ushered in a new era in the management of HCV therapy. Up to 80% of HCV genotype 1 patients achieved SVR with 24–48 weeks of therapy with a protease inhibitor plus peginterferon/ribavirin.[18–21] However, their development has been described as a "holding strategy;" regimens composed of injected interferon plus first-generation protease inhibitors are still 24–48 weeks in duration; have a complex dosing schedule, requiring intensive on-treatment monitoring required for efficacy and futility; have a high pill burden; and carry undesirable side effects—including cytopenias, depression, autoimmunity, and rash—that undermine the benefits of these DAAs.[22] In addition, boceprevir and telaprevir were approved for use in only genotype 1 HCV patients; 24 weeks of peginterferon/ribavirin remained the recommended treatment for patients infected with HCV genotype 2 or 3.

Peginterferon/ribavirin-containing regimens are associated with significant side effects and suboptimal response rates, and are less tolerable in patient populations with the greatest clinical need, including the elderly and patients with cirrhosis.[23,24] The toxicity and tolerability issues associated with peginterferon-containing regimens, including those regimens that contain a first-generation protease inhibitor, left many patients unable or unwilling to be treated, including those with the most advanced liver disease.[25]

There was a clear unmet medical need for shorter, safer, simplified HCV treatment regimens that were effective across all HCV genotypes and that eliminated or reduced the need for peginterferon. New treatment options were especially crucial for patients who could not tolerate interferon or for whom interferon was not recommended, including patients who had not responded to prior HCV therapy.

Sofosbuvir was discovered in 2007 by a team at Pharmasset, Inc., led by Michael Sofia, who was the principal inventor of the drug. The compound, which was originally designated PSI-7977 and renamed GS-7977 when Gilead Sciences acquired Pharmasset at the end of 2011, received approval from the USAN Council for the non-proprietary name "sofosbuvir" in May 2012.

Preclinical development of sofosbuvir

The HCV genome encodes three structural and seven non-structural proteins.[26,27] The nonstructural protein NS5B polymerase replicates the viral RNA genome, and its catalytic site is highly conserved across HCV genotypes, making this protein an attractive target for the development of nucleoside/nucleotide inhibitors.[27,28] Sofosbuvir is a novel HCV NS5B–directed inhibitor discovered to confer potent inhibition of HCV RNA replication *in vitro*.

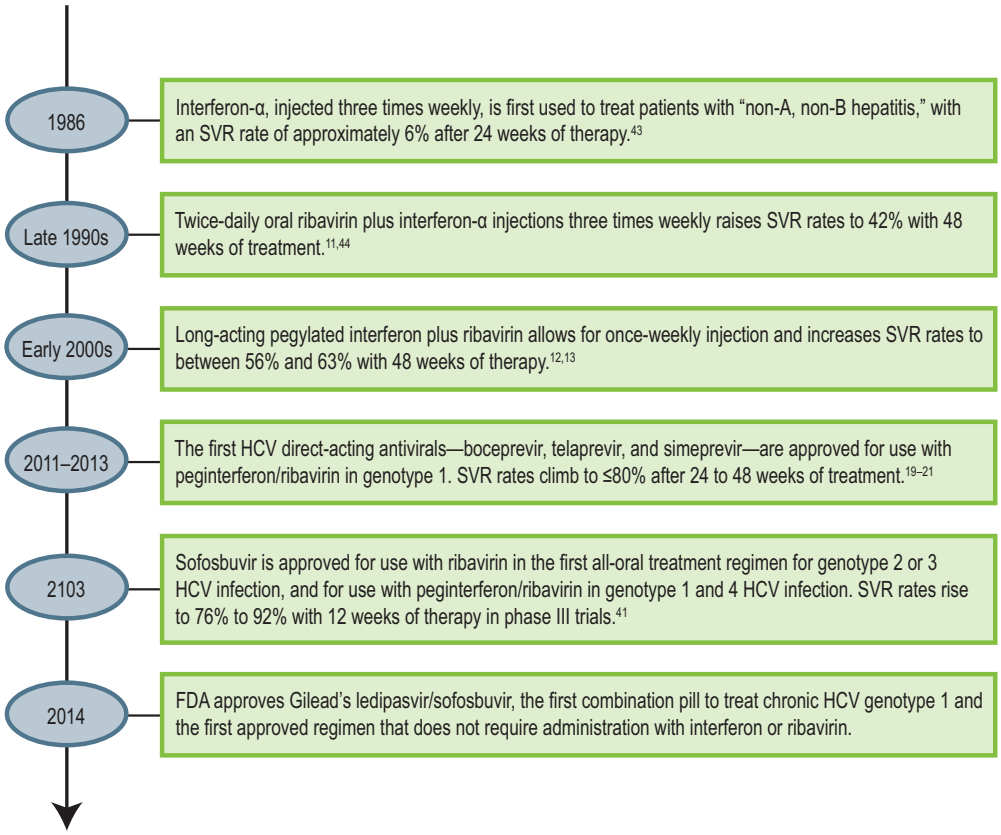

Figure 1. **Milestones in chronic hepatitis C treatment. SVR denotes sustained virologic response.**

In preclinical studies, sofosbuvir demonstrated activity against stable full-length genotype 1a, 1b, 2a, 3a, and 4a HCV replicons at sofosbuvir EC_{50} values of 0.040–0.11 μM. Potent antiviral activity was also observed against chimeric genotype 1b replicons encoding NS5B from genotypes 2b, 5a, and 6a (EC_{50} = 0.014–0.015 μM).[9]

In addition, sofosbuvir was active against genotype 1a and 2a HCV cell culture infectious virus (EC_{50} = 0.03 and 0.02 μM in genotype 1a and 2a, respectively). No significant differences were observed in sofosbuvir EC_{50} or EC_{95} values in the presence or absence of human serum or human serum albumin. Overall, sofosbuvir demonstrated significant broad-genotypic inhibition of HCV replication *in vitro*, and was selected for clinical development.[9] *In vitro* studies identified the S282T substitution in NS5B as conferring resistance to sofosbuvir.[29] This variant is rare, however, and has low replicative fitness *in vitro* and *in vivo*.[30]

Sofosbuvir clinical development program

The clinical development program for sofosbuvir included 13 phase I studies, five phase II trials,[31–35] five phase III registration studies, one pretransplant trial, one HCV/HIV coinfection study, and three collaborative trials.

The phase II and III studies evaluated sofosbuvir in combination with ribavirin with or without peginterferon for treatment in HCV genotypes 1–6. Nearly 3000 patients received at least one dose of sofosbuvir in phase II or III trials. Additional studies were conducted in special HCV populations, including patients awaiting liver transplantation and patients with HIV/HCV coinfection.

A unique feature of the sofosbuvir development program was the broad number of "in need" patient populations studied. A significant proportion of the patients included in the sofosbuvir clinical studies would have been excluded from participation in

trials with interferon-containing regimens, such as those with advanced age, patients with higher body mass index, those receiving opiate replacement therapy, patients with advanced liver disease or cirrhosis, and those with relative or absolute contraindications to interferon therapy.

Registrational phase III trials

The phase III program that led to regulatory approval of sofosbuvir for the treatment of HCV included the FISSION, POSITRON, FUSION, and NEUTRINO clinical trials (NCT01497366, NCT01604850, NCT01542788, and NCT01641640) (Table 1). Four studies (FISSION, FUSION, POSITRON, and VALENCE) assessed sofosbuvir/ribavirin in patients infected with HCV genotype 2 or 3. The NEUTRINO study assessed sofosbuvir plus peginterferon/ribavirin in treatment-naive genotype 1, 4, 5, or 6 HCV-infected patients. The VALENCE trial (NCT01682720) studied sofosbuvir/ribavirin for treatment-naive or previously treated HCV genotype 2 and 3 patients, and the PHOTON-1 study evaluated sofosbuvir/ribavirin in patients with HIV/HCV coinfection.

NEUTRINO

As reported by Lawitz, *et al.*,[36] the single-arm NEUTRINO trial evaluated the efficacy and safety of sofosbuvir-containing regimens in 327 previously untreated patients with HCV genotype 1, 4, 5, or 6. The primary end point was sustained virologic response 12 weeks after therapy (SVR12).

All participants received sofosbuvir plus peginterferon/ribavirin for 12 weeks. Sofosbuvir was administered orally at a dose of 400 mg once daily; ribavirin was administered orally as a divided dose according to body weight (1000 mg daily in patients with a body weight <75 kg 1200 mg daily in those weighing ≥75 kg). Peginterferon was administered subcutaneously once weekly at a dose of 180 μg.

All patients had rapid and substantial decreases in serum HCV RNA levels after the beginning of treatment, with no substantial differences in the rate or degree of decrease by HCV genotype, race, IL28B genotype, or presence or absence of cirrhosis. By week 2 of treatment, 91% of patients had HCV RNA levels below the lower limit of quantification (LLOQ, 25 IU/mL) (Table 2). By week 4, the proportion of patients with HCV RNA levels <LLOQ reached 99%. A total of 295 of 327 (90%, 95% CI, 87–93) patients achieved the primary outcome of SVR12.

Twenty-eight patients in the NEUTRINO study had virus relapse after the end of treatment. However, deep-sequencing analysis of samples collected at post-treatment visits showed no new resistance-associated HCV variants developed.

Treatment discontinuation due to adverse events was uncommon (2%), and rates of serious and severe adverse events were low. Fatigue, headache, nausea, and insomnia were the most common adverse events.

FISSION

In the randomized, open-label, active-control FISSION study, 499 previously untreated patients with HCV genotype 2 or 3 were randomly assigned in a 1:1 ratio to receive either 12 weeks of sofosbuvir/ribavirin or 24 weeks of peginterferon/ribavirin.[36] As in the NEUTRINO trial, all patients had serum HCV RNA levels of ≥10,000 IU/mL at screening. The primary end point was SVR12.

Sofosbuvir was administered orally at 400 mg once daily. For patients receiving sofosbuvir, ribavirin was administered orally as a divided dose according to body weight (1000 mg daily in patients with a body weight <75 kg 1200 mg daily in those weighing ≥75 kg). The ribavirin dose for patients in the peginterferon/ribavirin group was 800 mg daily in two divided doses, in alignment with product labeling.[37] Randomization was stratified by HCV genotype, HCV RNA level at study screening, and presence or absence of cirrhosis.

Sofosbuvir/ribavirin therapy was shown to be non-inferior to peginterferon/ribavirin. All patients receiving sofosbuvir/ribavirin saw rapid and significant reductions in serum HCV RNA levels. By week 2 of treatment, 92% of patients in the sofosbuvir/ribavirin group had HCV RNA levels <LLOQ, compared with 32% of those receiving peginterferon/ribavirin (Table 2). By week 4, just under 100% of patients receiving sofosbuvir/ribavirin had HCV RNA levels <LLOQ, compared with 67% of those in the peginterferon/ribavirin arm.

The SVR12 rate was 67% among patients receiving 12 weeks of sofosbuvir/ribavirin (97% of those with genotype 2 and 56% of those with genotype 3) and 67% among those receiving 24 weeks of peginterferon/ribavirin (Table 2). There was high

Table 1. Overview of sofosbuvir pivotal phase III studies

Study number	Design	Study objectives	Population	Number of subjects by treatment	Duration of treatment
Genotypes 1, 4, 5, or 6					
NEUTRINO	Phase III, open-label, multicenter study	Assess the efficacy (proportion of patients with SVR12) and safety of SOF+PEG+RBV in treatment-naive patients with genotype 1, 4, 5, or 6 HCV infection	Treatment-naive adult subjects with chronic genotype 1, 4, 5, or 6 HCV infection; up to 20% of participants may have cirrhosis	Overall: 327 treated; 301 completed through SVR12 assessment	12 weeks
Genotypes 2 or 3					
FISSION	Phase III, randomized, active-controlled, open-label, multicenter study	Assess the efficacy (proportion of patients with SVR12) and safety of SOF+RBV administered for 12 weeks compared with PEG+RBV administered for 24 weeks in treatment-naive patients with genotype 2 or 3 HCV infection	Treatment-naive adult subjects with chronic genotype 2 or 3 HCV infection; up to 20% of participants may have cirrhosis	Overall: 499 treated; 464 completed through SVR12 assessment SOF+RBV group: 256 treated; 239 completed PEG+RBV group: 243 treated; 225 completed	SOF+RBV group: 12 weeks PEG+RBV group: 24 weeks
POSITRON	Phase III, randomized, double-blind, placebo-controlled, multicenter study	Assess the efficacy (proportion of patients with SVR12) and safety of SOF+RBV compared with placebo administered for 12 weeks in patients with genotype 2 or 3 HCV infection who are unable or unwilling to take IFN	Adult subjects with chronic genotype 2 or 3 HCV infection who are unable or unwilling to take IFN; up to 20% of participants may have cirrhosis	Overall: 278 treated; 171 completed through SVR12 assessment SOF+RBV group: 207 treated; 171 completed Placebo group: 71 treated; 0 completed	12 weeks
FUSION	Phase III, randomized, double-blind, multicenter study	Assess the efficacy (proportion of patients with SVR12) and safety of SOF+RBV administered for 12 weeks compared with 16 weeks in patients with genotype 2 or 3 HCV infection who failed prior treatment with IFN	Treatment-experienced adult subjects with chronic genotype 2 or 3 HCV infection; up to 30% of participants may have cirrhosis	Overall: 201 treated; 127 completed through SVR12 assessment SOF+RBV 12-week group: 103 treated; 54 completed SOF+RBV 16-week group: 98 treated; 73 completed	SOF+RBV 12-week group: 12 weeks SOF+RBV 16-week group: 16 weeks

SOF, sofosbuvir; RBV, ribavirin; PEG, pegylated interferon; IFN, interferon; SVR, sustained virologic response.

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