

Medication of Chronic Hepatitis C: A Review on Sofosbuvir as a New Antiviral Drug

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Abstract

In the last decade, standard of care (SOC) anti-HCV treatment has been represented by the combination of Peginterferon (Peg-IFN) plus ribavirin (Rbv). The main disadvantages of Peg-IFN plus Rbv therapy were suboptimal rates of sustained virological response (SVR) in difficult-to-treat patients and side effects profile resulting in poor tolerability. Therefore, strategies to improve SVR rates and limited side effects have been an important issue for clinical physicians. Recently, direct antiviral agents (DAAs) opened the gate for a new era for management of HCV and have revolutionized the treatment of chronic hepatitis c (CHC) patients. Furthermore, the approval of sofosbuvir by FDA represents the first key step towards the new era in the management of CHC patients, since it is the first approved DAAs with excellent tolerability and favorable pharmacokinetic profile, limited potential for drug interactions, higher specificity, shorter treatment durations, low side effects, oral administration, potent antiviral activity and high genetic barrier against all HCV genotypes and acts as a chain terminator during the HCV replication process. Sofosbuvir efficacy and safety was demonstrated in many large and well-designed phase 2 and phase 3 clinical trials like PROTON, ATOMIC, NEUTRINO, ELECTRON, QUANTUM, FISSION, FUSION, POSITRON and LONSTAR studies. In this concept, sofosbuvir represents an almost ideal backbone, especially in difficult-to-treat CHC patients with cirrhosis or liver impairment, liver transplant recipients, and co-infection with HIV. This review summarizes the development of different anti-HCV agents, and also provides an overview of sofosbuvir clinical efficacy and discussing key results and potential future developments.

Keywords: Peginterferon; chronic hepatitis C; ribavirin; direct antiviral agents; sofosbuvir.

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1. Introduction

Chronic hepatitis C virus (HCV) infection is currently a major global health problem that affects 170 million people worldwide and is one of the main causes of chronic liver cirrhosis and hepatocellular carcinoma [1]. Epidemiological studies indicate that each year approximately 3-4 million people worldwide are affected and > 350000 individuals die due to liver disease [2]. A smaller but significant number of infected patients also show extrahepatic complications such as mixed cryoglobulinemia, glomerulonephritis, arthritis, and some varieties of B-cell lymphoma [3]. Genotypes 1-3 have a worldwide distribution with genotypes 1a and 1b accounting for nearly 60% of global hepatitis C infections [4]. Central and East Asia and North Africa/Middle East are estimated to have the highest prevalence of hepatitis C virus (HCV) infection (>3.5%) [5]. In USA, the HCV prevalence is estimated to range between 1.3% and 1.9% [6]. In addition, untreated chronic HCV infection imposes a considerable financial burden with average individual lifetime costs estimated at around \$65,000 US dollars and total costs of approximately \$6.5 billion US dollars [7]. In European countries, the HCV prevalence varies from 0.4% in Sweden, Germany and the Netherlands to over 2%-3% in some Mediterranean countries and even over 5% in some communities in Italy [8]. In Egypt, HCV is considered the most common etiology of chronic liver disease [9].

HCV is classified into 6 major genotypes. Some genotypes have a restricted geographical distribution (genotypes 4-6), while others (genotypes 1-3) are more broadly disseminated. Genotype 1 (subtypes 1a and 1b) is the most prevalent genotype in the world. Genotype 2 is found in clusters in the Mediterranean region, genotype 3 is most prevalent among intravenous drug users and genotype 4 is found mostly in Egypt, while genotypes 5 and 6 are less frequent [10]. The clinical presentation and management of infection arising from viral genotypes has advanced rapidly. In contrast, genotype 4, 5 and 6 have not been adequately studied, therefore, the management strategies for patients infected with these genotypes are not as well developed [11].

HCV is a small, 9,500- nucleotide, plus- strand ribonucleic acid (RNA) virus that replicates in the cytoplasm with a single open – reading frame [12]. Similar to other positive – strand RNA viruses, the genomic RNA of HCV serves as messenger RNA (mRNA) for the translation of viral proteins. HCV encodes a polyprotein of ~3,000 amino acids that is cleaved into at least 10 mature proteins by cellular and viral proteases [13]. During viral replication the poly protein is cleaved by viral as well host enzymes into three structural proteins (core, E1, E 2) and seven non-structural proteins (p7, NS3, NS4A, NS4B, NS5A, and NS5B) [14]. Once the virus is released into the cell, the viral polyprotein is translated and cleaved by host proteases and the viral NS3-4A protease into ten mature proteins. Next, viral RNA is replicated into progeny RNA by the viral NS5B polymerase [15].

The C-terminal component of the polyprotein contains the non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B). NS2 and NS3 are viral proteases required for the processing of the HCV non-structural portion of the polyprotein [16]. NS3 is a multifunctional enzyme, with serine protease, helicase and nucleotide triphosphatase activities, that forms a stable heterodimeric complex with its NS4A cofactor, essential for protein folding and stabilization. Moreover, the NS3A/NS4A complex cleaves the junctions between NS3/4A, NS4A/NS4B, NS4B/NS5A and NS5A/NS5B. NS3 has also helicase activity necessary for the unwinding of the

HCV genome during replication of the RNA [17]. NS4B, a hydrophobic transmembrane protein, is the presumed central organizer of the HCV replicase complex and a main inducer of intracellular membrane rearrangements that constitute the membranous web. Additionally, NS5A is an RNA-binding phosphoprotein required for RNA replication and assembly of infectious virus particles whilst NS5B is the RNA-dependent RNA-polymerase required for viral replication. The nonstructural proteins mentioned above have been the target for DAAs or Specifically Targeted Antiviral Therapy for hepatitis C (STAT-C) [18].

2. Current treatment regimens available for HCV

The goal of HCV treatment is to achieve sustained virological response (SVR) defined as undetectable HCV RNA at 24 weeks or recently at 12 weeks after the end of therapy [19]. Also, the HCV treatment aimed to decrease the risk of virus-related conditions such as cirrhosis, hepatocellular carcinoma (HCC), decompensated liver disease, liver transplant, or death from other liver-related causes. SVR is associated with improved outcomes in the form of reduction in the rate of hepatocellular carcinoma (HCC) and liver decompensation, and improved survival; patients who achieve SVR are considered to be cured [20].

2.1. Early antiviral therapy

2.1.1. Interferon monotherapy

Early treatment of acute hepatitis C with interferon alpha (α -IFN) for 24 weeks prevents chronic infection in almost all patients. Type I α -IFN was the first effective antiviral agent approved for use against chronic HCV infection in the early 1990s, although response rates were generally <20% [21]. Pegylated interferons (PEG-IFN) have replaced conventional interferon in the therapy of chronic hepatitis C. Several reports have documented improved SVR with PEG-IFN monotherapy. In a study of patients with chronic hepatitis C, it was found that PEG-IFN alfa-2a administered once per week was associated with a higher rate of virologic response than IFN alfa-2a at 6 million U subcutaneously administered 3 times per week for 12 weeks followed by 3 million U 3 times per week for 36 weeks. Findings were 69% versus 28% at week 48 of therapy and 39% versus 19% at week 72 of therapy [22]. In a controlled trial of persons with cirrhosis, it reported an SVR rate of 30% after 48 weeks of therapy with PEG-IFN alfa-2a, compared with 8% for patients treated with standard IFN alfa. Adverse effects were not significantly higher with the pegylated product [23].

Side effects of interferon or peg-interferon include predictable constitutional or “flu-like” symptoms of fever, chills, headache, and myalgia, nausea, anorexia, and less commonly, diarrhea, skin dryness, pruritus, rash, hair thinning, exacerbation of immune-mediated disorders, such as thyroiditis, inflammatory bowel disease, atopic dermatitis, or psoriasis. Moreover, the effect of IFN on bone marrow results in decreased granulocytes and thrombocytes during treatment [24].

2.1.2. PEG-IFN therapy with ribavirin

In the last decade, standard of care anti-HCV treatment has been founded on the combination of Peg-interferon (Peg-IFN) plus ribavirin (RBV). In a study of ribavirin in combination with either PEG-IFN alfa-2b or PEG-IFN

alfa-2a for the treatment of chronic HCV infection, it reported a higher SVR rate with PEG-IFN alfa-2a than with PEG-IFN alfa-2b (68% versus 54.4%) [25]. In a study of patients coinfecting with HCV and HIV with compensated cirrhosis, it was found that SVR to PEG-IFN plus ribavirin significantly reduced the incidence of liver-related decompensations and overall mortality [26]. Furthermore, [27] reported a significantly higher SVR rate in patients given higher-dose PEG-IFN alfa-2b plus ribavirin than in patients given lower-dose PEG-IFN alfa-2b plus ribavirin or given IFN alfa-2b plus ribavirin. Adverse effect profiles in the 3 treatment groups were similar (Figure 1)

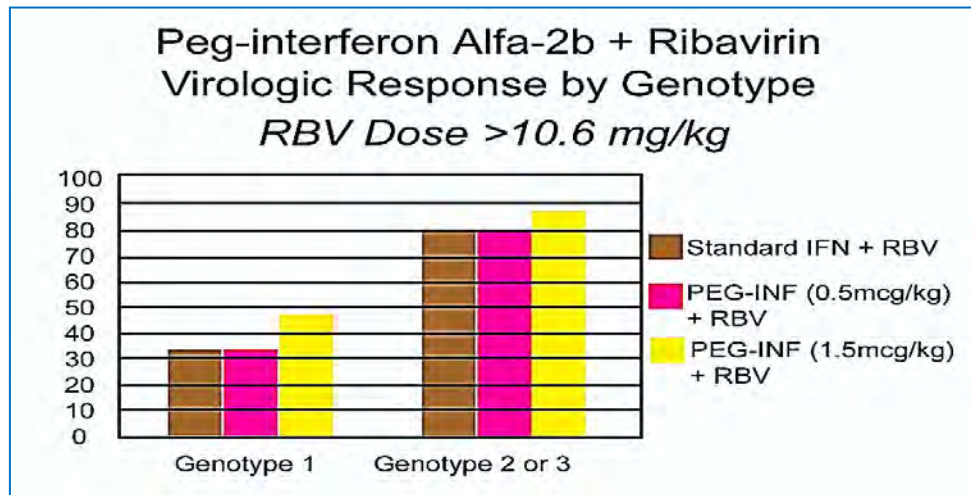


Figure 1: Peg-IFN+Rbv virologic response by genotype [27].

In addition, the patients who received PEG-IFN alfa-2a plus ribavirin had a significantly higher SVR rate than patients who received IFN alfa-2b plus ribavirin (56% vs 44%) or PEG-IFN alfa-2a alone (56% vs 29%)[28]. The SVR rates for patients with HCV genotype 1 were 46%, 36%, and 21%, respectively, for the 3 regimens. Moreover, [29] reported that in patients infected with HCV genotype 1, treatment for 48 weeks was statistically superior to 24 weeks, and standard-dose ribavirin was statistically superior to low-dose ribavirin.

A major barrier to successful treatment is the association of peginterferon/ribavirin therapy with frequent and sometimes serious adverse effects. In clinical trials, approximately 10-15% of treated patients discontinue peginterferon and ribavirin due to adverse events; however, in routine clinical practice, the rate of treatment discontinuation has been reported to be substantially higher, and the side effects of peginterferon/ribavirin therapy affect virtually all organ systems [30].

2.2. A new era of HCV management's

In the last decade, standard of care anti-HCV treatment has been founded on the combination of Peginterferon (Peg-IFN) plus ribavirin (Rbv), whose main disadvantages were suboptimal rates of sustained virological response (SVR) in difficult-to-treat patients (HCV genotype 1–4, advanced liver fibrosis) and, most of all, side effects profile resulting in poor tolerability and treatment contraindication in some patient subsets (decompensated liver disease and autoimmune disorders)[31]. Therefore, strategies to improve SVR rates have

been an important issue for clinical physicians. During the last years, intensive efforts focused on the development of direct acting antiviral agents (DAAs) that can block the activity of viral enzymes targeting either NS3-4A serine protease, which block HCV polyprotein processing, or HCV replication [32]. DAAs of all the major classes were designed to directly inhibit viral enzymes and proteins. The NS proteins NS3/4A protease–helicase and NS5B RdRp and the NS5A protein all perform crucial activities for the viral life cycle and potentially, each step of the viral cycle is a target for drug development. The new group of protease inhibitors has improved SVR to a great extent and various other groups of drugs nucleotide and non-nucleoside NS5B inhibitors, NS5A inhibitors and others have certainly opened the flood gates, with various drugs standing in line for approval [33].

Moreover, direct acting antivirals (DAAs) promised to open a new era in treating chronic HCV infection by increasing SVR rates, providing shortened and simplified regimens while also minimizing treatment-related side effects. Three major classes of DAA dominated the scenario at different stages of development and clinical practice: **1-NS3/4A protease inhibitors**, **2-NS5A inhibitors** and **3-NS5B polymerase inhibitors**, which can be subdivided into nucleoside inhibitors (NIs) or nonnucleoside inhibitors (NNIs) [34] (Table 1).

Table 1: Comparisons between the major direct-acting antiviral drugs in safety and efficacy [34]

Characteristic	NS3/4A	NIs	NNIs	NS5A inhibitors
Potency	High for GT1 Variable for GT2–4	Moderate to high	Variable among GTs	High; multiple among GT
Barrier to resistance	Low; GT1a<1b	Very high; GT1a=1b	Very low; GT1a<1b	Low; GT1a<1b
Drug interaction	High	Low	Variable	Low to moderate
Toxicity	High for first generation Rash, anemia, hyperbilirubinemia	Variable mitochondrial nuclear interactions	Variable	Variable
Agents approved	Telaprevir, boceprevir, simeprevir	Sofosbuvir	None	None
Examples in pipeline	Faldaprevir (BI-201335) MK-5172 Danoprevir (RG7227/ITMN-191) Veruprevir (ABT-450/r) Vaniprevir-MK-7009	VX-135 Mercitabine	ABT-072 Dasabuvir (ABT-333) BMS-791325	Daclatsavir (BMS-790052) Ledipasvir (GS-5885) Samatasvir (IDX21437) Ombitasvir (ABT-267)

Abbreviations: GT, genotype; NIs, nucleotide polymerase inhibitors; NNIs, non-nucleoside inhibitors; NS3/4A

protease inhibitors.

2.2.1. NS3/4A protease inhibitors

The NS3 protease is an enzyme that catalyzes the post-transcriptional processing of proteins important for viral replication. NS4A is a cofactor that works with NS3 to expedite this process. The serine protease inhibitors telaprevir is a linear peptidomimetic HCV NS3/4A serine protease inhibitor, and boceprevir is a protease inhibitor that binds to the HCV NS3 active site [35]. Newer, NS3/4A inhibitors under development are mainly macrocyclic compounds (simeprevir-TMC-435, danoprevir-R7227, and vaniprevir-MK-7009) and the linear asunaprevir (BMS-650032). In addition, newer first generation as well as second- and third-generation NS3/4A inhibitors (faldaprevir-BI 201335, GS-9256, ABT-450, etc.) are expected to have better pharmacokinetics for once daily dosage, less complicated treatment algorithms and less side effects compared to boceprevir and telaprevir [18] (Table 1).

2.2.1.1. Telaprevir and boceprevir

Two first-generation, linear NS3/4A PIs, boceprevir and telaprevir, were approved in the USA and Europe in 2011 for clinical use in patients with genotype 1, while numerous new NS3/4 PIs are currently under evaluation in clinical trials. However, these first generation DAAs have a low genetic barrier to resistance, their efficacy is limited to HCV genotype 1 and co-administration of peg-IFN and RBV is needed. In addition, the triple regimens are associated with unfavorable tolerance and safety profiles, particularly in patients with underlying cirrhosis [32].

2.2.1.2. Simeprevir

Simeprevir (formerly **TMC435**; trade name **Olysio**) is a new direct acting antiviral drug and a second generation NS3/4A serine protease inhibitor. Food and Drug Administration (FDA) has approved Olysio (simeprevir), a hepatitis C virus (HCV) NS3/4A protease inhibitor, in combination with sofosbuvir as an all-oral, interferon- and ribavirin-free treatment option for genotype 1 chronic hepatitis C (CHC) infection in adult patients as part of a combination antiviral treatment regimen. It was introduced by Janssen and Medivir for the oral treatment of patients with genotype 1 and/or genotype 4 chronic HCV infections [36]. In vitro, it is active against all six genotypes with lesser efficacy against genotype 3a [37]. Simeprevir (SMV) prevents viral maturation through inhibition of protein synthesis. SMV is also a substrate and mild inhibitor of P-glycoprotein which may help predict other drug interactions [38]. SMV is metabolized mainly by the cytochrome P450 3A (CYP3A) pathway in the liver and can cause drug-drug interactions with inhibitors and inducers of CYP3A. The all-oral, interferon-free treatment regimen with simeprevir and sofosbuvir resulted in an overall SVR12 rate of 92%, consistent SVR12 rates regardless of METAVIR score, and was an effective and well-tolerated therapeutic regimen in both treatment-naïve and prior null-responder patients [39]. Moreover, simeprevir proved to have several advantages such as its high efficacy, short treatment duration (12–24 weeks), better safety profile than first generation drugs and the decreased pill burden being taken once daily [40].

2.2.1.3. Faldaprevir;

Faldaprevir is a second-generation HCV NS3/4A protease inhibitor with significant improvement in potency and adverse event profile compared with first-generation protease inhibitors. Faldaprevir has an acceptable tolerability and safety at all dose levels [41]. Several studies used it with different drug combinations. SILEN-C1 (for naïve G1 HCV patients) and SILEN-C2 (for prior nonresponders) were enrolled on 429 and 290 patients respectively. Of interest, faldaprevir in combination with pegylated interferon and ribavirin, and interferon-free treatment with faldaprevir in combination with deleobuvir plus ribavirin provides high sustained virological response rates for HCV genotype 1 infection % respectively [42]. SILEN-C3 is a phase II trial for 160 naïve G1 infected patients. Faldaprevir was taken for 12–24 weeks with 24–48 weeks of pegylated interferon and ribavirin. SVR rates were 67% (if 12 weeks) and 74% (if 24 weeks) [41].

2.2.2. NS5A inhibitors;

The NS5A protein is a regulator of replication. NS5A inhibitors have high antiviral activity against different genotypes, but a low genetic barrier to resistance. Resistance variants to NS5A inhibitors are not associated with impaired viral replication fitness and they do not disappear after the end of treatment. Persistence of NS5A resistance mutations was detected up to one year after stopping treatment [43]. Other NS5A inhibitors such as ACH-3102 samatasvir and GS-5816 BMS-824393, PPI-461, ledipasvir (GS-5885) and ABT-267 are still under clinical development [44], [45], [46] (Table 1).

2.2.2.1. Daclatasvir

Daclatasvir (BMS-790052), produced by Bristol-Myers Squibb, is the first-in-class NS5A inhibitor that demonstrated a satisfactory multiphase rapid HCV RNA decline without significant adverse events. It is highly effective against genotypes 1–4 [47]. Several clinical trials assessed the efficacy of daclatasvir with different compounds. Using daclatasvir with interferon and ribavirin, Suzuki et al. managed HCV patients who were treatment naïve, prior null or partial responders. Two Different concentrations of daclatasvir were used (10 and 60 mg) versus a placebo group. SVR24 reached 66.7–90% in naïve group versus 62.5% in placebo group and much less satisfactory results in prior null responders (22–33.3%) [48]. Naïve group had SVR24 89–100% while null responders group 50–78% [49].

2.2.2.2. Ledipasvir

Ledipasvir is another NS5A inhibitor that showed promising results in different trials that evaluated its combination to sofosbuvir. It provided high potency against HCV genotypes 1a, 1b, 4a and 6a while it was less efficacious against genotypes 2a and 3a [50]. It cannot be used alone due to quick development of resistance [51].

2.2.2.3. Cyclophilin A inhibitors

Cyclophilins are host proteins involved in protein folding. They play an important role in the HCV lifecycle as

regulators of replication. The cyclophilin inhibitor alisporivir (DEB-025) is a cyclosporine analog without its immunosuppressive properties that has shown pan-genotypic antiviral activity and has been used both alone and in combination with PEG-IFN and RBV with promising results [52].

2.2.3. NS5B polymerase inhibitors

There are two categories of NS5B polymerase inhibitors: nucleos(t)ide (NIs) and non-nucleoside inhibitors (NNIs) (Table 1). NIs mimic the naturally occurring nucleos(t)ides and thus are incorporated into the nascent RNA chain causing premature chain termination [53]. NIs are considered to have a high genetic barrier to resistance, although single amino acid substitutions are able to confer drug resistance *in vitro*. NIs have antiviral activity against all HCV genotypes (pan-genotype activity) as the active site of NS5B is well conserved across genotypes [54]. NS5A inhibitors showed promising results among the different DAA drug studies due to their multigenotypic efficacy, high potency but low to intermediate barrier to resistance.

Several nonnucleoside inhibitors (NNIs) of the HCV polymerase can prevent either de novo initiated RNA synthesis, primer-extended RNA synthesis, or both. These NNIs inhibitors achieve polymerase inhibition by binding to one of the at least four allosteric enzyme sites [55]. Most of them have a genotype-specific activity and they may select rapidly drug-resistant variants if HCV replication is not completely suppressed [56].

2.2.3.1. Dasabuvir

Dasabuvir (ABT-333) is a NS5B non-nucleoside polymerase inhibitor (NNPIs). Dasabuvir containing regimens achieve high rates of sustained virologic response in HCV genotype 1a and 1b–infected patients when combined with other DAAs, namely paritaprevir (ABT-450), ritonavir and ombitasvir (ABT-267). In the populations studied, dasabuvir seems to be well tolerated and safe and the major limitations of this novel drug are its genotype-restricted activity, the necessity to include ribavirin for HCV genotype 1a and the emergence of resistance if not combined with other DDAs [57].

2.2.3.2. Sofosbuvir

Sofosbuvir (Sovaldi®, Gilead Sciences) is a new drug, formerly named GS-7977, the first NS5B HCV nucleotide polymerase inhibitor (NPIs), whose US Food and Drug Administration (FDA) and European Medicines Agency (EMA) approvals have been granted at the end of 2013 and at the beginning of 2014, respectively [58]. Sofosbuvir with the chemical name L-Alanine, N-[[P(S),2'R]-2'-deoxy-2'-fluoro-2'-methyl-P-phenyl-5'-uridylyl]-, 1-methyl-ethyl ester and a molecular formula of C₂₂H₂₉FN₃O₉P. It is administered at a dosage of 400 mg once daily, taken with or without food, and has a potent activity against all HCV genotypes [59]. Sofosbuvir is used in combination with other medicines. Several varieties (genotypes) of hepatitis C virus exist, and the duration of treatment with Sofosbuvir will depend on the genotype of the virus and on which medicines are used together with Sofosbuvir. Metabolism of sofosbuvir and the other related drugs of the same family have no relation with the CYP3A4 pathway [60].

The price of sofosbuvir, quoted in various media sources as \$84,000 to \$168,000 for a course of treatment in the

U.S., £35,000 for 12 weeks in the UK, has engendered considerable controversy. In September 2014, Gilead announced that it would permit generic manufacturers to sell sofosbuvir in 91 developing countries and that it would sell a name brand version of the product in India for approximately \$300 per course of treatment [61].

2.2.4. Mechanism of actions of antiviral drugs

2.2.4.1. Anti-HCV mechanisms of interferon

These are glycoproteins of low molecular weight produced by fibroblasts, epithelial cells hepatocytes and predominantly dendritic cells in response to various pathogens like virus, tumor cells, bacteria, parasites. It is the first drug approved by FDA to treat hepatitis C (IFN- α got FDA approval in 1991). There are several types of IFNs grouped into three types (types I, II and III), type I (α and β) and type II (gamma) exhibits significant activity and hence IFN- α 2a and 2b produced by recombinant DNA technology used commercially [33]. Pegylated (PEG) IFN- α 2a is absorbed slowly and sustained twice longer in plasma compared to IFN- α 2b. IFN receptors have JAK-STAT (Janus Kinase-signal transduction and transducer mechanism) signaling pathway. The phosphorylated STAT binds with p48 protein and forms an ISGF-3 (IFN-stimulated gene factor-3) which in turn enters into nucleus, stimulating IFN-stimulated regulatory element leading to synthesis of various proteins which interfere in viral penetration, synthesis of viral m-RNA, assembly of viral particles and its release (Figure 3) [62].

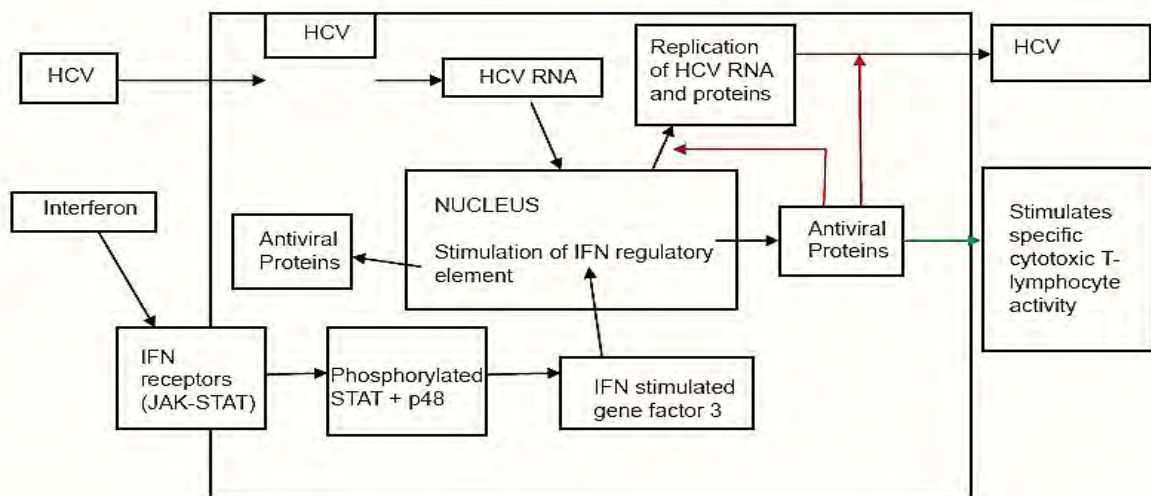


Figure 2: Mechanism of action of IFN in HCV infection (Red arrow = inhibition, Green arrow = stimulation).

2.2.4.2. Mechanism of Action of Ribavirin

The addition of ribavirin to IFN- α -based regimens produces dramatic improvement in SVR rates among patients with chronic hepatitis C. Clinically; ribavirin appears both to increase the end-of-treatment response rate and to decrease the subsequent relapse rate (Figure 3). It is a synthetic guanosine analog with a broad spectrum of antiviral activity, administered along with IFN at a dose of 800-1200 mg/day with an oral bioavailability of 50% and an elimination half-life of >10 days [33]. When combined with standard IFN- α ,

ribavirin appears to increase the second phase decline in HCV RNA levels; however, this effect is slight and is not observed when standard IFN is given daily or peg-interferon is used. These results suggest that ribavirin does not have direct antiviral activity against HCV. Although ribavirin is an IMPDH inhibitor, there is little clinical data to support this mechanism of action. Other IMPDH inhibitors have failed to demonstrate antiviral activity *in vivo* against HCV either alone or in combination with ribavirin [63].

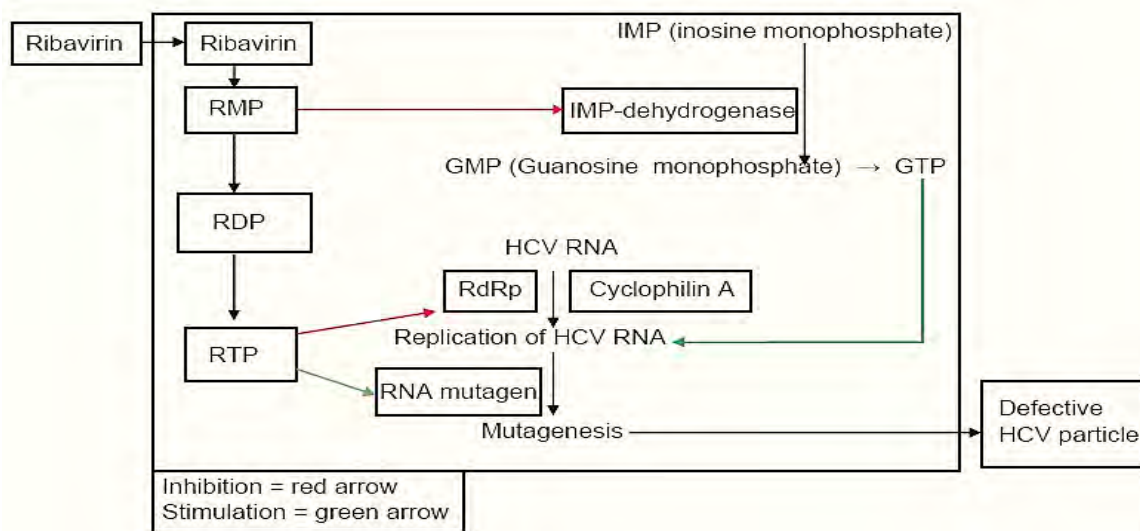


Figure 3: Mechanism of action of Ribavirin in HCV infection (Red arrow = inhibition, Green arrow = stimulation).

2.2.4.3. Mechanism of action of sofosbuvir

Sofosbuvir is a prodrug metabolized to the active antiviral agent 2'-deoxy-2'- α -fluoro- β -C-methyluridine-5'-triphosphate. The triphosphate serves as a defective substrate for the NS5B protein, which is the viral RNA polymerase, thus acts as an inhibitor of viral RNA synthesis [64]. The active substance in sofosbuvir, blocks the action of an enzyme called 'NS5B RNA-dependent RNA polymerase' in the hepatitis C virus, which is essential for the virus to multiply. This stops the hepatitis C virus from multiplying and infecting new cells. NS5B is one of the non-structural proteins essential for viral RNA replication, and has been found to be a valuable target for directly acting antiviral agents (DAAs) [65]. Prior to the discovery of sofosbuvir, a variety of nucleoside analogs had been examined as anti-hepatitis C treatments, but these exhibited relatively low potency. This low potency arises in part because the enzymatic addition of the first of the three phosphate groups of the triphosphate is slow. The design of sofosbuvir, based on the prodrug approach, avoids this slow step by building the first phosphate group into the structure of the drug during synthesis. Additional groups are attached to the phosphorus to temporarily mask the two negative charges of the phosphate group, thereby facilitating entry of the drug into the infected cell [66]. Moreover, sofosbuvir and other nucleotide inhibitors of the HCV RNA polymerase exhibit a very high barrier to resistance development. This is an important advantage relative to HCV drugs that target other viral enzymes such as the protease, for which rapid resistance development has proved to be an important cause of therapeutic failure [67].

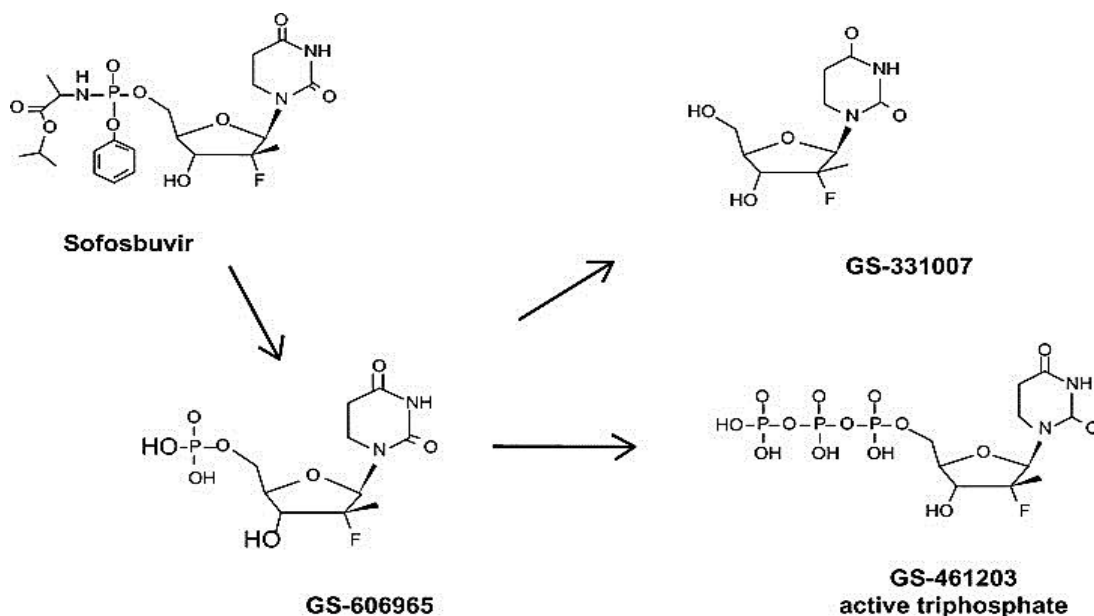


Figure 4; The activation and metabolic pathway of sofosbuvir [69].

2.2.5. Pharmacokinetics of sofosbuvir

The main goals in SOF development for historically “easy-to-treat” genotypes were to shorten treatment duration, but most of all, to improve tolerability through an IFN-free regimen [68]). Sofosbuvir has a beneficial pharmacokinetic profile, being effective orally as a single daily dose, and has rather limited potential for drug-drug interactions. However, some issues remain to be elucidated including the reasons for HCV relapse after stopping a sofosbuvir-based regimen, the possible impact of host and viral factors associated with HCV eradication, the optimal duration in difficult-to-treat population, such as prior non-responders with cirrhosis and its use in patients with advanced decompensated cirrhosis [69]. SOF is a second-generation NI, for treatment of HCV genotypes 2 and 3 in combination with RBV, and for genotypes 1 and 4 in combination with PR, based on data from nearly 3,000 patients studied in Phase II and III trials [70]. It enters the hepatocytes as a prodrug of uridine monophosphate and is phosphorylated within the cells to active triphosphate form. Dephosphorylation of the active molecule results in the formation of the metabolite GS-331007, which lacks anti-HCV activity; GS-331007 is the main circulating metabolite of SOF and undergoes renal elimination. The median half-lives of SOF and GS-331007 are 0.4 and 27 hours, respectively. Population pharmacokinetic (PK) models developed for GS-331007 and SOF revealed that demographic variables such as age, sex, BMI, race, common concomitant medications, and cirrhosis did not influence GS-331007 or SOF exposure (Fig. 4) [71].

2.2.5.1. Efficacy and safety;

The efficacy and safety of sofosbuvir in patients with different HCV genotypes and with various combinations of drugs have been tested in numerous clinical trials. A dose of 400 mg of sofosbuvir has been found to be most effective, with treatment durations ranging from 12 to 24 weeks, in various combinations of PEG-IFN and ribavirin. Sofosbuvir has been studied in different combinations [72]. The value of adding sofosbuvir to the combination of Peg-IFN α in genotype 1 patients was first addressed in two phase II trials (PROTON and

ATOMIC) (Table 2).

The first study **PROTON** was a double-blind, randomized, placebo-controlled, dose-ranging phase II study that demonstrated that sofosbuvir was highly effective against genotypes 1, 2 and 3 HCV when used in combination with peg-interferon and ribavirin as 12-week triple therapy, followed by additional peg-interferon and ribavirin in the genotype 1 patients, results greater than 90% in all sofosbuvir-containing arms of the study [73].

The other trial (**ATOMIC**) evaluated the role of maintenance therapy after the initial 12 week response. The **ATOMIC** study explored shorter treatment durations of sofosbuvir-based triple therapy, and randomized 316 treatment-naïve patients with genotype 1 HCV into three treatment arms that included sofosbuvir 400 mg plus peg-interferon and ribavirin therapy of 12 or 24 weeks duration, and one arm who received sofosbuvir triple therapy for 12 weeks, and then subjects were randomized to receive a further 12 weeks of sofosbuvir alone or with ribavirin. SVR rates remained greater than 90% in all arms of this study, with minimal differences in SVR seen in patients with factors traditionally associated with reduced response to interferon-based therapy such as high baseline viral load, patients with non-CC IL28B genotypes or bridging fibrosis on liver biopsy [74].

Following on from **PROTON** and **ATOMIC**, the **ELECTRON** study evaluated sofosbuvir in interferon-sparing and interferon-free regimens for the treatment of HCV infection in noncirrhotic patients. The **ELECTRON** trial was the first Phase II study in treatment-naïve patients to explore an IFN-free arm of SOF/Rbv for 12 weeks compared to three arms of variable PegIFN duration (4/8/12 weeks), with 100% SVR in both the PegIFN-based and IFN-free arms [75]. The **ELECTRON** trial also demonstrated that the key role of Rbv could not be superseded, as a SOF monotherapy 12-week arm reported SVR of 60% due to a 40% virological relapse rate. Based on these results, the SOF/Rbv combination administered for 12 weeks was chosen to be further evaluated in Phase III trials. Studies that tested for sofosbuvir in genotype 1 included **QUANTUM** and **ELECTRON** studies. Fifty naïve patients were treated with sofosbuvir and ribavirin for 12 weeks. SVR12 rates were 56% and 88% respectively [79].

NEUTRINO was the first SOF Phase III trial in naïve patients with the aim to evaluate the safety and efficacy of the combination regimen SOF/PegIFN/Rbv for 12 weeks. The **NEUTRINO** trial included 28 CHC patients with genotype 4 and 7 patients with genotype 5 or 6 who were treated with sofosbuvir plus pegIFN and weight-based RBV for 12 weeks. The SVR rates were 96% in genotype 4 and 100% in genotype 5-6 patients [71]. The combination of SOF/PegIFN/Rbv for 12 weeks demonstrated high rates of on-treatment response, with 91% of patients achieving HCV-RNA undetectability at week 2 and 99% at week 4, which resulted in an overall SVR rate of 90%. Concerning safety profile, the most common side effects reported were fatigue (59%), headache (36%), nausea (34%), and insomnia (25%), which are mainly consistent with Rbv or PegIFN safety profiles. Anemia with Hb <10 g/dL was observed in 23%, while only 2% of patients had Hb <8.5 g/dL [77].

In a phase III non-inferiority trial study named **FISSION**, nearly 500 genotype 2 or 3 naïve CHC patients were randomized to receive sofosbuvir plus weight-based RBV for 12 weeks (n=256) or SOC, i.e. pegIFN plus RBV (800 mg daily) for 24 weeks (n=243). Approximately 20% had cirrhosis and 29% had interleukin-28B (IL28B) CC genotype. The combination of sofosbuvir plus RBV achieved SVR in 97% of genotype 2 (78% with SOC)

and 56% of genotype 3 patients (63% with SOC). SVR rates under sofosbuvir and RBV were lower in patients with cirrhosis (genotype 2: 91%, genotype 3: 34%) compared to those without cirrhosis (genotype 2: 98%, genotype 3: 61%) without significant difference from the SVR achieved by SOC in any subgroup [77].

The efficacy of the SOF/Rbv IFN-free combination in patients with a previous treatment failure to PegIFN/Rbv was investigated in the **FUSION** trial. Overall SVR in treatment experienced patients with SOF/Rbv was significantly lower in the 12-week compared to 16-week arms (50% vs 73%). Cirrhosis and HCV genotype 3 were confirmed as predictors of treatment failure: indeed, in the two treatment arms (12/16 weeks) SVR rates were respectively 96%/100% in non-cirrhotic vs 60%/78% in cirrhotic HCV-2 patients, while in HCV-3 patients, SVR decreased to 37%/63% in non-cirrhotics vs 19%/61% in cirrhotics [70].

In another phase III, placebo-controlled trial named **POSITRON**, 278 patients with CHC genotype 2 or 3 who were interferon intolerant or ineligible or had refused pegIFN therapy were randomized to receive sofosbuvir plus weight-based RBV (n=207) or placebo (n=71) for 12 weeks. SVR was achieved in 78% of patients in the sofosbuvir arm and 0% in the placebo arm. SVR rate was significantly higher in genotype 2 than genotype 3 patients (93% vs. 61%, P<0.001). The presence of cirrhosis did not affect the SVR rates in genotype 2 patients (94% vs. 92%), but it had a great impact on the SVR rates in genotype 3 patients (21% vs. 68%, P=0.002) [70]. Concerning safety, the combination of SOF/Rbv showed an optimal tolerability profile: the most frequent adverse events were fatigue (44%), nausea (22%), headache (21%), insomnia (19%), and pruritus (11%), mainly consistent with Rbv. Hemoglobin decline <10 g/dL occurred in only 7% of patients (<8.5 g/dL in 1%), while no reduction in platelets and neutrophil values were reported [70].

In a third phase III trial named VALENCE including genotype 2 and 3 naïve CHC patients, sofosbuvir plus weight-based RBV was given for 12 weeks in genotype 2 (n=32) and for 24 weeks in genotype 3 patients (n=105). SVR was achieved in 97% of genotype 2 (non-cirrhotics: 97%, cirrhotics: 100%) and 93% of genotype 3 patients (non-cirrhotics: 94%, cirrhotics: 92%). The rates of discontinuation were negligible without evidence of viral resistance among the patients who relapsed after treatment [78].

2.2.5.2. SOF in combination with other DAAs

Overall, SOF appears to be an attractive choice to serve as a backbone for future DAA combinations. The possibility of developing an IFN-free regimen for difficult-to-treat genotypes requires the combination of different DAA classes to provide high antiviral efficacy as well as a high barrier to resistance. In another open-label, phase II study named LONESTAR, one tablet co-formulation of sofosbuvir and ledipasvir (NS5A inhibitor) with or without weight-based RBV were given for 8 or 12 weeks in 100 genotype 1 CHC patients who were treatment-naïve or had failed a course with triple combination including a 1st generation protease inhibitor. The SVR rates were 95-100% in all subgroups regardless of previous treatment history or presence of cirrhosis. Adverse events were rare and always mild (nausea, anemia and headache), while one patient in the RBV-containing arm developed severe anemia [79]. In a single arm phase II trial named LONESTAR-2, such a triple combination offered SVR rates of 96% (22/23) in genotype 2 and of 83% (20/24) in genotype 3 patients [80].

The combination of 12 or 24 weeks of SOF plus the NS3 inhibitor simeprevir was evaluated by the **COSMOS** Phase II study, where an overall 93% SVR12 rate in non-cirrhotic HCV-1 previous null-responder to PegIFN/Rbv was obtained. Interim analysis of a second cohort including cirrhotic HCV-1 naïve and null-responder patients showed 100% SVR4 with the 12-week regimen [39].

The two DAA drug combinations, regardless of whether RBV was included, performed impressively and led to SVR rates between 79% and 100%. Recently, the FDA granted priority review to Gilead’s New Drug Application for a once-daily fixed-dose combination of SOF (400 mg) and LDV (90 mg) for the treatment of chronic hepatitis C genotype 1 infection in adults called **Harvoni**. Furthermore, it now appears that SOF in combination with LDV for 8 weeks may be just as effective as 12 weeks in genotype 1 patients without cirrhosis, as established by LONESTAR and ION-3 data. SOF + DCV were tested against genotypes 2 and 3. In particular, 8-12 week courses with the combination of sofosbuvir with a potent NS5A inhibitor (e.g. ledipasvir or daclatasvir) or NS3 protease inhibitor (e.g. simeprevir) have been shown to achieve SVR in almost all genotype 1 patients without safety and tolerability concerns [81].

Its excellent safety profile makes sofosbuvir an optimal choice for patients with decompensated cirrhosis and liver transplant recipients as well as for patients who cannot tolerate interferon and/or RBV. The safety profile was good in all the patients, thus suggesting that no dosage or interval modification was required in patients with moderate-to-severe hepatic impairment [20]. Safety profile did not differ in patients with well compensated liver cirrhosis who were included in the phase III sofosbuvir trials and no hepatic decompensation was observed while on any of the IFN α -free treatment arms [81].

Table (2): Different sofosbuvir efficacy studies [72].

Name of study	Design	Genotype	SVR (%)
PROTON	Sof, IFN/RBV	1	91
ATOMIC	Sof, IFN/RBV	1,4,6	90–94
NEUTRINO	Sof, IFN/RBV	1,4,5,6	89–100
ELECTRON	Sof, RBV	1	88
	Sof, RBV, Ledipasvir		100
	Sof, RBV, GS-9669		92
QUANTUM	Sof, RBV	1	56
FISSION	Sof, RBV	2,3	67
FUSION	Sof, RBV	2,3	56–73
POSITRON	Sof, RBV	2,3	61–93
LONESTAR	Sof, RBV, Ledipasvir	1	95–100

2.2.5.3. Potential for sofosbuvir in liver transplantation

One obvious clinical need is for data regarding safety and efficacy of sofosbuvir in patients who have decompensated chronic liver disease, are peri-transplant or post-liver-transplant. The excellent safety data to

date and the lack of significant drug interactions makes sofosbuvir an appealing choice to be studied in these groups. To date there is one case report published of a patient with severe recurrent cholestatic hepatitis C, 6 months post-transplant, who was effectively rescued and achieved SVR with treatment with sofosbuvir and daclatasvir in combination [83]. This is promising, and results of future trials of sofosbuvir in these types of patient groups are awaited with interest. Moreover, [66] reported that sofosbuvir and ribavirin combination therapy for 24 weeks is an effective and well-tolerated interferon-free treatment for post-transplantation HCV infection.

2.2.5.4. Resistance to sofosbuvir

SOF has been studied in several thousand patients to date, and effective in vivo drug resistance leading to viral escape and relapse in response to therapy appears to be, at best, a rare event. In the registration Phase III studies, no genotypic or phenotypic resistance was detected. In Phase II trials, S282T mutation was detected in only one relapse patient, and this occurred after SOF monotherapy [75]. Overall, SOF has eliminated most of the treatment barriers for patients that were present in the IFN era. Nevertheless, making treatment decisions among patients still requires thoughtful consideration, as host factors such as cirrhosis and viral factors such as genotype still have significant influences on treatment times and, in some cases, outcome. An important principle is that monotherapy with SOF is not recommended [34].

Cross-resistance studies have been conducted using panels of replicons with mutations in the NS3/4A protease, NS5A, and NS5B, which remained susceptible to sofosbuvir (except for HCV type 1b S282T), thus indicating that sofosbuvir can be combined with other directly acting antiviral agents. It has been suggested that additional mutations with amino acids change in both the finger and palm domains and are required to compensate for poor HCV fitness, resulting from S282T mutation, in order to confer resistance to sofosbuvir [20]. The S282T mutation has so far only been detected in one patient, with HCV type 2b; a relapse has been seen in this patient after sofosbuvir monotherapy. Genotype or subtype-specific resistance has not been seen with sofosbuvir [92]. In the clinical studies, although relapse leading to treatment failure was seen in a few patients, no virological resistance was detected in these patients receiving sofosbuvir 400 mg monotherapy or in combination with ribavirin, PEG-INF or both [84].

2.2.5.5. Adverse events of sofosbuvir

Adverse events associated with ribavirin therapy (fatigue, insomnia and anemia) were commonly reported, and headache was also frequently reported. Sofosbuvir has shown a good safety profile in clinical trials; a small decrease in the Hb levels (0.54 mg/dl) and reduction in the cumulative events in comparison to interferon-containing regimens is seen. Common adverse events observed include: Headache, insomnia, fatigue, nausea, dizziness, pruritis, upper respiratory tract infections, rash, back pain, grade 1 anemia, and grade 4 lymphopenia [70]. Depression is a common side effect of interferon therapy, and in the FISSION study, occurred in 14% of patients receiving peginterferon, as compared with 5% of patients receiving sofosbuvir plus ribavirin. In a recent analysis of the impact of HCV treatment on quality of life, in the FISSION and POSITRON trials, sofosbuvir plus ribavirin was associated with better health-related quality of life than peginterferon plus ribavirin, and was

similar to patients not receiving active treatment [85]. In the monotherapy treatment groups, nausea and fatigue seemed to be the only adverse events possibly correlated to sofosbuvir. An overall improved tolerability was seen with sofosbuvir compared to the interferon-based regimens.

In March 2015, Gilead Sciences said warnings to health care providers about nine patients that began taking its hepatitis C drugs Harvoni (ledipasvir/sofosbuvir) or Sovaldi (sofosbuvir) along with the heart treatments amiodarone, Bristol-Myers Squibb's Daklinza (daclatasvir), or Olysio Johnson & Johnson's (simeprevir) developed abnormally slow heartbeats and one died of cardiac arrest. Three required a pacemaker to be inserted. Gilead said the combinations aren't recommended and product labels will be updated [86].

2.2.5.6. Drug interactions

Sofosbuvir is a substrate of P-glycoprotein, a transporter protein that pumps drugs and other substances from intestinal epithelium cells back into the gut. Therefore, inducers of intestinal P-glycoprotein, such as rifampicin could reduce the absorption of sofosbuvir. In addition, coadministration of sofosbuvir with anticonvulsants, antimycobacterials, and the HIV protease inhibitor tipranavir is expected to decrease sofosbuvir concentration. Thus, coadministration is not recommended [87]. Studies have shown no clinically significant interactions between sofosbuvir and the following drugs: Cyclosporine, tacrolimus, methadone, efavirenz, rilpivirine, darunavir/ritonavir, raltegravir, and tenofovir. No dose adjustments are required in patients receiving these drugs along with sofosbuvir [88].

3. Conclusions and future perspectives

From the above data, it seems that clinical research in the field of new treatments for chronic hepatitis C (HCV) has been devoted to developing regimens based on direct-acting antivirals (DAAs), with the goal of increasing treatment efficacy and improving tolerability and safety. A new era of successful interferon-free DAA therapy for HCV is emerging, with potential to broaden treatment of HCV to include patient groups who have either avoided or not been suitable for previous interferon-based therapy, and it is likely that sofosbuvir will form the backbone of this treatment approach. Sofosbuvir considered a promising therapy for chronic HCV infection, as it offers several advantages over the existing therapies, particularly in dealing with patients with decompensated liver disease and patients who cannot tolerate interferon-containing therapies. Large post-marketing studies, including pharmacovigilance and pharmacoepidemiological studies, can solve many unanswered questions for the future of this novel drug. Hepatitis C virus (HCV) continues to be a global problem but with the arrival of new drugs era it is expected to move towards exile and it is likely that sofosbuvir will form the cornerstone of this treatment approach. On the other hand, sofosbuvir commercialization will be the key moment to address some open questions with these new regimens, as the huge raise in treatment costs will represent the first problem to solve for national health care services. Consequently, affordability could be the driving force for the development of new strategies in treatment individualization and the approval of drugs from several pharmaceutical companies and the ensuing competition may result in lower drug costs in the future. Moreover, the future will evidence the development of much more compounds that will provide 100% efficacy within very

short periods of therapy, simplification of treatment management, lower costs and the dream of HCV eradication seems to be possible in the near future.

Acknowledgment

We are grateful to the staff members of Zoology departments, Faculty of science, Beni Suef University, for providing facilities to finish this work.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

Source of Support

Nil

Conflict of interest

The authors have declared no conflict of interest.

References

- [1] Mohd HK, Groeger J, Flaxman AD, Wiersma ST,(2013), Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence, *Hepatology*, 57,1333–42.
- [2] Naggie S, Patel K, McHutchison J, (2010),Hepatitis C virus directly acting antivirals: current developments with NS3/4A HCV serine protease inhibitors, *J Antimicrob Chemother*. 65, 2063–69.
- [3] Perz JF, Armstrong GL, Farrington LA, Hutin YJ, Bell BP,(2006), The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide, *Hepatology*, 45 (4), 529–38.
- [4] Kayali Z, Labrecque DR, Schmidt WN, (2006),Treatment of hepatitis C cryoglobulinemia: mission and challenges, *Curr Treat Options Gastroenterol*, 9,497–507.
- [5] Messina JP, Humphreys I, Flaxman A, Brown A, Cooke GS, et al.,(2015), Global Distribution and Prevalence of Hepatitis C Virus Genotypes, *Hepatology*, 61, 77-87.
- [6] Armstrong GL, Wasley A, Simard EP, McQuillan GM, Kuhnert WL, Alter MJ, (2002),The prevalence of hepatitis C virus infection in the United States, 1999 through 2002, *Ann Intern Med*, 144,705-14.
- [7] Razavi H, El-Khoury AC, Elbasha E, Estes C, Pasini K, Poynard T, Kumar R, (2013),Chronic Hepatitis C Virus (HCV) Disease Burden and Cost in the United States, *Hepatology*, 57(6), 2164–70
- [8] Hatzakis A, Wait S, Bruix J, Buti M, Carballo, M, et al, (2011),The state of hepatitis B and C in Europe : report from the hepatitis B and C summit conference, *J Viral Hepatol*, 18(1), 1-16.
- [9] Papatheodoridis, G , Hatzakis, A ,(2012), Public health issues of hepatitis C virus infection, *Best Practice &Research Clinical Gastroenterology*, 26,371-80.

- [10] European Association for the Study of the Liver (EASL),(2011), clinical practice guidelines: management of hepatitis C virus infection, *J Hepatol*, 55,245–64.
- [11] Nguyen LH, Ko S, Wong SS, Tran PS, Trinh HN, et al.,(2011), Ethnic differences in viral dominance patterns in patients with hepatitis B virus and C virus dual infection, *Hepatology* ,53,1839-45.
- [12] Lindenbach, BD, Rice, CM, (2005), Unraveling hepatitis C virus replication from genome to function, *Nature*, 436,933–38.
- [13] Shimotohno K1, Tanji Y, Hirowatari Y, Komoda Y, Kato N, Hijikata M,(1995), Processing of the hepatitis C virus precursor protein, *J Hepatol* , 22(1 Suppl),87-92.
- [14] Branch AD, Stumpp DD, Gutierrez JA,(2005), The hepatitis virus alternate reading frame (ARF) and its Family of navel products: the alternate reading frame protein/ F-protein, the double- frame shift protein, and others, *Semin Liv Dis*, 25,105-17.
- [15] Varaklioti A, Vassilaki, N , Georgopoulou, U ,(2002), Alternate translation occurs within the core coding region of the hepatitis C viral genome, *J Biol Chem*, 277(20), 17713-21.
- [16] Appel N, Schaller T, Penin F, Bartenschlager R,(2006), From structure to function: new insights into hepatitis C virus RNA replication, *J Biol Chem*, 281,9833–36.
- [17] Ma Y, Yates J, Liang Y, Lemon SM, Yi M,(2008), NS3 helicase domains involved in infectious intracellular hepatitis C virus particle assembly, *J Virol*, 82,7624–39.
- [18] Alexopoulou, A , Karayiannis, P ,(2015), Interferon-based combination treatment for chronic hepatitis C in the era of direct acting antivirals, *Ann Gastroenterol* , 28(1), 55–65.
- [19] Martinot-Peignoux M, Stern C, Maylin S, Ripault MP, Boyer N, et al.,(2010), Twelve weeks post-treatment follow-up is as relevant as 24 weeks to determine the sustained virologic response in patients with hepatitis C virus receiving pegylated interferon and ribavirin, *Hepatology*, 2010, 51,1122-26.
- [20] Gentile I, Borgia F, Buonomo AR, Castaldo G, Borgia G ,(2013), A novel promising therapeutic option against hepatitis C virus: An oral nucleotide NS5B polymerase inhibitor sofosbuvir, *Curr Med Chem*, 20,3733–42.
- [21] Hoofnagle JH, Mullen KD, Jones DB, Rustgi V, Di Bisceglie A, et al.,(1986), Treatment of chronic non-A, non-B hepatitis with recombinant human alpha interferon, A preliminary report, *N Engl J Med.*, 315,1575–78.
- [22] Zeuzem S, Feinman SV, Rasenack J, Heathcote EJ, Lai, MY, et al.,(2000), Peg-interferon alfa-2a in patients with chronic hepatitis C, *N Engl J Med.*, 343(23),1666-72.
- [23] Heathcote EJ, Shiffman ML, Cooksley WG, Dusheiko GM, Lee SS, et al.,(2000), Peg-interferon alfa-2a in patients with chronic hepatitis C and cirrhosis, *N Engl J Med*, 343(23),1673-80.
- [24] Fried, MW,(2002), Side effects of therapy of hepatitis C and their management.*Hepatology*, 36, S237-44.
- [25] Ascione A, De Luca M, Tartaglione MT, Lampasi F, Di-Costanzo GG ,et al.,(2010), Peg-interferon Alfa-2a Plus Ribavirin is More Effective than Peg-interferon Alfa-2b Plus Ribavirin for Treating Chronic Hepatitis C Virus Infection, *Gastroenterology*, 138(1),116–22.
- [26] Berenguer J, Ivarez-Pellicer JA, Marti'n PM, Opez-Aldeguer IL, Von-Wichmann MA,(2009), Sustained Virological Response to Interferon Plus Ribavirin Reduces Liver-Related Complications and Mortality in Patients Coinfected with Human Immunodeficiency Virus and Hepatitis C Virus,

- Hepatology, 50(2),407-13.
- [27] Manns MP, Mc-Hutchison JG, Gordon SC,(2001), Peg-interferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomized trial, *Lancet*, 358,958–65.
- [28] Fried MW, Shiffman ML, Reddy KR,(2002), Peg-interferon alfa-2a plus ribavirin for chronic hepatitis C virus infection, *N Engl J Med*, 347,975–82.
- [29] Hadziyannis SJ, Sette H J, Morgan TR, (2004),Peg-interferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med*, 140,346–355.
- [30] Hunyady B, Kovács B , Battyáni Z ,(2011), Side-effects of pegylated interferon plus ribavirin therapy with or without protease inhibitor direct acting antiviral agents during treatment of chronic hepatitis C virus infection, *Orv Hetil* , 152(50),1997-09.
- [31] European Association for the Study of the Liver (EASL),(2011), Clinical Practice Guidelines: management of hepatitis C virus infection, *J Hepatol* , 55,245–264.
- [32] Alexopoulou A, Papatheodoridis GV,(2012), Current progress in the treatment of chronic hepatitis C, *World J Gastroenterol*, 18,6060–69.
- [33] Anantharamu A, Sharma S, Sharma AK, Gupta AK, Dahiya N, Brashier DB,(2014), A review of the current anti-HCV therapy: Are we finally ready for interferon-free regimens, *I J of Nutr Pharm Neurolog Dise*, 4(5), 6-11.
- [34] Kayali Z, Schmidt WN ,(2014), Finally sofosbuvir: an oral anti-HCV drug with wide performance capability, *Pharmgenomics Pers Med*, 7,387–98.
- [35] Chatel-Chaix L, Baril M, Lamarre D ,(2010),Hepatitis C Virus NS3/4A Protease Inhibitors: A Light at the End of the Tunnel, *Viruses* , 2(8), 1752–65.
- [36] Vaidya A , Perry CM ,(2013), Simeprevir: first global approval, *Drugs*, 73 (18) , 2093–06.
- [37] Gottwein JM, Scheel TK, Jensen TB, Ghanem L, Bukh J ,(2011), Differential efficacy of protease inhibitors against HCV genotypes 2a, 3a, 5a, and 6a NS3/4A protease recombinant viruses. *astroenterology*, 141(3),1067-79.
- [38] US Food and Drug Administration, Simeprevir prescribing information, Silver Spring, [accessed 2014 May 5], Available at: http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/205123s001lbl.pdf
- [39] Lawitz E, Sulkowski MS, Ghalib R, Rodriguez-Torres M, Younossi ZM, et al.,(2014), Simeprevir plus sofosbuvir, with or without ribavirin, to treat chronic infection with hepatitis C virus genotype 1 in non-responders to pegylated interferon and ribavirin and treatment-naïve patients: the COSMOS randomized study, *Lancet*, 384, 1756–65,
- [40] Asselah T, Marcellin P, (2014), Second-wave IFN-based triple therapy for HCV genotype 1 infection: simeprevir, faldaprevir and sofosbuvir, *Liver Int*, 34 (1), 60–68.
- [41] Sulkowski MS, Bourlie`re M, Bronowicki GP, Asselah T, Pawlotsky GM, et al.,(2013),Faldaprevir combined with pegylated interferon alfa-2a and ribavirin in treatment-naïve patients with chronic genotype 1 HCV, SILEN-C1 trial, *Hepatology*, 57 (6), 2143–54.
- [42] Kanda T, Yokosuka O, Omata M,(2015), Faldaprevir for the Treatment of Hepatitis C, *Int J Mol Sci* , 16(3),4985-96.

- [43] Mc-Phee F, Hernandez D, Yu F, Ueland J, Monikowski A, et al.,(2013), Resistance analysis of hepatitis C virus genotype 1 prior treatment null responders receiving daclatasvir and sunaprevir, *Hepatology*, 58,902–11
- [44] Marcellin P, Manns MP, Janczewska E, Muir AJ, Wu X, Trenkle JD, et al. ,(2013), 12 weeks response- guided treatment with the NS5A inhibitor, GS-5885, the NS3 protease inhibitor, GS-9451, plus pegylated interferon and ribavirin in treatment naive genotype 1 hepatitis C infected patients, *J Hepatol*. 58(2), S355.
- [45] Vince B, Hill JM, Lawitz EJ, O’Riordan W, Webster LR, Gruener DM, et al.,(2014), A randomized, double-blind, multiple-dose study of the pan-genotypic NS5A inhibitor samatasvir in patients infected with hepatitis C virus genotype 1, 2, 3 or 4, *J Hepatol*, 60 (5), 920–27
- [46] German P, Mathias A, Pang P, Yang C, Yang C, Han L, et al.,(2013), Healthy volunteer first-in-human evaluation of GS-5816, a novel second generation broad-genotypic NS5A inhibitor with potential for once-daily dosing, In: Abstract presented at the 48th annual meeting of the European association for the study of the liver, Amsterdam (The Netherlands).
- [47] Guedj J, Dahari H, Rong L, Sansone ND, Nettles RE, Cotler SJ, et al.,(2013), Modeling shows that the NS5A inhibitor daclatasvir has two modes of action and yields a shorter estimate of the hepatitis C virus half-life, *Proc Natl Acad Sci USA*, 110 (10), 3991–96
- [48] Suzuki F, Toyota J, Ikeda K, Chayama K, Mochida S, Hayashi N, et al. ,(2014),A randomized trial of daclatasvir with peginterferon alfa-2b and ribavirin for HCV genotype 1 infection, *Antivir Ther*, 19, , 491– 99
- [49] Izumi N, Yokosuka O, Kawada N, Osaki Y, Yamamoto K, Sata M, et al.,(2014), Daclatasvir combined with peginterferon alfa-2a and ribavirin in Japanese patients infected with hepatitis C genotype 1, *Antivir Ther*, 19,501– 10
- [50] Wong KA, Worth A, Martin R, Svarovskaia E, Brainard DM, Lawitz E, et al.,(2013), Characterization of hepatitis C virus resistance from a multiple dose clinical trial of the novel NS5A inhibitor GS-5885 *Antimicrob Agents Ch*, 57 (12),6333–40
- [51] Lawitz EJ, Gruener D, Hill JM, Marbury T, Moorehead L, Mathias, A, et al. ,(2012), A phase 1, randomized, placebo-controlled, 3-day, dose-ranging study of GS-5885, an NS5A inhibitor, in patients with genotype 1 hepatitis C, *J Hepatol*, 57 (1), 24–31
- [52] Flisiak R, Pawlotsky JM, Crabbe R, Calistru PI, Kryczka W, et al.,(2011), Once-daily alisporivir (DEB025) plus peginterferon alfa-2a/ribavirin results in superior sustained virologic response in chronic hepatitis C genotype 1 treatment-naive patients, *J Hepatol*, 54(1), S2
- [53] Koch, U, Narjes, F,(2007), Recent progress in the development of inhibitors of the hepatitis C virus RNA-dependent RNA polymerase, *Curr Top Med Chem*, 7(13),1302-29.
- [54] Kwong AD, McNair L, Jacobson I, George S ,(2008),Recent progress in the development of selected hepatitis C virus NS3.4A protease and NS5B polymerase inhibitors, *Curr Opin Pharmacol* , 8,522–31.
- [55] Belkacem AA, Guichou JF, Brillet R, Ahnou N, Hernandez E ,et al.,(2014), Inhibition of RNA binding to hepatitis C virus RNA-dependent RNA polymerase: a new mechanism for antiviral intervention, *Nucl Acids Res*, 42 (14), 9399-09.
- [56] Legrand-Abrevanel F, Nicot F, Izopet J ,(2010), New NS5B polymerase inhibitors for hepatitis

- C, Expert Opin Invest Drugs, 19,963-75
- [57] Trivella JP1, Gutierrez J, Martin P,(2015), Dasabuvir : a new direct antiviral agent for the treatment of hepatitis C, Expert Opin Pharmacother, 16(4),617-24.
- [58] Pawlotsky JM1,(2013), NS5A inhibitors in the treatment of hepatitis C, J Hepatol, 59(2),375-82.
- [59] Hassanein T, Lawitz E, Crespo I, Davis M, DeMicco MP, Nelson DR, et al.,(2012), Once daily sofosbuvir (GS-7977) plus PEG/RBV: high early response rates are maintained during post-treatment follow-up in treatment-naive patients with HCV genotype 1, 4, and 6 infections in the ATOMIC study, Hepatology, 56 ,307A
- [60] Mathias MA, Cornpropst D, Clemons J, Denning WT,(2012), No clinically significant pharmacokinetic drug–drug interactions between sofosbuvir (GS-7977) and the immunosuppressants, cyclosporine a or tacrolimus in healthy volunteers, Hepatology, 56, 1063A–64A
- [61] Ron W,"Gilead's HCV drug sofosbuvir approved by the FDA but accessible for how many?". Doctors of the World, 2014.
- [62] Wodak A , An overview of Hepatitis C; Clinical Management in Opiate Pharmacotherapy Settings Australian Society for HIV Medicine Inc (ASHM), National Viral Hepatitis Education Program, 2009,1-4.
- [63] Smith MW, Walters KA, Korth MJ, Fitzgibbon M, Proll S, Thompson JC, et al.,(2006), Gene expression patterns that correlate with hepatitis C and early progression to fibrosis in liver transplant recipients, Gastroenterology, 130,179–87.
- [64] Fung A, Jin Z, Dyatkina N, Wang G, Beigelman L, Deval J ,(2014), Efficiency of incorporation and chain termination determines the inhibition potency of 2'-modified nucleotide analogs against hepatitis C virus polymerase, Antimicrobial Agents and Chemotherapy, 58 (7), 3636–45.
- [65] Lam AM, Espiritu C, Bansal S, Micolochick-Steuer HM, Niu C, Zennou V, et al.,(2012), Genotype and subtype pro-ling of PSI-7977 as a nucleotide inhibitor of hepatitis C virus, Antimicrob Agents Chemother, 56,3359-68.
- [66] Murakami E, Tolstykh T, Bao H, Niu C, Steuer HM, Bao D, et al.,(2010), Mechanism of activation of PSI-7851 and its diastereoisomer PSI-7977, J Biol Chem, 285 (45), 34337–47.
- [67] Chae HB, Park SM, Youn SJ(2013),, Direct-acting antivirals for the treatment of chronic hepatitis C: open issues and future perspectives, Scientific World Journal, 704912.
- [68] Degasperi E, Aghemo A ,(2014),Sofosbuvir for the treatment of chronic hepatitis C: between current evidence and future perspectives, Hepat Med. 6, 25–33.
- [69] Cholongitas E, Papatheodoridis GV,(2014), Sofosbuvir: a novel oral agent for chronic hepatitis C, Ann Gastroenterol, 27(4), 331–337.
- [70] Jacobson IM, Gordon SC, Kowdley KV, Kowdley KV , Yoshida EM ,et al. ,(2013), Sofosbuvir for hepatitis C genotype 2 or 3 in patients without treatment options, N Engl J Med, 368(20),1867–77.
- [71] Kirby B, Gordi T, Symonds WT, Kearney BP ,Mathias A,(2013), Population pharmacokinetics of sofosbuvir and its major metabolite (GS-331007) in healthy and HCV-infected adult subjects; 64th Annual Meeting of the American Association for the Study of Liver Diseases, November 1–5, Washington, DC.
- [72] Elbaza T, El-Kassasb M, Esmata G,(2014), New era for management of chronic hepatitis C virus

- using direct antiviral agents: A review. J Adv Res, [doi:10.1016/j.jare.2014.11.004](https://doi.org/10.1016/j.jare.2014.11.004)
- [73] Lawitz E, Lalezari J, Hassanein T, Kowdley K, Poordad F, Sheikh A, et al., (2013), Sofosbuvir in combination with peginterferon alfa-2a and ribavirin for non-cirrhotic, treatment-naïve patients with genotypes 1, 2, and 3 hepatitis C infection: a randomized, double-blind, phase 2 trial, *Lancet Infect*, 13, 401–408
- [74] Kowdley K, Lawitz E, Crespo I, Hassanein T, Davis M, DeMicco M, et al. (2013), Sofosbuvir with pegylated interferon alfa-2a and ribavirin for treatment-naïve patients with hepatitis C genotype-1 infection (ATOMIC): an open-label, randomised, multicentre phase 2 trial, *Lancet*, 381, 2100–07
- [75] Gane E, Stedman C, Hyland R, Pang P, Ding X, Symonds W, et al., (2013), All-oral sofosbuvir-based 12-week regimens for the treatment of chronic HCV infection: the ELECTRON study, *J Hepatol*, 58(S1–S24), A14
- [76] Lalezari JP, Nelson DR, Hyland RH, Lin M, Rossi SJ, Symonds WT, et al., (2013), Once daily sofosbuvir plus ribavirin for 12 and 24 weeks in treatment-naïve patients with HCV infection: the QUANTUM study, *J Hepatol*, 58, S236
- [77] Lawitz E, Mangia A, Wyles D, Rodriguez-Torres M, Hassanein T, et al, Sofosbuvir for previously untreated chronic hepatitis C infection, *N Engl J Med*. 2013, 368,1878–87.
- [78] Zeuzem S, Dusheiko G, Salupere R, Mangia A, Flisiak R, et al., (2013), Sofosbuvir plus ribavirin for 12 or 24 weeks for patients with HCV genotype 2 or 3: the VALENCE trial, *Hepatology*, 58(4), 733A.
- [79] Lawitz E, Poordad FF, Pang PS, Hyland RH, Ding X, Mo H, et al., (2014), Sofosbuvir and ledipasvir fixed-dose combination with and without ribavirin in treatment-naïve and previously treated patients with genotype 1 hepatitis C virus infection (LONESTAR): an open-label, randomised, phase 2 trial, *Lancet*, 383,515–23.
- [80] Lawitz E, Poordad F, Brainard D, Brainard DM, Robert H, et al, (2013), Sofosbuvir in combination with Peg-IFN and ribavirin for 12 weeks provides high SVR rates in HCV-infected genotype 2 or 3 treatment experienced patients with and without compensated cirrhosis: results from the LONESTAR-2 study, *Hepatology*, 58(Suppl 4),1380A.
- [81] Gane EJ, Hyl RH, An D, Pang PS, Symonds WT, et al, (2014), Sofosbuvir/Ledipasvir fixed dose combination is safe and effective in difficult-to-treat populations including genotype-3 patients, decompensated genotype-1 patients, and genotype-1 patients with prior Sofosbuvir treatment experience, *J Hepatol*, 60:S3-S4.
- [82] Fontana R, Hughes E, Bifano M, Appelman H, Dimitrova D, Hindes R, et al., (2013), Sofosbuvir and daclatasvir combination therapy in a liver transplant recipient with severe recurrent cholestatic hepatitis C, *Am J Transplant*, 1601–05
- [83] Charlton M, Gane E, Manns MP, Brown RSJ, Curry MP, et al., (2015), Sofosbuvir and ribavirin for treatment of compensated recurrent hepatitis C virus infection after liver transplantation. *Gastroenterology*, 148(1):108-17.
- [84] Rodriguez-Torres M, Lawitz E, Kowdley KV, Nelson DR, Dejesus E, Mc-Hutchison JG, et al, (2013), Sofosbuvir (GS-7977) plus peg-interferon/ribavirin in treatment-naïve patients with HCV genotype 1: A randomized, 28-day, dose-ranging trial, *J Hepatol*. 58,663–68.
- [85] Younossi Z, Stepanova M, Mir H, Jacobson I, Gane E, Nader F, et al. (2013), Minimal impact of

sofosbuvir + ribavirin on health related quality of life (HRQL) compared to pegylated interferon + ribavirin for chronic hepatitis C: results from FISSION + POSITRON trials, *J Hepatol*, 58(S567–S577), Abstract 1431

[86] US Food and Drug Administration,(2015), FDA Drug Safety Communication.

<http://www.fda.gov/Drugs/DrugSafety/ucm439484.htm>

[87] Karageorgopoulos DE, El-Sherif O, Bhagani S, Khoo SH,(2014) Drug interactions between antiretrovirals and new or emerging direct-acting antivirals in HIV/hepatitis C virus coinfection, *Curr Opin Infect Dis*, 27(1), 36-45.

[88] Levin J (2012), GS-7977 and HIV ARTs PK - No Clinically Significant Pharmacokinetic Interactions between Sofosbuvir (GS-7977) and HIV Antiretrovirals Atripla, Rilpivirine, Darunavir/Ritonavir, or Raltegravir in Healthy Volunteers. AASLD- 63rd Annual Meeting of the American Association for the Study of Liver Diseases.