

Hepatitis C therapies: A chronology of Past, Present and Future Strategies

Tariq khan

Tariq khan, Department of Biotechnology, University of Malakand, House 172, Street 28, Sector C, township, Kanju, Tehsil and District Swat, Pakistan

Correspondence to: Tariq khan, Department of Biotechnology, University of Malakand, House 172, Street 28, Sector C, township, Kanju, Tehsil and District Swat, 19200, Pakistan. tariqkhansaqi@yahoo.com

Telephone: +00923465552060

Received: September 6, 2012 Revised: October 13, 2012

Accepted: October 16, 2012

Published online: December 21, 2012

ABSTRACT

The key role of antiviral therapy of patients with chronic HCV is the sustained eradication of HCV. Different therapeutic plans such as herbal formulations against hepatitis symptoms were put forward for hundreds of years. However, lesser efficacy and, adverse effects constrained the large scale use of herbal medicines. The modern approach, thrived after the discovery of HCV made use of “interferon-based” regimens in different dose, duration of treatment and combination. After, ribavirin used in combination with interferon alpha became the centerpiece, pegylation of interferon alpha also aided in enhancing the efficacy of therapeutic plans. Keeping in view the genotype of HCV, viral load and virologic response to treatment, pegylated interferon alpha in combination with ribavirin soon became the standard of care for treating hepatitis C. Adverse effects and lower sustained virologic response rates associated with “interferon-based” regimen made indispensable the search for novel HCV therapies. The direct interference of some drugs in the HCV life cycle opened new gates to the treatment strategies beyond interferon. HCV NS3/4A protease inhibitors, the first developed class of Direct Acting Antivirals provided the modern world with Telaprevir and Boceprevir like drugs, that increased the SVR rates up to twice or thrice as that of Standard of Care alone. Many other important direct acting antivirals including polymerase inhibitors, alpha-glucosidase and cyclophilin inhibitors, Nitazoxanide and immunomodulatory therapies proved effective in increasing the upshot rates of HCV therapies. Recent advancement saw the upstart of direct acting antiviral combination therapies and more direct acting drugs under different phases of clinical trials.

© 2012 Thomson research. All rights reserved.

Key words: Antiviral therapy; SOC (standard of care); Sustained virologic response (SVR); DAAs

khan T. Hepatitis C therapies: A chronology of Past, Present and Future Strategies. *Journal of Gastroenterology and Hepatology Research* 2012; 1(11): 289-296 Available from: URL: <http://www.ghrnet.org/index./joghr/>

INTRODUCTION

Hepatitis C has been reported as a serious common transmissible disease, having hepatic and non-hepatic manifestations such as hemorrhage in the digestive tract, fibrosis, cirrhosis, and liver cancer^[1]. Having a different prevalence ratio in different regions of the world, HCV is a globally incident pathogen impacting about 130-170 million people worldwide^[2,3]. 70% of all the infected individuals develop chronic HCV infection and 20% adopt liver cirrhosis which may ultimately lead to hepatic carcinoma^[4]. The standard ‘interferon-based’ regimens had evolved as the backbone of standard of care (SOC) against HCV^[5]. “Interferon-based” regimens evolved from IFN monotherapy to the combination of ribavirin and ultimately the pegylation of interferon. The treatment outcome i.e. SVR associated with IFN based therapy increased with its evolution thus pegylated interferon having greater SVR rates associated with it^[6]. However, “interferon-based” therapy has several limitations in terms of adverse effects and lower response rates in the majority of patients^[7]. Therefore, the focus shifted towards the development of therapies ahead of interferon resulting in the discovery of a wide array of anti-HCV drugs that stops further generation of the virion by blocking various indispensable steps of viral replication and assembly^[8,9]. This review focuses on the present, future assessments in the field of antiviral treatment and finally on intensive efforts for the designing and optimizing new anti-HCV drugs.

HISTORICAL PERSPECTIVES: TRADITIONAL APPROACH TOWARDS HEPATITIS C TREATMENT

Traveling from the folk ways of treatment towards the advanced therapeutic strategies, it might be anticipated that the long-term

approaches must have been in practice for thousands of years for hepatic disorders^[10]. Ayurveda, that is in existence for about 5 000 years was used to treat different types of maladies. It is a natural technique of treatment which works on the principle of prevention followed by cure practices. It involves spiritual and mental healing for promotion of good health and treats a disease by natural medicines. It recommends a specific way to normalize the liver which includes lifestyle changes, dietary advices, yoga and use of medicinal herbs. Ayurvedic medicines proved to be great hepato-protective agents^[10]. Many other herbs were used by the system to treat the liver ailments caused by hepatitis C or other viral infections. Some herbs with products obtained are *Silybum marianum* (Milk Thistle), *Berberis aristata/vulgari* (Berberine), *Andrographis paniculata* (Kalmegh), *Angelica archangellica*, and *Boerhavia diffusa* etc.^[11]. *Silybum marianum* is however controversial according to recent studies as some experiments has proved its inefficacy against HCV^[12].

With the passage of time, focus shifted towards the Chinese herbal medicines which are in practice for the past 1000 years approximately^[10,13]. Traditional Chinese Medicines (TCM), the Chinese herbs proved very effective and are still in practice with efficiency greater than the modern western medicines. In the international symposium held in April 1991, in Beijing, more than a hundred researchers approved the traditional Chinese medicines (TCM) to be most effective against the hepatic diseases including hepatitis C^[13]. Ergil at the Fifth Symposium of the Society for Acupuncture Research Conference in 1998 stated that more than 55 herbal formulas are in use against hepatitis C according to a 1995 literature review^[14]. Acupuncture is an important tool of Chinese medicines in which sterilized fine needles are inserted in the body at various points. Acupuncture therapy is a balancing tool which helps the human body to recover from various syndromes by regulating the blood flow^[13]. A research paper by Cohen *et al*, (1998) presented at the 11th International Conference on AIDS, in Geneva Switzerland in 1998, referred to a study carried in 1995 on co-infection with HIV and hepatitis (B or C), inferring that acupuncture alone proved effective in regulating the production of hepatic enzymes^[15].

The TCM also includes the use of herbs as drugs. Porter (2004) stated in an article that there are many types of herbs approved by the National Centre for Complementary and Alternative Medicines (NCCAM). Some are Kava, Blue-green Algae, Sassafras (*Sassafras albidum*), Dong Quai (*Angelica polymorpha*), Jin Bu Huan (*Lycopodium serratum*), Comfrey (*Symphytum officinale* and *S. uplandicum*), Pennyroyal (*Mentha pulegium*), and Shark Cartilage etc. However, information regarding the use of herbs for hepatitis C is by no means complete. There are evidences about the disorders related to the simultaneous use of herbs with antiviral drugs e.g. the use of *Bupleurum* along with antivirals resulted in 16 deaths in Japan^[16].

Similarly, toxic hepatitis is an adverse reaction resulting from the simultaneous use of herbal medicine with hepatotoxic ingredients such as acetaminophen and non-steroidal anti-inflammatory agents^[17].

Natural medicines against HCV have been widely applied both solely and in combination with modern therapies i.e. Western medicines (discussed later). Medicines used in the therapies are classified into three categories called as Western medicines ("interferon-based" regimens), complementary and alternative medicines (Ayurvedic and Chinese medicines) and a combination of these two called the Integrative medicine^[10]. Natural medicines only act mostly as hepato-protective agents. Herbs act as healers of the damage caused by the notorious rival. They do so by regulating the activities of several enzymes in the liver. *Andrographis paniculata*, for example regulates glutathione reductase, superoxide dismutase, catalase, and, glutathione peroxidase. This and many others have been reported as a good antioxidant and promising hepatoprotective

agents^[18,19]. Similarly, many other herbal formulations such as silymarin have improved the disease state while acting as hepato-protective agents. For instance, 650 mg/d of silymarin for 6 months in patients with chronic hepatitis C resulted in improved serum HCV-RNA titer, serum aminotransferases and patients' quality of life. Silymarin has been demonstrated as anti-inflammatory and anti-oxidants, which aid in reducing hepatic inflammation and damage caused by HCV^[17].

However, herbal medicines solely are less efficient in eliminating the virus from the body. So far, herbs are unsuccessful in viral load reduction and preventing relapse of the disease. Studies have shown that a very low proportion of subjects treated with herbal formulation alone reported an increase in serum HCV RNA. This calls for the need for further research and expertise in the area^[20].

PRESENT PERSPECTIVES: WESTERN APPROACH TOWARDS HEPATITIS C TREATMENT

The discovery and Evolution of Interferon

Sir Alick Isaacs in 1957, started a new era when he found a human protein block the process of viral replication and was called as interferon^[21]. Cantell's group of Finland derived clean and fairly sufficient amount of IFN, which soon got a pivotal role in antiviral activities^[22]. A therapy is said to be successful when the HCV becomes undetectable after 24 weeks of the treatment. The viral load should be 100 times less than the amount present before treatment. The viral load should be 100 times less than the amount present before treatment. This is known as the response of the virus to treatment^[23].

Hoofnagle *et al*^[16,23], (1986) first reported the enthusiastic anti-HCV properties of interferon. Lau *et al*^[24] (1998) also affirmed that interferon-a was used for treatment of Non-A non-B hepatitis even before the incredible discovery of HCV in 1989. In their follow-up study conducted upon the patients who have received interferon a treatment between the year 1984 and 1986, they confirmed the efficacy of interferon-a for chronic hepatitis C patients^[25]. Moreover, the meta-analytic studies of Tine *et al*, (1991) gave a relative percentage of 20-25% sustained virologic response shown to interferon treatment^[26].

After its approval by the FDA in 1991, more trials were made on the 'interferon-based' therapies of hepatitis C. Its standard dose was set to be 3 MU (million units), three times in a week for 6 months^[27,28]. Interferon alpha-2b resulted in about 47% ALT normalization response and about 40% histological response in treated patients^[27]. The studies of Poynard *et al*, (1996) favored 3 MU three times a week for 12 months for treating chronic hepatitis C, while it should be 3 MU three times a week for 3 months for acute hepatitis C^[29].

The second type of interferon, which is known as Alpha-2a interferon (Roferon-A), approved by the FDA in 1996, bears only one amino acid difference from alpha-2b interferon and its standard dose was set as 3 MU three times in a week for 12 months. The overall efficacy of Roferon-A is the same as that of Intron-A^[27,30]. Another type of interferon approved in 1997, called as consensus interferon showed a greater in vitro biological activity as compared to interferon 1^[27,31]. However, Tong *et al*, (1997) suggested that consensus interferon is only slightly efficient as compared to the interferon 1^[32].

"Interferon-based" therapy has many delimits associated with it such as thrombocytopenia, neutropenia, neuropsychiatric disorders, headache, fever, chills, pain in joints, and myalgia^[33-35]. Interferon is involved in amending the immunology of the recipients, resulting in autoimmune disorders such as thyroiditis, reported in 20% of patients subjected to IFN therapies^[36,37].

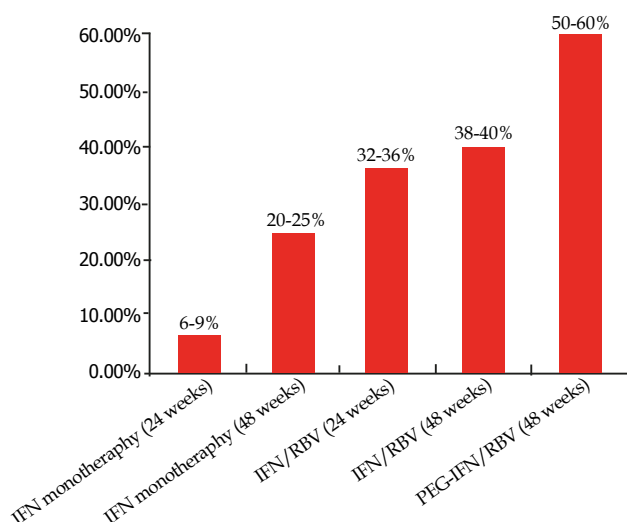


Figure 1 pegylation improved the efficacy of interferon based regimen by showing greater SVR rate at different dose and duration of treatment.

Emergence of Ribavirin and Combination therapy

With more advances, a synthetic guanosine analogue Ribavirin, was used with interferon-alpha in combination to enhance the effect of treatment^[38]. Ribavirin that was approved by the FDA in 1998, was used against HCV as Rebetron (combination of Interferon alpha and Ribavirin) and its monotherapy was not favored^[27,39,40]. Schalm, S. (1998) performed randomized trials on the coalescent therapy of interferon alpha and ribavirin to check its efficacy and safety in HCV patients. They concluded that ribavirin in combination with interferon resulted in a greater percentage of SVR i.e. 36% of patients in combination as compared to 18% in interferon monotherapy, when 3 mega units of interferon three times a week plus ribavirin 1-1.2 grams per day for 24 weeks was administered^[41].

Pegylation improved the efficacy of therapy

Due to its quick elimination from the blood, practitioners recommend three doses of interferon in a week but, this resulted in a burden on the patient. Furthermore, this regimen may induce viral resistance because of irregularity in treatment protocol^[42,43]. Researchers found that the attachment of a 40 KD polyethylene glycol (PEG) chain can result in greater half-life of interferon alpha^[44].

One of the two major types of pegylated interferon is the alpha-2b, which was the first PEG-IFN approved in 2001. It is a 12 kDa PEG molecule bound to the histidine-34 of interferon alpha-2b by an unstable urethane bond^[42,43,45,46]. Peg-Interferon (Peg-intron) is generally administered in accordance with body weight of a person i.e. 1.0 µg/kg once weekly for one year in case of monotherapy and 1.5 µg/kg when administered in combination with ribavirin^[27,46]. Lindsay *et al.*, (2001) reported that 49% of patients treated with 1.5 mg/kg per week of pegylated interferon alpha-2b achieved a response, vs. 24% with the standard formulation. 35-49% SVR was predicted in patients with HCV genotype 2 or 3 depending on the dose and 10% to 14% SVR was shown by patients with genotype 1^[47].

The combined use of pegylated interferon with ribavirin led to the approval of peg-intron plus rebetol (ribavirin) in the same year 2001^[27]. Manns *et al.*, (2001) performed a randomized trial and reported that administration of Peg-IFN alpha-2b (FDA approved dose: 1.5 µg/kg) in coalescence with ribavirin (800 mg) resulted in a greater SVR rate as compared to monotherapy. The overall SVR rates shown to this combination therapy were 42% in patients with HCV genotype 1 and 81% in patients with genotype 2 or 3^[41,48].

The 2nd useful type, pegylated interferon alpha-2a was

demonstrated to have sustained absorption, a slower rate of clearance and an extended serum half-life as compared to the peg-intron and intact interferon Alpha. The overall sustained virologic response rates were shown to be 36-39% with peg-IFN-alpha-2a monotherapy^[49]. Pegasys was approved in October 2002 and the recommended dose for using Pegasys monotherapy is set to be 180 micrograms per week for 48 weeks subcutaneously^[44].

Many randomized and multicentre analyses were performed to check the efficacy of the coalescent use of Pegasys plus ribavirin and finally approved in 2002^[27]. Fried *et al.*, (2001) affirmed the efficacy of combined therapy of Pegasys plus Copegus and showed 46% SVR rates for genotype 1 and 76% for genotypes 2 and 3^[50]. Pegylation really helped improve the Overall SVR rates associated with “interferon-based” regimens^[51] as shown in the figure 1.

Optimization strategy for treatment

Concerning the efficacy of treatment protocol, the dose (interferon and ribavirin) and duration of therapy are kept into considerations. Improved efficacy can be achieved by administering 80% of the interferon dose, 80% of the ribavirin dose, and at least 80% of the standard 48-week duration of therapy. This standard was called as the 80-80-80 rule^[52]. It helps to improve adherence with therapy of hepatitis C and eliminate the various side effects associated with therapy^[53].

HEPATITIS C TREATMENT AHEAD OF INTERFERON

So far, there are no effective vaccines against the HCV and the standard therapy is the combined administration of pegylated IFN and ribavirin (12-72 weeks). But a sustained virological response (SVR) was shown by only 40%–50% of patients infected with HCV genotypes 1 or 4 as compared SVR shown by 80% by individuals infected with genotypes 2 or 3. Moreover, several side effects are associated with the combination therapy^[54].

The realization that insight into the viral life cycle will ultimately develop a new approach to treatment of HCV, motivated scientists to successfully design a cell culture system for HCV. For instance the HCV JFH-1 based cell culture system designed in 2005 unraveled various steps in the life cycle of HCV, which promised the development of novel anti-virals against HCV^[55-57].

Improved insight into the viral life cycle, favored the development of novel agents beyond interferon, termed as direct-acting antiviral (DAA) agents. These agents were observed to target HCV replication and post-translational processing specifically^[8,9].

HCV PROTEIN PROCESSING INHIBITORS

NS3/4A protease inhibitors

The NS3/4A protease aids in polyprotein processing by catalyzing the cleavage of the downstream region of genome-encoded polyprotein into 4 functional nonstructural proteins, including the HCV polymerase (NS5B) Therefore, NS3/4A protease has been documented as a key target for anti-HCV therapy^[54,58].

Francesco and Carfi (2007) classified protease inhibitors in two major classes on the basis of bonding. The first group is that of non-covalent inhibitors, such as ciluprevir (PI limited due to adverse effects). The second group is represented by covalent reversible inhibitors, also termed as serine-trap inhibitors. Telaprevir (TVR) and boceprevir are the most potent drugs in the later class^[59].

Telaprevir against HCV

Telaprevir (TVR), a NS3/4A PI, is an orally administered linear keto-amide protease inhibitor studied in monotherapy carried out

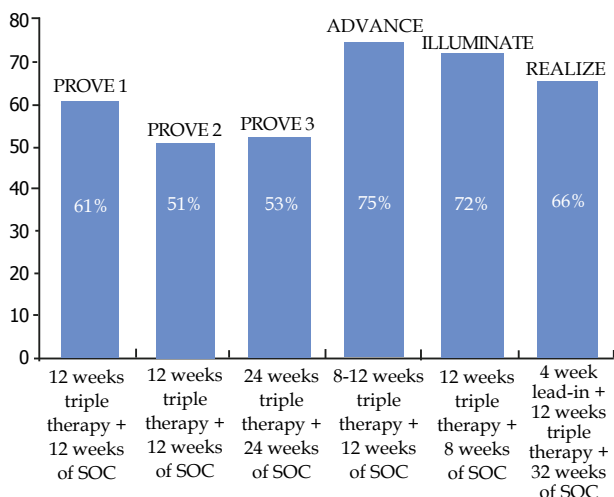


Figure 2 Telaprevir results in different SVR rate.

for 14 days in patients having HCV genotype 1^[60]. TVR therapy demonstrated a spectacular reduction of viral RNA from the replicon cells^[61]. Vertex Pharmaceuticals, a global biotechnology company initiated two types of trials in 2006 named, the Protease Inhibition for Viral Evaluation (PROVE-1 and PROVE-2) studies^[54,62]. These trails respectively conducted on patients from the United States and Europe, reported the results such that about 67-69% of patients showed sustained virologic response (SVR) in the telaprevir-containing study as compared to the 41-46% SVR rates in patients who received the standard treatment^[63,64]. Phase 3 studies, ADVANCE and ILLUMINATE, conducted in about 1500 treatment naïve individuals infected with HCV genotype 1. In the ADVANCE trials, when 6 pills of TVR were given per day along with the SOC (PEG-IFN + RBV) for 12 weeks, 75% SVR was achieved while the SVR was 69% for 8 week regimen. Side effects such as severe rash, itchy skin, anemia, nausea, vomiting, and diarrhea were associated with the therapy. In the ILLUMINATE trials, 6 pills/day of TVR for 24-48 weeks with 12 weeks of triple therapy followed by 8 weeks of PEG-IFN/RBV resulted in overall SVR rates of 72%, while SVR was 92% and 88% at 24 and 48 weeks, respectively for patients who have shown extended Rapid Virological Response (eRVR). eRVR means undetectable HCV RNA at week 4 of treatment and that remains undetectable at week 12. For people who did not have eRVR, SVR after 48 weeks was 64%. It means that no benefits in terms of long duration were obtained in ILLUMINATE study^[65].

Another phase 3 study of telaprevir, named REALIZE resulted in SVR rates of 64%, 66% and 17%, respectively when either a 12-week triple combination treatment regimen followed by 36 weeks of SOC was applied, or prior therapy for the 4-week with SOC, followed by triple combination therapy for 12 weeks, and finally therapy with SOC for 32 weeks of treatment-experienced genotype 1 patients or only SOC for 48 weeks^[55]. The FDA approved telaprevir with the brand name Incivek very recently in May, 2011 with a recommended dose as the 750 mg telaprevir given as two 375-mg tablets three times daily for 12 weeks^[66,67].

Boceprevir against HCV

Boceprevir is an orally administered peptidomimetic α -ketoamide, which has been tested in three phases of trials for its efficacy against HCV serine protease enzyme^[58].

In an initial phase 1 study, 400 mg daily of boceprevir was given in combination with pegylated IFN to patients infected with HCV genotype 1 and who were non-responders to SOC; this type of therapy

resulted in an average fall of 2.16 log₁₀ UI/mL viral HCV RNA level in serum, with undetectable HCV RNA levels in 40% individuals within 2 weeks^[68,69]. But unfortunately, due to improper dosage of boceprevir (100 mg-400 mg) or an improper dose of boceprevir (800 mg three times a day) with pegylated interferon a-2b without ribavirin, the study resulted in lower overall SVR rates of 2-14%^[70].

In January 2006, Schering Corporation initiated a pair of phase II trials for boceprevir called HCV SPRINT-1 (Serine Protease Inhibitor Therapy-1) in treatment-naïve patients^[71-73]. The results showed that, with triple therapy for 28 weeks, the SVR rates were 54% and a 4-week lead-in treatment with PEG-IFNa-2b/RBV followed by 44 weeks of triple therapy resulted in 75% SVR, as compared with 38% in the control group^[74].

Schering Corporation initiated two phase 3 trials named SPRINT-2 and RESPOND-2 for treatment naïve and treatment-experienced patients respectively. Poordad and colleagues trialed 1097 treatment naïve patients having HCV genotype 1 divided into three groups each one receiving a different treatment regimen. All three groups received lead-in treatment with peg-IFN alpha-2b plus ribavirin for 4 weeks. One of the groups, the control group received the SOC for 44 weeks and the 2nd group was treated with boceprevir plus SOC for 24 weeks, while group 3 was treated with boceprevir plus SOC for 44 weeks. SVR rates of 63-66% were observed with boceprevir plus SOC as compared to 38% in SOC (control group) alone^[75].

In phase 3 RESPOND-2 trials performed by Bacon and colleagues, 403 previously treated patients were analyzed for treatment boceprevir. HCV RESPOND-2 trials also included a lead-in treatment phase of 4 weeks. SVR rates obtained with RESPOND-2 trials were impressive (59-66%) as compared with the 21% SVR shown by control group^[76]. Consequently, the FDA approved the protease inhibitor boceprevir in May, 2011 with the trade name Victrelis for use against HCV genotype 1^[66]. The recommended dose is set to be Victrelis (800 mg) administered orally three times daily (every 7-9 hours) with food. Moreover, it is also strongly recommended that Victrelis must be administered in combination with peginterferon Alpha and ribavirin^[71].

Some of the most interesting PIs that are tested in phase I and phase-II trials are danoprevir (RG 7227/ITMN191), narlaprevir, vaniprevir (MK-7009), BI 201335^[77-80].

Replication specific Inhibitors

NS5B polymerase inhibitors: Polymerase inhibitors were previously observed as antiviral drugs in HIV, herpes and HBV infections. Researchers therefore, focused on the use of this antiviral agent against HCV. They are mostly analogous to nucleotides thus can inhibit nucleic acid synthesis by conversion into nucleotide phosphate to which they are analogous^[78,81]. NS5B polymerase inhibitors can be distinguished into two main classes: nucleoside or nucleotide analogue inhibitors, and non-nucleoside analogue inhibitors^[54].

Nucleoside or nucleotide analogue polymerase inhibitors:

These inhibitors are observed to show similar activity against all HCV genotypes due to conserved regions of NS5B. Valopicitabine (2'-C-methylcytidine) and R1626 (4'azidocytidine) emerged as remarkable antiviral agents alone or in coalescence with PEG-IFN-alpha-2a/RBV at high doses. But unfortunately, severe side effects hindered its further development^[82-84].

The first successful (Phase IIa) trials used a combination of R1626 (a nucleoside analogue and polymerase inhibitor introduced by Roche pharmaceuticals) with pegylated interferon a-2a and ribavirin. The results of this study, presented at the 2008 EASL meeting by Nelson and associates, showed that high RVR (Rapid Virological Response i.e. 100-fold or greater drop in HCV viral load by week 4 of

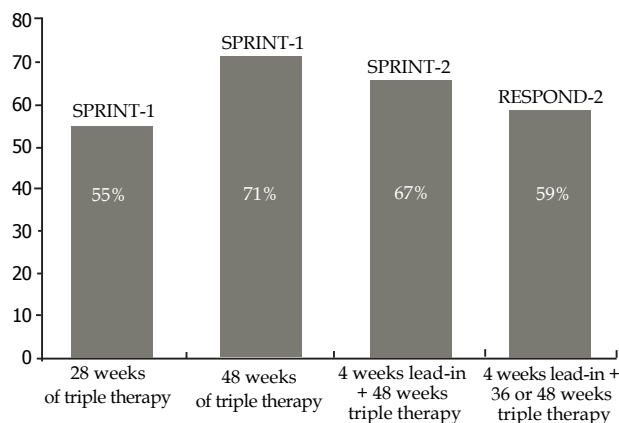


Figure 3 Boceprevir shows promising results.

treatment) can be obtained by using R1626 with SOC^[85,86]. Phase IIb trials conducted by Roche are also underway to identify an efficient regimen of R1626 by using 500, 1 000, or 1 500 mg/d, and 90 or 180 µg of pegylated interferon plus weight-based ribavirin. A very low resistance level is shown to R1626 monotherapy or coalescent therapy with PEG-IFN/RBV^[84]. Many other nucleotide analogue inhibitors such as R7128, VCH-759, PSI-7977 and IDX184 etc. are under clinical trials^[87-90].

Non-Nucleoside analogue polymerase inhibitors: S5B polymerase enzyme is a 'right-hand motif' of finger, palm and thumb domains having 5 allosteric sites A, B, C, D, and E targeted by non-nucleoside inhibitors. Four of these sites benzothiadiazine (psalm 1) -binding site, a benzofuran (psalm 2) -binding site, a benzimidazole (thumb 1) -binding site, and a thiophene (thumb 2) -binding site is important targets^[54,91,92]. Many novel inhibitors for each site are developed e.g. BI 207127 blocks the polymerase site 1, VX-222 for site 2, ANA598 for site 3, and HCV-796 (nesbuvir) is a site 4 inhibitor^[93-96].

NS5A inhibitors: Inhibitors directed against the non-structural NS5A protein, are found to be active against almost all genotypes. One such inhibitor BMS-790052 forms a bond with a crucial regulatory domain of the NS5A protein. After phase I studies, which resulted in promising responses, this NS5A inhibitor is under phase 2 clinical trials, whose interim data has predicted that different doses of BMS-790052 in combination with SOC resulted in 83% and 92% rate of rapid virological response in patients who respectively received 10 and 60 mg BMS-790052 once in a day^[97].

COMBINATION OF DAAS: THE UPSTART

To overcome these side effects still associated with combination therapies, researchers focused on the coalescent use of two or more DAAs. In a study named INFORM-1, combined use of a polymerase inhibitor (RG 7128) and the NS3/4A protease inhibitor danoprevir (RG 7227/ITMN191) in both treatment-naïve and treatment-experienced patients with HCV genotype 1 for up to 2 weeks, resulted in undetectable RNA levels of treatment-naïve patients in approximately 63% after 2 weeks of combination therapy. Levels of undetectable RNA were lower in treatment experienced patients^[98]. Many DAA combinations such as polymerase-polymerase, polymerase-protease, and protease-protease inhibitor combinations are under clinical trials^[99]. However, drug resistance, safety and suitable combination of DAA are the key factors that should be addressed in future trials^[100].

INTERFERON FREE THERAPIES

After a long time of tough and timid research, the sole dominion of interferon seems to be vanishing. Fortunately, after the successful trials of interferon free dose of Gilead's GS-7977 also called as PSI-7977, a pyrimidine nucleotide analogue, it has been proved that some of the DAAs has a potential role against HCV and can cure HCV up to a great extent without the use of interferon as SOC. Almost 100% SVR rates have been shown with this DAA against genotype 2 and 3^[101,102].

Although, the results of phase 3 trials upon an interferon free regimen of GS-7977 formerly called PS-7977 are still awaited that will conclude its efficacy and safety, yet, many recent studies have demonstrated almost 100% efficacy of GS-7977 in combination with Ribavirin and other antivirals like Daclatasvir (NS5A Inhibitor)^[103-105].

More such DAAs like the combination of asunaprevir (a protease inhibitor) and Daclatasvir has also shown promising result against HCV without the use of interferon^[106,107].

NOVEL THERAPIES UNDERWAY

Cyclophilin B Inhibitor as Alisporivir (Debio-025) resulted in a decrease in HCV RNA in patients with genotype 1 or 4^[108]. HCV Alpha-Glucosidase Inhibitor such as celgosivir resulted in a greater eVR (Early virological response i.e. 100-times or greater drop in HCV viral load by week 12 of treatment) when applied in combination with SOC for 2 weeks^[99]. Furthermore, Immunomodulatory therapies like Toll-like receptors (TLRs) agonists such as ANA245 (a guanine nucleoside analogue) and human monoclonal antibody as HCV-AB68 resulted in a promising response and are under clinical trials^[109,110]. Another novel drug, Nitazoxanide (alinia), a thiazolidine, resulted in about 79% SVR rate in treatment naïve patients with HCV genotype 4^[111]. Nucleic-acid-based antiviral agents as antisense oligonucleotides (mRNA binding fragments), ribozymes (molecular scissors) and, more recently, siRNAs are also being explored as therapeutic agents against HCV^[110]. Novel interferons such as Hanferon, Interferon 5, Interferon-a-2b XL, Interferon lozenges, Locteron interferon and Albinterferon a-2b (Alb-IFN) are also becoming a part of the HCV pipeline^[112]. Spirulina platensis, a cyanobacterium is also under study and has been proved worthy in chronic hepatitis and can be of great use in future^[113].

SUMMARY AND CONCLUSIONS

After studying the remedial actions of folk medicines, it can be concluded that several of them have a convincing role against HCV but with side effects and less knowledge about their use. Moreover, pegylation improved the value of interferon against HCV and the use of ribavirin with pegylated interferon added zest to the therapy. Moreover, new strategies beyond "interferon-based" therapies have also proved well in several classes of patients. The most well developed and recently approved direct acting drugs, Telaprevir and Boceprevir are the most promising among DAAs regarding its SVR outcome and safety. However, monotherapy with many DAAs is still not recommended, it indicates that "interferon-based" regimens remains the backbone of therapeutic strategies against HCV. Moreover, most of the DAAs have targeted HCV genotype 1 so far.

REFERENCES

- 1 Poynard T. Hepatitis B and C: Management and Treatment, Second Edition: Taylor & Francis; 2004
- 2 Idrees M, Lal A, Naseem M, Khalid M. High prevalence of hepatitis C virus infection in the largest province of Pakistan. *Journal of Digestive Diseases* 2008; 9: 95-103

- 3 World Health Organization. Factsheet. 2011. <http://www.who.int/mediacentre/factsheets/fs164/en/index.html>
- 4 Koziel MJ, Peters MG. Viral Hepatitis in HIV Infection. *New England Journal of Medicine* 2007; **356**: 1445-1454
- 5 Munir S, Saleem S, Idrees M, Tariq A, Butt S, Rauff B, Hussain A, Badar S, Naudhani M, Fatima Z, Ali M, Ali L, Akram M, Aftab M, Khubaib B, Awan Z. Hepatitis C treatment: current and future perspectives. *Viral Journal* 2010; **7**: 296
- 6 Shariieff KA, Duncan D, Younossi Z. Advances in treatment of chronic hepatitis C: 'pegylated' interferons. *Cleveland Clinic Journal of Medicine* 2002; **69**: 155-159
- 7 Rafique S, Idrees M, Ilyas M, Hussain A, Ali M, Ali L, Butt S, Afzal S, Ur Rehman I, Saleem S. Positional effect of phosphorylation sites 266 and 267 in the cytoplasmic domain of the E2 protein of hepatitis C virus 3a genotype: Interferon Resistance analysis via Sequence Alignment. *Virology Journal* 2011; **8**: 204
- 8 Moradpour D, Penin F, Rice CM. Replication of hepatitis C virus. *Nat Rev Microbiol* 2007; **5**: 453-463
- 9 Sarrazin C, and Zeuzem S. Resistance to Direct Antiviral Agents in Patients With Hepatitis C Virus Infection. *Gastroenterology* 2010; **138**(2): 447-462
- 10 John TS, and Sandt L. Hepatitis C Choices: *Caring Ambassadors Program*; 2008
- 11 Khabiya R, and Joshi A. "A review on hepatoprotective medicinal plants. *Inter J Curr Trends Sci Tech* 2010; **1**: 16-27
- 12 Cutler N. Three Reasons to Be Wary of Recently Publicized Milk Thistle Doubt 2011 November, 2011. Available from: <http://www.hepatitis-central.com/mt/archives/2011/11/>
- 13 Lee CH, Wang JD, Chen PC. Case-crossover study of hospitalization for acute hepatitis in Chinese herb users. *J Gastroenterol Hepatol* 2008; **23**: 1549-55
- 14 Ergil K. "Fifth Symposium of the Society for Acupuncture Research Conference. In: panel Hsar, editor. Society for Acupuncture Research Conference; Palo Alto, California. 1998
- 15 Cohen M, Wilson CJ, and Surasky A. Acupuncture Treatment In HIV+ Peoples With HCV/HBV co-infection And Elevated Transaminase. XIIIth International Conference on AIDS; Geneva Switzerland 1998
- 16 Porter L. herbs and hepatitis C: Hepatitis C Support Project; 2004. Available from: <http://www.hcvadvocate.org>
- 17 Del Prete A, Scalera A, Iadevaia MD, Miranda A, Zulli C, Gaeta L, Tuccillo C, Federico A, Loguercio C. Herbal products: benefits, limits, and applications in chronic liver disease. *Evid Based Complement Alternat Med*. 2012; 2012: Article ID 837939: 19 pages
- 18 Mishra SK, Ravi B, Vinjamury S. Ayurvedic Medicines. In: John TMS, Sandt L, editors. Hepatitis C Choices: *Caring Ambassadors Program, Inc.* 2008; 153-165
- 19 Jain NK, Singhai AK. Hepatoprotective activity of Chenopodium album Linn: in vitro and in vivo studies. *J Exp Integr Med* 2012; **2**: 331-336
- 20 Liu JP, Manheimer E, Tsutani K, Gluud C. Medicinal Herbs for Hepatitis C Virus Infection: A Cochrane Hepatobiliary Systematic Review of Randomized Trials Medicinal Herbs for HCV Infection. *The American Journal of Gastroenterology* 2003; **98**: 538-544
- 21 Isaacs A, Lindenmann J. Virus interference. I. The interferon. *Proc R Soc Lond B Biol Sci.* 1957; **147**: 258-267
- 22 Cantell K, Hirvonen S, Koistinen V. [71] Partial purification of human leukocyte interferon on a large scale. In: Sidney P, editor. *Methods in Enzymology*: Academic Press; 1981. 499-505
- 23 Ghany MG, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; **49**: 1335-1374
- 24 Lau DT, Kleiner DE, Ghany MG, Park Y, Schmid P, Hoofnagle JH. 10-Year follow-up after interferon-alpha therapy for chronic hepatitis C. *Hepatology* 1998; **28**: 1121-1127
- 25 Hoofnagle JH, Mullen KD, Jones DB, Rustgi V, Di Bisceglie A, Peters M, Waggoner JG, Park Y, Jones EA. Treatment of chronic non-A, non-B hepatitis with recombinant human alpha interferon. A preliminary report. *N Engl J Med* 1986; **315**: 1575-1578
- 26 Tine F, Magrin S, Craxi A, Pagliaro L. Interferon for non-A, non-B chronic hepatitis: A meta-analysis of randomised clinical trials. *Journal of Hepatology* 1991; **13**: 192-199
- 27 Franciscus A. A Brief History of Hepatitis C. Hepatitis C fact sheet [Internet]. 2009. Available from: www.hcvadvocate.org
- 28 Carithers RL, Jr., Emerson SS. Therapy of hepatitis C: meta-analysis of interferon alfa-2b trials. *Hepatology* 1997; **26**: 83S-88S
- 29 Poynard T, Leroy V, Cohard M, Thevenot T, Mathurin P, Opolon P, et al. Meta-analysis of interferon randomized trials in the treatment of viral hepatitis C: effects of dose and duration. *Hepatology* 1996; **24**: 778-89
- 30 Lee WM. Therapy of hepatitis C: interferon alfa-2a trials. *Hepatology* 1997; **26**: 89S-95S
- 31 Blatt LM, Davis JM, Klein SB, Taylor MW. The biologic activity and molecular characterization of a novel synthetic interferon-alpha species, consensus interferon. *J Interferon Cytokine Res* 1996; **16**: 489-499
- 32 Tong MJ, Reddy KR, Lee WM, Pockros PJ, Hoefs JC, Keeffe EB, et al. Treatment of chronic hepatitis C with consensus interferon: a multicenter, randomized, controlled trial. Consensus Interferon Study Group. *Hepatology* 1997; **26**: 747-754
- 33 McHutchison JG, Dusheiko G, Shiffman ML, Rodriguez-Torres M, Sigal S, Bourliere M, et al. Eltrombopag for Thrombocytopenia in Patients with Cirrhosis Associated with Hepatitis C. *New England Journal of Medicine* 2007; **357**: 2227-2236
- 34 Shiffman ML, Hofmann CM, Luketic VA, Sanyal AJ. Use of granulocyte macrophage colony stimulating factor alone or in combination with interferon-alpha-2b for treatment of chronic hepatitis C. *J Hepatol* 1998; **28**: 382-389
- 35 Schäfer M, S. Mauss. Management of adverse events and drug interactions of interferon based therapy for chronic hepatitis C. In: S. M, editor. *Hepatology-A Clinical Textbook*. Germany: Flying Publsiher; 2010. 171-196
- 36 Wesche B, Jaekel E, Trautwein C, Wedemeyer H, Falorni A, Frank H, et al. Induction of autoantibodies to the adrenal cortex and pancreatic islet cells by interferon alpha therapy for chronic hepatitis C. *Gut* 2001; **48**: 378-383
- 37 Lisker-Melman M, Di Bisceglie AM, Usala SJ, Weintraub B, Murray LM, Hoofnagle JH. Development of thyroid disease during therapy of chronic viral hepatitis with interferon alfa. *Gastroenterology* 1992; **102**: 2155-2160
- 38 Telfer PT, Garson JA, Whitby K, Grant PR, Yardumian A, Hoffbrand AV, et al. Combination therapy with interferon alpha and ribavirin for chronic hepatitis C virus infection in thalassaemic patients. *British Journal of Haematology* 1997; **98**: 850-855
- 39 Rebetol A. Summary of Product Characteristics. Europe: Schering Plough; 1999
- 40 Pharmaceuticals R. Roferon-A. Summary of Product Characteristics. Roche Products Ltd; 2000
- 41 SCHALM SW. Adding ribavirin to interferon α -2b for chronic hepatitis C infection increased virological response and nausea. *Gut* 1998; **43**: 602
- 42 Glue P, Fang JW, Rouzier-Panis R, Raffanel C, Sabo R, Gupta SK, Salfi M, Jacobs S. Pegylated interferon-[alpha]2b: Pharmacokinetics, pharmacodynamics, safety, and preliminary efficacy data[ast]. *Clin Pharmacol Ther* 2000; **68**: 556-567
- 43 Perry CM, Jarvis B. Peginterferon-alpha-2a (40 kD): a review of its use in the management of chronic hepatitis C. *Drugs* 2001; **61**: 2263-2288
- 44 Algranati NE, Sy S, Modi M. A branched methoxy 40 kDa polyethylene glycol (PEG) moiety optimises the pharmacokinetics (PK) of peg interferon a-2a (PEG-IFN) and may explain its enhanced efficacy in chronic hepatitis C (CHC). *Hepatology* 1999; **40**: 190
- 45 Franciscus A. HCV Treatment: Interferon and Ribavirin. Hepatitis C Support Project [Internet]. 2009 Accessed 22 June 2011. Available from: <http://www.hcvadvocate.org>
- 46 Foster GR. Past, Present, and Future Hepatitis C Treatments. *Semin Liver Dis* 2004; **24**: 97-104
- 47 Schering Corporation. PEG-Intron product label. 2005
- 48 Lindsay KL, Trepo C, Heintges T, Shiffman ML, Gordon SC,

- Hoefs JC, Schiff ER, Goodman ZD, Laughlin M, Yao R, Albrecht JK; Hepatitis Interventional Therapy Group. A randomized, double-blind trial comparing pegylated interferon alfa-2b to interferon alfa-2b as initial treatment for chronic hepatitis C. *Hepatology* 2001; **34**: 395-403
- 49 Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *The Lancet* 2001; **358**: 958-965
- 50 Zeuzem S, Feinman SV, Rasenack J, Heathcote EJ, Lai MY, Gane E, et al. Peginterferon alfa-2a in patients with chronic hepatitis C. *N Engl J Med* 2000; **343**: 1666-1672
- 51 M.Fried, M.L.Shiffman, al KRe. Pegylated (40 Kda) interferon alfa-2a (PEGASYS) in combination with Ribavirin: efficacy and safety results from a phase III, randomized, actively controlled, multicenter study. *BMC Gastroenterology* 2001; **120**: (A-55)
- 52 Cornberg M, Manns M, Wedemeyer H. Hepatitis C Standard of care. In: al SMe, editor. *Hepatology-A Clinical Textbook*. 2nd ed. Germany: Flying; 2010; 171-196
- 53 McHutchison JG, Manns M, Patel K, Poynard T, Lindsay KL, Trepo C, Dienstag J, Lee WM, Mak C, Garaud JJ, Albrecht JK; International Hepatitis Interventional Therapy Group. Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. *Gastroenterology* 2002; **123**: 1061-1069
- 54 Fried M, Hadziyannis S. Treatment of chronic hepatitis C infection with peginterferons plus ribavirin. *Semin Liver Dis* 2004; **24**: 2447-2454
- 55 Kato T, Wakita T. Production of infectious hepatitis C virus in cell culture. *J Virol*. 2005; **55**: 287-95
- 56 Wakita T, Pietschmann T, Kato T, Date T, Miyamoto M, Zhao Z, Murthy K, Habermann A, Kräusslich HG, Mizokami M, Bartenschlager R, Liang TJ. Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. *Nat Med* 2005; **11**: 791-796
- 57 Zhong J, Gastaminza P, Cheng G, Kapadia S, Kato T, Burton DR, Wieland SF, Uprichard SL, Wakita T, Chisari FV. Robust hepatitis C virus infection in vitro. *Proc Natl Acad Sci U S A* 2005; **102**: 9294-9299
- 58 Soriano V, Peters MG, Stefan Z. New Therapies for Hepatitis C Virus Infection. *Clinical Infectious Diseases* 2009; **48**: 313-320
- 59 Vermehren J, Sarrazin C. New HCV therapies on the horizon. *Clin Microbiol Infect* 2011; **17**: 122-134
- 60 De Francesco R, Carfi A. Advances in the development of new therapeutic agents targeting the NS3-4A serine protease or the NS5B RNA-dependent RNA polymerase of the hepatitis C virus. *Adv Drug Deliv Rev* 2007; **59**: 1242-1262
- 61 Reesink HW, Zeuzem S, Weegink CJ, Forestier N, van Vliet A, van de Wetering de Rooij J, McNair L, Purdy S, Kauffman R, Alam J, Jansen PL. Rapid Decline of Viral RNA in Hepatitis C Patients Treated With VX-950: A Phase Ib, Placebo-Controlled, Randomized Study. *Gastroenterology*. 2006; **131**: 997-1002
- 62 Lin K, Perni RB, Kwong AD, Lin C. VX-950, a novel hepatitis C virus (HCV) NS3-4A protease inhibitor, exhibits potent antiviral activities in HCV replicon cells. *Antimicrob Agents Chemother* 2006; **50**: 1813-22
- 63 Vertex Pharmaceuticals Inc. inventor; Protease Inhibition for Viral Evaluation 2006
- 64 McHutchison JG, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, et al. Telaprevir with Peginterferon and Ribavirin for Chronic HCV Genotype 1 Infection. *New England Journal of Medicine* 2009; **360**: 1827-1838
- 65 Hézode C, Forestier N, Dusheiko G, Ferenci P, Pol S, Goreser T, et al. Telaprevir and Peginterferon with or without Ribavirin for Chronic HCV Infection. *New England Journal of Medicine* 2009; **360**: 1839-1850
- 66 Vertex Pharma. 65% of people whose prior treatment for hepatitis C was unsuccessful achieved SVR (viral cure) with telaprevir-based therapy in phase 3 REALIZE study. Vertex Pharmaceuticals Inc., 2010
- 67 FDA. FDA approved telaprevir against HCV. Food and Drug Administration, 2011
- 68 Vertex. Boceprevir in combination with Peg-Interferon. In: Vertex Pharmaceuticals, editor. 2011
- 69 Lopez-Labrador F-X. Hepatitis C Virus NS3/4A Protease Inhibitors. *Recent Patents on Anti-Infective Drug Discovery* 2008; **3**: 157-167
- 70 Zeuzem S, Sarrazin C, Wagner F. The HCV NS3 protease inhibitor SCH-503034 in combination with PEG-IFNa-2b in the treatment of HCV-1 PEG-IFNa-2b non-responders: antiviral activity and HCV variant analysis. *Hepatology* 2006; **42**: 35
- 71 Schiff E, Poordad F, Jacobson I, Flamm S, Bacon B, Lawitz E, et al. 104 Boceprevir (b) combination therapy in null responders (nr): response dependent on interferon responsiveness. *Journal of Hepatology* 2008; **48**: S46
- 72 Schering Corporation, Serine Protease Inhibitor Therapy-1. 2006
- 73 Kwo, P. Y. et al. Interim results from HCV SPRINT-1: RVR/EVR from phase 2 study of boceprevir plus Pegintron (peginterferon alfa-2b)/ribavirin in treatment-naïve subjects with HCV genotype 1 CHC. *natap* 2008; **48**: 372
- 74 Swan T. Hepatitis C Treatment Pipeline Report. 2008
- 75 Kwo PY, Lawitz EJ, McCone J, Schiff ER, Vierling JM, Pound D, Davis MN, Galati JS, Gordon SC, Ravendhran N, Rossaro L, Anderson FH, Jacobson IM, Rubin R, Koury K, Pedicone LD, Brass CA, Chaudhri E, Albrecht JK; SPRINT-1 investigators. Efficacy of boceprevir, an NS3 protease inhibitor, in combination with peginterferon alfa-2b and ribavirin in treatment-naïve patients with genotype 1 hepatitis C infection (SPRINT-1): an open-label, randomised, multicentre phase 2 trial. *Lancet* 2010; **376**: 705-716
- 76 Poordad F, McCone J, Bacon BR, Bruno S, Manns MP, Sulkowski MS, et al. Boceprevir for Untreated Chronic HCV Genotype 1 Infection. *New England Journal of Medicine* 2011; **364**: 1195-1206
- 77 Bacon BR, Shiffman ML, Lim JK. Phase 2 randomized, double blind, placebo-controlled study of nitazoxanide plus peginterferon and ribavirin in HCV genotype 1 naïve patients: week 12 sustained virologic response rate. *Hepatology* 2010; **52**: 463
- 78 Terrault NA, Cooper C, Balart LA. Phase II randomised, partially-blind, parallel-group study of oral danoprevir (RG7227) with PegIFNalpha-2a (PEGASYS) plus ribavirin in treatment-naïve genotype 1 patients with CHC: results of planned week 12 interim analysis of the ATLAS study. *Hepatology*. 2010; **52**: 335A
- 79 de Bruijne J, Bergmann JF, Reesink HW, Weegink CJ, Molenkamp R, Schinkel J, Tong X, Li J, Treitel MA, Hughes EA, van Lier JJ, van Vliet AA, Janssen HL, de Knegt RJ. Antiviral activity of narlaprevir combined with ritonavir and pegylated interferon in chronic hepatitis C patients. *Hepatology* 2010; **52**: 1590-1599
- 80 Manns MP, Gane EJ, Rodriguez-Torres M et al. Sustained viral response (SVR) rates in genotype 1 treatment-naïve patients with chronic hepatitis C (CHC) infection treated with vaniprevir (MK-7009), a NS3/4A protease inhibitor, in combination with pegylated interferon alfa-2a and ribavirin for 28 days. *Hepatology*. 2010; **52**: 361
- 81 Ulkowski MS, Ferenci P, Emanoil C. SILEN-C1: early antiviral activity and safety of BI 201335 combined with peginterferon alfa-2a and ribavirin in treatment-naïve patients with chronic genotype 1 HCV infection. *Hepatology* 2009; **50**: 2
- 82 Pawlowsky JM. Treating hepatitis C in "difficult-to-treat" patients. *N Engl J Med* 2004; **351**: 422-423
- 83 Lawitz E, Nguyen T, Younes Z, et al. Clearance of HCV RNA with valopicitabine (NM 283) plus peg-interferon in treatment-naïve patients with HCV-1 infection: Results at 24 and 48 weeks. *Hepatology* 2007; **46**: S9
- 84 Pockros PJ, Nelson D, Godofsky E, Rodriguez-Torres M, Everson GT, Fried MW, Ghaliab R, Harrison S, Nyberg L, Shiffman ML, Najera I, Chan A, Hill G. R1626 plus peginterferon Alfa-2a provides potent suppression of hepatitis C virus RNA and significant antiviral synergy in combination with ribavirin. *Hepatology* 2008; **48**: 385-397

- 85 Roberts SK, Cooksley G, Dore GJ, Robson R, Shaw D, Berns H, Hill G, Klumpp K, Najera I, Washington C. Robust antiviral activity of R1626, a novel nucleoside analog: a randomized, placebo-controlled study in patients with chronic hepatitis C. *Hepatology* 2008; **48**(2): 398-406
- 86 Pogarm S, Sessaadri A, Kosaka A. A high barrier to resistance may contribute to the robust antiviral effect demonstrated by R1626 in HCV genotype 1-infected treatment-naive patients," 58th Annual Meeting of the American Association for the Study of Liver Diseases; November 2-6; Boston, MA2007
- 87 Nelson D, Pockros PJ, Godofsky E, Rodriguez-Torres M, Everson. High end-of-treatment response (84%) after 4 weeks of R1626, peginterferon alfa-2a and ribavirin followed by a further 44 weeks of peginterferon alfa-2a and J.ribavirin. *Hepatology* 2008; **48**: 371
- 88 McHutchison JG, Reddy R, Rodriguez-Torres M. Potent antiviral activity of the nucleoside HCV inhibitor, R7128, in prior IFN non-responders. *Frontiers in Drug Development in Viral Hepatitis (HEP-DART)* 2007: 9-13
- 89 Cooper C, Lawitz EJ, Ghali P. Antiviral activity of the non-nucleoside polymerase inhibitor, VCH-759, in chronic Hepatitis C patients: results from a randomized, double-blind, placebo-controlled, ascending multiple dose study. 58th Annual Meeting of the American Association for the Study of Liver Diseases; November 2-6; Boston, MA2007
- 90 Lalezari JP, Riordan W, Poordad F. A phase IIa study of IDX184 in combination with pegylated interferon (pegIFN) and ribavirin (RBV) in treatment-naive HCV genotype 1-infected subjects. *Hepatology*. 2010; **52**: 337
- 91 Lawitz E, Lalezari JP, Rodriguez-Torres M. High rapid virologic response (RVR) with PSI-7977 qd plus PEG-IFN/RBV in a 28-day phase 2a trial. *Hepatology* 2010; **52**: 706
- 92 Lesburg CA, Cable MB, Ferrari E, Hong Z, Mannarino AF, Weber PC. Crystal structure of the RNA-dependent RNA polymerase from hepatitis C virus reveals a fully encircled active site. *Nat Struct Biol* 1999; **6**: 937-943
- 93 Beaulieu PL. Non-nucleoside inhibitors of the HCV NS5B polymerase: progress in the discovery and development of novel agents for the treatment of HCV infections. *Curr Opin Investig Drugs* 2007; **8**(8): 614-634
- 94 Erhardt A, Deterding K, Benhamou Y, Reiser M, Fornis X, Pol S, Calleja JL, Ross S, Spangenberg HC, Garcia-Samaniego J, Fuchs M, Enríquez J, Wiegand J, Stern J, Wu K, Kukulj G, Marquis M, Beaulieu P, Nehmiz G, Steffgen J; BILB 1941 Study Group. Safety, pharmacokinetics and antiviral effect of BILB 1941, a novel hepatitis C virus RNA polymerase inhibitor, after 5 days oral treatment. *Antivir Ther* 2009; **14**: 23-32
- 95 Lawitz E, Rodriguez-Torres M, Rustgi VK. Safety and antiviral activity of ANA598 in combination with pegylated interferon alfa-2a plus ribavirin in treatment-naive genotype-1 chronic HCV patients. *Hepatology* 2010; **52**: 334
- 96 Rodriguez-Torres M, Lawitz E, Conway B. Safety and antiviral activity of the HCV non-nucleoside polymerase inhibitor VX-222 in treatment-naive genotype 1 HCV-infected patients. *Hepatology* 2010; **52**: 14
- 97 Kneteman NM, Howe AY, Gao T, Lewis J, Pevear D, Lund G, Douglas D, Mercer DF, Tyrrell DL, Immermann F, Chaudhary I, Speth J, Villano SA, O'Connell J, Collett M. HCV796: A selective nonstructural protein 5B polymerase inhibitor with potent anti-hepatitis C virus activity in vitro, in mice with chimeric human livers, and in humans infected with hepatitis C virus. *Hepatology* 2009; **49**: 745-752
- 98 Pol S, Everson GT, Ghalib R. Once-daily NS5A inhibitor (BMS-790052) plus peginterferon-alpha-2a and ribavirin produces high rates of extended rapid virologic response in treatment-naive HCV-genotype 1 subjects: phase 2a trial. *Hepatology* 2010; **52**: 462
- 99 Gane EJ, Roberts SK, Stedman CA, Angus PW, Ritchie B, Elston R, Ipe D, Morcos PN, Baher L, Najera I, Chu T, Lopatin U, Berrey MM, Bradford W, Laughlin M, Shulman NS, Smith PF. Oral combination therapy with a nucleoside polymerase inhibitor (RG7128) and danoprevir for chronic hepatitis C genotype 1 infection (INFORM-1): a randomised, double-blind, placebo-controlled, dose-escalation trial. *The Lancet* 2010; **376**: 1467-1475
- 100 Franciscus A. Hepatitis C Treatments in Current Clinical Development. Hepatitis C Support Project [Internet]. 2011. Available from: www.hcvadvocate.com
- 101 Rockstroh JK. Will there be an interferon-free HCV therapy for all: upcoming reality or just a vision? Conference Reports for NATAP [Internet]. 2012 30/Aug/2012. Available from: http://www.natap.org/2012/EASL/EASL_96.htm
- 102 Nature Reviews. Trial watch: An all-oral and interferon-free future for HCV therapy? *Nat Rev Drug Discov* 2011; **10**: 891
- 103 Levin J. Potent Viral Suppression With the All-Oral Combination of Daclatasvir (NS5A Inhibitor) and GS-7977 (Nucleotide NS5B Inhibitor), +/- Ribavirin, in Treatment-Naive Patients With Chronic HCV GT1, 2, or 3 (100% SVR gt1, 91% gt2) EASL 47th Annual Meeting; 2012; Barcelona, Spain
- 104 Gane E, Stedman C, Hyland R, Sorensen R, Symonds W, Hindes R, et al. Once Daily GS-7977 Plus Ribavirin in HCV Genotypes 1-3: The ELECTRON Trial. 47th Annual Meeting of the European Association for the Study of the Liver; Barcelona, Spain2012
- 105 Open-Label Study of GS-7977+ Ribavirin for 12 Weeks in Subjects With Chronic HCV Infection [database on the Internet]. National Institute of Health. 2012. Available from: http://www.clinicaltrials.gov/ct2/show/NCT01625338?term=hepatitis+c&no_unk=Y&rcv_s=06%2F01%2F2012&rcv_e=06%2F30%2F2012&lup_s=06%2F01%2F2012&lup_e=06%2F30%2F2012&rank=4
- 106 Suzuki F, Ikeda K, Toyota J, Karino Y, Ohmura T, Chayama K, et al. Dual oral therapy with the NS5A inhibitor daclatasvir (BMS-790052) and NS3 protease inhibitor asunaprevir (BMS-650032) in HCV genotype 1b-infected null responders or ineligible/intolerant to peginterferon. 47th Annual Meeting of the European Association for the Study of the Liver (EASL 2012); Barcelona2012
- 107 Highleyman L. Hepatitis C meds GS-7977, daclatasvir and asunaprevir look good in interferon-free combos. New and experimental hepatitis c treatment [Internet]. 2012. Available from: <http://www.aidsmap.com/Hepatitis-C-meds-GS-7977-daclatasvir-and-asunaprevir-look-good-in-interferon-free-combos/page/2323144/>
- 108 Rong L, Dahari H, Ribeiro RM, Perelson AS. Rapid Emergence of Protease Inhibitor Resistance in Hepatitis C Virus. *Science Translational Medicine*. 2010, **2**(30):30ra32
- 109 Flisiak R, Feinman SV, Jablkowski M, Horban A, Kryczka W, Pawlowska M, Heathcote JE, Mazzella G, Vandelli C, Nicolas-Métral V, Grosgrurin P, Liz JS, Scalfaro P, Porchet H, Crabbé R. The cyclophilin inhibitor Debio 025 combined with PEG IFN α 2a significantly reduces viral load in treatment-naive hepatitis C patients. *Hepatology* 2009; **49**: 1460-1468
- 110 Kaita K, Yoshida E, Kunimoto D. PhII proof of concept study of celgosivir in combination with peginterferon alfa-2b and ribavirin in chronic hepatitis C genotype 1 non-responder patients. 42nd Annual Meeting of the European Association for the Study of the Liver 2007; p. 11-5
- 111 De Francesco R, Migliaccio G. Challenges and successes in developing new therapies for hepatitis C. *Nature* 2005; **436**: 953-960
- 112 Sookoian SC. New therapies on the horizon for hepatitis C. *Annals of Hepatol* 2003; **2**: 164-170
- 113 Yakoot M, Salem A. Spirulina platensis versus silymarin in the treatment of chronic hepatitis C virus infection. A pilot randomized, comparative clinical trial. *BMC Gastroenterology*. 2012; **12**: 32

Peer reviewers: Sandeep Khurana, Assistant Professor of Medicine, Division of Gastroenterology, University of Maryland School of Medicine, 22 South Green Street, N3W50, Baltimore, MD 2120, the United States; Rodney S. Russell, PhD, Assistant Professor, Division of BioMedical Sciences, Faculty of Medicine, Memorial University, 300 Prince Philip Drive, St. John's, NL, Canada.