Study on HCV direct acting drugs in treatment of chronic hepatitis C

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Abstract: Hepatitis C virus (HCV) infection is a serious public health concern that affects 170 million people worldwide for which no vaccine is available. The hepatitis C virus (HCV) is a small, enveloped, single-stranded, positive-sense RNA virus. It is a member of the Hepacivirus genus in the family Flaviviridae. There are seven major genotypes of HCV, which are known as genotypes one to seven. Among those infected approximately 20–30% develop severe liver disease, such as chronic hepatitis, liver cirrhosis, or hepatocellular carcinoma. The combined use of the nucleoside analog ribavirin and pegylated interferon alpha was the current standard of care. How-ever, success in treatment depends largely on the viral genotype. For instance, the rate of viral clearance upon current standard of care is only 50% with genotype 1, the most prevalent circulating strain in Western Europe and North America. Egypt's epidemiological and social situation differs from Western countries states that the prevalence of C virus is very high (estimated infection rate among adults by 10 and 20% in urban areas and rural areas, respectively). The origin of the epidemic due in the treatment of schistosomiasis control in rural areas in 1960 campaigns.Since then, the virus has continued to spread, mainly through intravenous injections and other medical procedures. But there is an important difference about the epidemic in Egypt is that more than 95% of infections are genotype 4 (HCVg4). Currently there are more than 8 million people infected in Egypt and the incidence of new infections is still the highest in all parts of the world. In addition, this combined regimen of treatment has been associated with many serious side effects such as fatigue, depression, nausea and bone marrow depression. All of these issues justify the need to develop novel, more efficacious and safer anti-HCV drugs. The development of new models and tools has led to the discovery and clinical development of a large number of new anti-hepatitis C virus (HCV) drugs.telaprevir and boceprevir, both first-wave, first-generation NS3-4A protease inhibitors Two others in 2013/2014: Simeprevir, a second-wave, first-generation NS3-4A protease inhibitor. And Sofosbuvir, a nucleotide analogue inhibitor of the viral polymerase. Numerous other drugs have reached phase II or III clinical development. Beginning in 2015 and beyond and cure rates have increased to more than 90 percent.
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Keyword: HCV, viral hepatitis, direct acting anti viral, DAAS.

Introduction

Hepatitis C virus (HCV) infection is a serious public health concern that affects 170 million people worldwide ***(Shepard et al., 2005***) for which no vaccine is available. Among those infected approximately 20–30% develop severe liver disease, such as chronic hepatitis, liver cirrhosis, or hepatocellular carcinoma ***(Alter and Seeff., 2000)***. The combined use of the nucleoside analog ribavirin and pegylated interferon alpha was the current standard of care. How-ever, success in treatment depends largely on the viral genotype ***(Nakano, T et al., 2011)***.

The hepatitis C virus (HCV) is a small, enveloped, single-stranded, positive-sense RNA virus. It is a member of the Hepacivirus genus in the family Flaviviridae. There are seven major genotypes of HCV, which are known as genotypes one to seven. ***(Nakano, T; et al 2011).*** The genotypes are divided into several subtypes with the number of subtypes depending on the genotype ***(Nakano, T., et al 2011).***

For instance, the rate of viral clearance upon current standard of care is only 50% with genotype 1, the most prevalent circulating strain in Western Europe and North America ***(Russo and Fried., 2003).***

In Egypt the epidemiological and social situation differs from that of Western countries. HCV prevalence is very high (estimated among adults at 10 and 20% in urban and rural areas, respectively). The origin of the epidemic has been attributed to mass campaigns of parenteral anti-schistosomiasis treatment in rural areas in the 1960s–70s ***(Zeuzem et al 2000).***

All of these issues justify the need to develop novel, more efficacious and safer anti-HCV drugs (***Jean-Michel Pawlotsky., 2014).***

The development of new models and tools has led to the discovery and clinical development of a large number of new anti-hepatitis C virus (HCV)

drugs, including direct-acting antivirals and host-targeted agents. Surprisingly, curing HCV infection appears to be easy with these new drugs, provided that a potent drug combination with a high barrier to resistance is used. HCV infection cure rates can be optimized by combining drugs with synergistic antiviral effects, tailoring treatment duration to the patients' needs, and/or using ribavirin. Two HCV drugs have been approved in 2011—telaprevir and boceprevir, both first-wave, first-generation NS3-4A protease inhibitors ***(Jean-Michel Pawlotsky, 2014).***

Two others in 2013/2014:

Simeprevir, a second-wave, first-generation NS3-4A protease inhibitor.

And Sofosbuvir, a nucleotide analogue inhibitor of the viral polymerase.

Numerous other drugs have reached phase II or III clinical development. From 2015 and onwards, interferon-containing regimens will disappear, replaced by interferon-free regimens yielding infection cure rates over 90%. These therapies will raise new issues, including the need for broad-scale screening and access to care. ***(Jean-Michel Pawlotsky 2014).***

When hepatitis C virus (HCV) was discovered in the late *1980s*, ***(Choo QL et al., 1989)*** the only available treatment for what was known as chronic "non-A, non-B" hepatitis was standard interferon-alpha (IFN-α), 3 million units three times per week subcutaneously for 24 weeks. Only 6% of patients achieved a sustained virological response (SVR), i.e. a cure of HCV infection, with this regimen. The initial choice of IFN-α as a potential treatment for chronic hepatitis C was an empirical one.

Alpha interferon has already been shown to inhibit replication of several human hepatitis viruses, including hepatitis A virus (in cell culture), hepatitis B virus and the hepatitis delta agent." Prolonging IFN-α administration to 48 weeks increased the SVR rate up to 12 to 16%.

Ribavirin was introduced in 1991 ***(Reichard O et al., 1991)*** with the following justification: "Ribavirin is a noninterferon-inducing nucleotide analogue with a broad spectrum of activity against RNA and DNA viruses, including those from the flavivirus family.

In fact, ribavirin happened to be a very weak and transient direct inhibitor of HCV replication. ***(Pawlotsky JM et al., 2004).***

However, when added to IFN-α, ribavirin increased the SVR rates up to 40 to 45%, through mechanisms that remain unknown. An additional 10% increase in SVR rate was achieved when standard IFN-α was replaced by pegylated IFN-α PegIFN-α), that could be administered once per week. ***(Fried MW et al., 2002).***

New HCV drugs split into two groups: direct-acting antivirals **(DAAs)** that target viral actors of the HCV lifecycle, and host-targeted agents **(HTAs)** that target host-cell components involved in complex interactions with viral proteins that are essential to the HCV lifecycle. The field quickly learned how to use these new compounds to optimize HCV infection cure rates.

Antiviral Potency (First-Phase HCV RNA Decline)

The HCV lifecycle offers several potential targets for DAAs and HTAs with potent antiviral activity. The lifecycle starts with receptor binding, entry into cells, and fusion. Decapsidation of viral nucleocapsids liberates free positive-strand genomic RNAs in the cell cytoplasm, where they serve, together with newly synthesized RNAs, as messenger RNAs for synthesis of the HCV polyprotein. The polyprotein is then cleaved by host cell peptidases, the NS2 autocatalytic protease and the NS3-4A serine protease to generate three structural and seven nonstructural mature viral proteins. Replication is catalyzed by the HCV RNA-dependent RNA polymerase (RdRp), and the NS5A protein plays an important regulatory role in virus replication. HCV then uses the lipoprotein production pathway to generate mature viral particles and export them. ***(Rupp D et al., 2014)***

Thus far, drugs from all classes of DAAs in clinical development, including NS3-4A protease inhibitors, nucleoside/nucleotide analogue inhibitors of HCV RdRp, nonnucleoside inhibitors of RdRp, and NS5A inhibitors, have been shown to reduce HCV replication by 3 logs or more over 3 days of administration. HTAs, such as cyclophilin A inhibitors and the microRNA-122 (miR-122) antagonist, can also reduce viral replication by more than 3 logs within a week or 2 weeks ***(Flisiak R et al 2008).*** This antiviral effectiveness is sufficient to trigger the second-phase decline. ***(Delang L et al., 2012)***

New Approved HCV Drugs or in Clinical Development

Approved Drugs

Pegylated IFN-α2a and IFN-α2b and ribavirin are still available for triple or quadruple IFN-containing regimens with new HCV drugs, as discussed by ***Aronsohn*** and ***Jensen*** in this issue. Telaprevir and boceprevir are first-wave, first-generation NS3-4A protease inhibitors that were approved in 2011 for use in combination with PegIFN-α and ribavirin. They are specific for genotype 1 and have a low barrier to resistance. Their use was associated with frequent side effects, including cutaneous complications, anemia, gastrointestinal disorders and renal toxicity for telaprevir; and anemia, dysgeusia, and renal toxicity for boceprevir, as well as with frequent drug–drug interactions due to their metabolism by cytochrome P450 CYP34A that they inhibit. ***(Back D et al 2013).***

For these reasons, these drugs will no longer be used when better tolerated compounds are available.

Two new HCV DAAs have been approved in the United States in December 2013, in Europe in the first half of 2014 simeprevir and sofosbuvir. Simeprevir is a second-wave, first-generation NS3-4A protease inhibitor. Its genotypic coverage is broader than that of the first-wave drugs, including at least genotypes 1, 2, and 4; however, simeprevir is inactive against HCV genotype 3. Simeprevir has a low barrier to resistance, with extensive cross resistance with telaprevir and boceprevir and the other first-generation NS3-4A protease inhibitors. In addition, simeprevir preferentially selects resistant variants bearing the Q80K substitution in the NS3 protease sequence. The presence of detectable levels of these variants at baseline has been associated with failures of simeprevir-containing regimens ***(Jacobson I et al., 2013).*** Phase II and III clinical trials have shown an excellent tolerance profile for this compound. Simeprevir only modestly inhibits CYP34A.

Sofosbuvir is a first-generation uridine nucleotide analogue inhibitor of HCV RdRp, which is phosphorylated into its triphosphate form and incorporated into the RNA chain in formation, thus acting as a chain terminator. Sofosbuvir has pangenotypic antiviral activity, confirmed in vivo against genotypes 1 to 6.***(Lam AM et al., 2012)***

In vitro, sofosbuvir selects variants with an S282T substitution in the RdRp sequence. However, these variants have considerably impaired replication capacity, both in vitro and in vivo. As a result, they have never been associated with virological breakthroughs on treatment, and were exceptionally found in patients who relapsed after treatment withdrawal ***(Lawitz E et al., 2013).*** Thus, sofosbuvir has a high barrier to resistance. Sofosbuvir was well tolerated in phase II and III clinical trials that included several thousand patients. ***(Lawitz E et al., 2013).*** It is not metabolized by CYP450. Thus, sofosbuvir has few drug–drug interactions, except with potent P-glycoprotein and/or breast cancer resistance protein inducers.

Drugs in Clinical Development

NS3-4A Protease Inhibitors

NS3-4A protease inhibitors in development include second-wave, first-generation drugs that share simeprevir's properties, including broad genotypic coverage that excludes genotype 3 and a low barrier to resistance with cross-resistance with other first-generation inhibitors. The most frequently selected substitutions conferring resistance to first-generation NS3-4A protease inhibitors have been described at amino acid positions V36, T54, R155, A156, D168, and V170 ***(Pawlotsky et al., 2011).*** The Q80K substitution is preferentially selected by simeprevir. Drug–drug interactions have been reported for some of them.

Compounds in phase II or III clinical development include faldaprevir ***(Boehringer-Ingelheim, Ingelheim, Germany),*** likely approved in 2014 in combination with PegIFN-α and ribavirin; asunaprevir ***(Bristol-Myers Squibb, New York, NY),*** possibly approved in 2014 or 2015; ABT-450 ***(Abbvie, North Chicago, IL)*** boosted by ritonavir, likely approved in 2014 or 2015 as part of the first all-oral, IFN-free combination for HCV genotype 1 infection; vedroprevir ***(Gilead, Foster City, CA)***; IDX-320 (Idenix Pharmaceuticals, Cambridge, MA); sovaprevir ***(Achillion Pharmaceuticals, New Haven, CT),*** on clinical hold due to elevated alanine aminotransferase levels in a drug–drug interaction study with atazanavir; danoprevir ***(Hoffmann-La Roche, Basel, Switzerland),*** boosted by ritonavir; vaniprevir ***(Merck, White House Station, NJ),*** that will be developed in Japan only. Second-generation NS3-4A protease inhibitors have pan genotypic antiviral activity, including genotype 3, and a higher barrier to resistance than first-generation drugs. However, resistant HCV variants can be selected by these compounds. They include MK-5172 ***(Merck),*** possibly approved in 2015 or 2016, and ACH-2684 ***(Achillion).***

Nucleoside/Nucleotide Analogue Inhibitors of HCV RdRp

Few nucleoside/nucleotide analogues remain at the clinical developmental stage after several programs were halted due to serious, sometimes fatal toxicity. VX-135 ***(Vertex Pharmaceuticals, Cambridge, MA)*** is a pyrimidine nucleotide analogue on partial clinical hold following the observation of reversible elevated liver enzymes in patients receiving a high dose of the drug. Mericitabine ***(Roche)*** is a modestly potent cytidine nucleoside analogue still in development.

Nonnucleoside Inhibitors of HCV RdRp

Nonnucleoside inhibitors of HCV RdRp bind to one of four allosteric sites on the polymerase, thereby altering its catalytic function. Two sites are located in the "thumb" domain and two in the "palm" domain Nonnucleoside inhibitors of HCV RdRp are generally active against HCV genotype 1 only and they have a low barrier to resistance, with extensive cross resistance between drugs targeting the same allosteric site and possible cross resistance between drugs targeting different sites. Thumb-1 inhibitors include deleobuvir ***(Boehringer-Ingelheim),*** the development of which has been stopped in January 2014, BMS-791325 ***(Bristol-Myers Squibb),*** and TMC647055 ***(Janssen).*** The two latter belong to triple-combination regimens that will seek approval in 2015 or 2016. Thumb-2 inhibitors include filibuvir ***(Pfizer, New York, NY)***, the development of which has been

stopped, lomibuvir ***(Vertex)*** and GS-9669 ***(Gilead).*** Palm-1 inhibitors include setrobuvir ***(Roche),*** and ABT-333 ***(Abbvie),*** which will likely be approved in 2014 or 2015 in combination with other DAAs, and ABT-072 ***(Abbvie).*** Palm-2 inhibitors include tegobuvir ***(Gilead),*** the development of which has been stopped.

NS5A Inhibitors

NS5A inhibitors bind to domain 1 of the NS5A protein and block both replication and viral assembly and release ***(Guedj J et al., 2013).***

First-generation NS5A inhibitors in development include daclatasvir ***(Bristol-Myers Squibb),*** likely approved in 2014 or 2015 for use in IFN-containing and IFN-free combinations; ledipasvir ***(Gilead),*** available as a fixed-dose combination (one pill) with sofosbuvir, likely approved in 2015; ABT-267 ***(Abbvie),*** likely approved in 2014 or 2015 in combination with ritonavir-boosted ABT-450 and ABT-333; PPI-668 ***(Presidio Pharmaceuticals, San Francisco, CA);*** ACH-2928 ***(Achillion);*** GSK2336805 ***(Glaxo Smith Kline);*** BMS824393 ***(Bristol-Myers Squibb);*** and samatasvir ***(Idenix)***. All of them are currently being tested as part of double- or triple-combination IFN-free regimens.

Second-generation NS5A inhibitors have reached clinical development. They have pangenotypic activity and their barrier to resistance has been improved. However, they have been shown to select amino acid substitutions that confer resistance to first-generation compounds. They include MK-8742 ***(Merck),*** likely approved in 2015 or 2016 in combination with MK-5172; ACH-3102 ***(Achillion);*** and GS-5816 ***(Gilead),*** which may ultimately replace ledipasvir in the fixed-dose combination with sofosbuvir.

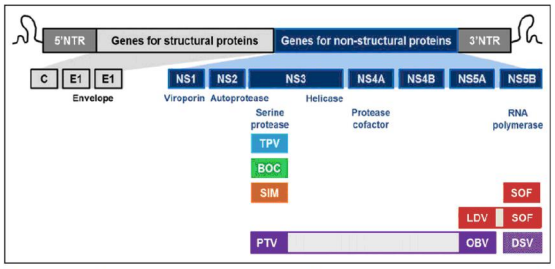

Figure 1) *Hepatitis C virus genome and the polyprotein targets of newly approved, direct-acting antiviral agents. Note: Sofosbuvir (SOF) is a nucleotide nonstructural protein (NS)5B polymerase inhibitor and dasabuvir (DSV) is a non-nucleoside polymerase inhibitor. BOC Boceprevir; LDV Ledipasvir; OBV Ombitasvir; PTV Paritaprevir; SIM Simeprevir; TPV Telaprevir*

Host-Targeted Agents

Two classes of host-targeted agents have reached clinical development. They include specific inhibitors of cyclophilin A peptidyl-prolyl cis/trans isomerase activity and antagonists of miR-122. These compounds have pangenotypic activity, a high barrier to resistance and they are well tolerated in the absence of IFN-α coadministration. Cyclophilin inhibitors in clinical development include Alisporivir ***(Novartis, Basel, Switzerland)*** and SCY-635 ***(Scynexis, Inc., Durham, NC).*** Alisporivir was put on clinical hold due to a fatal case of acute pancreatitis that occurred in combination with PegIFN-α and ribavirin. It is now back in development in all-oral, IFN-free combination regimens. The miR-122 antagonist miravirsen ***(Santaris Pharma, Copenhagen, Denmark)*** is available in an injectable form. Two weeks of administration reduced HCV replication by several logs. However, concerns have been raised as to the long-term hepatic effects of inhibiting miR-122 and the risk of steatohepatitis, fibrosis, and hepatocellular carcinoma.

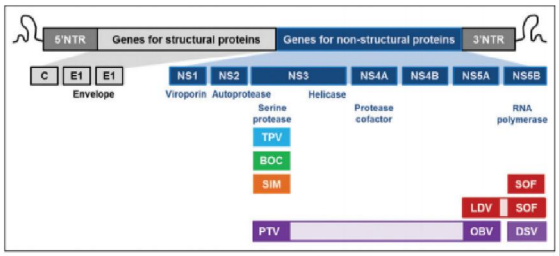

Figure 2) *Hepatitis C virus genome and the polyprotein targets of newly approved, direct-acting antiviral agents. Note: Sofosbuvir (SOF) is a nucleotide nonstructural protein (NS)5B polymerase inhibitor and dasabuvir (DSV) is a non-nucleoside polymerase inhibitor. BOC Boceprevir; LDV Ledipasvir; OBV Ombitasvir; PTV Paritaprevir; SIM Simeprevir; TPV Telaprevir*

HCV Treatment Strategies

Until 2014

For the past 15 years, the treatment of chronic hepatitis C has been based on the use of PegIFN-α and ribavirin. In 2011, telaprevir and boceprevir were approved in combination with PegIFN-α and ribavirin for patients infected with HCV genotype 1. This triple combination improved the SVR rates by 15 to 20% compared with PegIFN-α and ribavirin alone Easy-to-cure patients— patients with mild liver disease—were those with the highest SVR rates. However, frequent and often serious side effects were observed with this regimen, especially in patients with advanced liver disease who marginally benefited from the addition of the protease inhibitor. ***(Hézode C, et al., 2013)***. Because of the complexity of these therapies and of the arrival of new, more efficient, and better-tolerated treatments, most patients who could wait for new treatment regimens were not treated in 2013 in the United States and Europe.

2014–2015

Two HCV DAAs were approved in December 2013 in the United States and in the first half of 2014 in Europe: simeprevir and sofosbuvir. Four treatment options are available in 2014 for the treatment of chronic hepatitis C, including IFN-containing and IFN-free regimens. IFN-containing options include the triple combination of PegIFN-α, ribavirin, and

simeprevir 24 to 48 weeks for patients infected with HCV genotype 1 (excluding those infected with subtype 1a with a detectable Q80K substitution in the NS3-4A protease sequence at baseline), that yields SVR rates of the order of 80% ***(Poordad F et al., 2013)*** and the triple combination of PegIFN-α, ribavirin, and sofosbuvir for 12 weeks for patients infected with HCV genotypes 1 to 6 that yields SVR rates of the order of 90% or more. IFN-free options include the combination of sofosbuvir and ribavirin 12 weeks for patients infected with HCV genotype 2, that yields SVR rates over 95% (except in patients with cirrhosis who may need slightly longer treatment) the combination of sofosbuvir and ribavirin 24 weeks for patients infected with HCV genotype 3, that yields SVR rates of the order of 85% in non cirrhotic patients, but appears to be suboptimal (60%) in patients with cirrhosis. ***(Lawitz et al 2013)*** The combination of sofosbuvir and simeprevir, with or without ribavirin, can also be used in patients infected with HCV genotypes 1 and 4, based on results of a small-scale phase II trial including null responders to a prior course of treatment with extensive fibrosis or cirrhosis showing SVR rates of 95 to 100%. ***(Jacobson IM et al., 2013).***

2015 and Onward

IFN-containing regimens will progressively disappear, replaced by all-oral IFN-free regimens. Phase II trials of different drug combinations have shown SVR rates of the order of more than 90%. The recent release of preliminary results from phase III trials confirmed that most, if not all patients can achieve an SVR with these regimens, at least in clinical trials. The combination of the second-wave, first-generation protease inhibitor ABT-450 boosted by ritonavir, the NS5A inhibitor ABT-267, and the nonnucleoside inhibitor of HCV RdRp ABT-333 plus ribavirin for 12 weeks yielded SVR rates of 95% in subtype 1a and 98% in subtype 1b treatment-naïve patients, and 96% in subtype 1a and 97% in subtype 1b treatment-experienced patients (SAPPHIRE-1 and SAPPHIRE-2 phase III trials). In the ION-1, ION-2, and ION-3 phase III trials with the fixed-dose combination of sofosbuvir and ledipasvir in patients infected with HCV genotype 1, the SVR rates were 97.7% and 97.2% with or without ribavirin, respectively, after 12 weeks of therapy in treatment-naïve patients (including 16% with cirrhosis); 94.0%, 93.1%, and 95.4% without ribavirin for 8 weeks, with ribavirin for 8 weeks and without ribavirin for 12 weeks in treatment-naïve patients, respectively; 93.6% and 96.4% with or without ribavirin, respectively, after 12 weeks, and 99.1% and 99.1% with or without ribavirin, respectively, after 24 weeks of therapy in treatment-experienced patients (including 20% with cirrhosis***).(Gilead 2014)*** Other phase III trials are ongoing with the same and other drug combinations.

Aim of the work

To highlight the new HCV direct antiviral drugs in treatment of chronic hepatitis C.

Daclatasvir

Daclatasvir (USAN) (formerly **BMS-790052**, trade name **Daklinza**) is a drug for the treatment of hepatitis C (HCV). It is wasdeveloped by Bristol-Myers Squibb and was approved in Europe on 22 August 2014.

Daclatasvir inhibits the HCV nonstructural protein NS5A. ***(Gao, Min et al., 2010).*** Recent research suggests that it targets two steps of the viral replication process, enabling rapid decline of HCV RNA. ***(Guedj, J et al., 2013).***

Daclatasvir has been tested in combination regimens with pegylated interferon and ribavirin, as well as with other direct-acting antiviral agents including asunaprevir ***(New England Journal 2014)*** and sofosbuvir.***(Mark Sulkowski et al., 2014).***

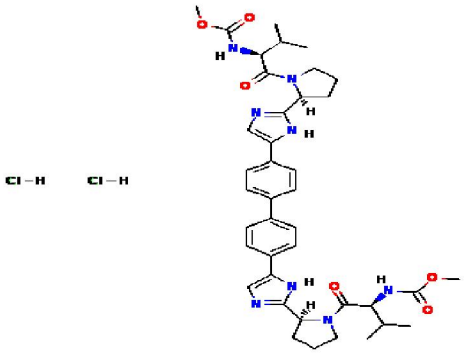

Fig. (16): daclatasvir chemical structure

Chemical Names:	Daclatasvir dihydrochloride; BMS790052 dihydrochloride; BMS 790052 dihydrochloride; 1009119-65-6; CHEBI:83800; BMS-790052-05
Molecular Formula:	$C_{40}H_{52}Cl_2N_8O_6$
Molecular Weight:	811.79688 g/mol

It is on the World Health Organization's List of Essential Medicines, a list of the most important medications needed in abasichealthsystem. ("www.who.in).

According to WHO Essential Medicines List Application DACLATASVIR:

Daclatasvir is a new direct acting antiviral (DAA) for HCV and was approved by the European Medicines Agency on 22 August, 2014 ***(European Medicines Agency Press Office, 2013).*** Thus, its use has been limited, although patients have been treated both as part of clinical trials and through a compassionate use programme. 798 patients have received daclatasvir as part of clinical trials, including compassionate use as of 16 October 2014, and an unknown but small number as part of market use ***(Bristol-Meyers Squibb Pharmaceutical Limited, 2014).***

The WHO 2014 HCV guidelines recommend HCV screening for all individuals who are part of a population with high background HCV seroprevalence or who have a history of HCV risk exposure or behavior. This includes people living with HIV, people who have received medical or dental procedures in settings where infection control practices are sub-standard as well as people who inject drugs or use drugs internasally. These guidelines also provide a strong recommendation that all patients with chronic HCV be assessed for potential HCV treatment ***(World Health Organization, 2014).*** According to WHO guidelines, all patients who are chronically infected with hepatitis C should be assessed for hepatitis C therapy, including HIV-HCV co-infected patients, people who use injected drugs and those with compensated cirrhosis. This recommendation includes treatment with DAAs such as daclatasvir. The European Association for the Study of the Liver (EASL) recommends treatment for all patients with moderate or severe liver fibrosis (METAVIR F2 or higher) and states that for patients with no or mild fibrosis, the decision to treat can be individualized. Daclatasvir is an oral NS5A inhibitor that is licensed by the European Medicines Association for the treatment of hepatitis C infection, in combination with other drugs active against HCV. The main goal of hepatitis C treatment is to cure HCV infection (as defined by those who achieve a sustained virological response (SVR) at 12 or 24 weeks post-treatment) and to prevent complications or mortality from liver cirrhosis and Hepatocellular Carcinoma (HCC) ***(Bruno et al., 2007)***. The current WHO HCV treamtent guidelines do not include daclatasvir as they were released before the approval of daclatasvir. However, these guidelines do include recommendations for the available DAAs, including sofosbuvir and simeprevir, which were on the market at the time of guideline publication and these closely reflect other expert guidelines such as the EASL and American Association for the Study of the Liver (AASLD) HCV treatment guidelines. The next update of the WHO guidelines is expected to include recommendations for use of 5 daclatasvir in treating patients with HCV (personal communication with Stefan Wiktor, team leader of the WHO's Global Hepatitis Program, and Philippa Easterbrook, senior scientist at the WHO's HIV department). EASL guidelines support the use of daclatasvir for treatment of HCV ***(European Association for the Study of the Liver, 2014):***

• **Genotype 1:** Daily daclatasvir (60 mg) plus daily sofosbuvir (400 mg) for 12 weeks for treatment naïve patients or 24 weeks for treatment-experienced patients.

• **Genotype 1b:** Daily daclatasvir (60 mg) plus weekly pegylated IFN-α plus daily weight-based ribavirin (1000 or 1200 mg in patients Contraindications to use of daclatasvir will include pregnancy, hypersensitivity to daclatasvir or hemodialysis ***(EASL, 2014; BMS, 2014).*** No data exist on the effect of daclatasvir on pregnancy, but daclatasvir has been associated with teratogenic and embryotoxic effects in animals ***(BMS, 2014).*** Women should use contraception during use and for 5 weeks thereafter. No dosage adjustments are needed for renal impairment (except dialysis), nor for moderate or several liver impairment ***(BMS, 2014).*** Daclatasvir is metabolized via the cytochrome P450 3A4. Thus it is recommended that with concomitant administration of strong inducers cytochrome P450 3A4 to increase the daclatasvir dose to 90mg or consider change therapy. It is recommended that with the concomitant use of strong inhibitors of cytochrome P450 3A4 to reduce the daclatasvir dose to 30 mg. Daclatasvir can be taken with or without meals ***(BMS, 2014).*** Summary of comparative effectivness in a variety of clinical settings: A systematic review was performed using Pubmed, Medline and Embase databases with the search term daclatasvir, BMS-790052 or Daklinza either as in the title or as a keyword. Additionally, abstracts of the 2013 and 2014 AASLD and EASL conferences and the journals Hepatology and Journal of Hepatology were searched with the same searchterms. 31 titles were identified of which 15 clinical trials with efficacy outcomes were included. Overall, the quality of the included studies was high and efficacy, as defined by SVR12 or 24, with daclatasvir-containing (both interferon-sparing and interferon-free) regimens is excellent, with large numbers of patients with GT 1 studied and smaller numbers of patients with GT2, 3 and 4 included. Overall, 79.2% (476 of 601) of patients in daclatasvir trials and eligible to be included in a long-term follow up assessment achieved SVR12. This follow up study of all trials including daclatasvir-containing regimens has also revealed that HCV cure is durable, with 99% (471 of 476) of patients who achieve SVR12

maintaining SVR at the most recent follow-up assessment ***(Reddy, 2014).*** Thirty genotype 4 patients were treated with either 20mg or 60mg of daclatasvir and PEG-RBV in the COMMAND-1 study. In the 20mg, 60mg and PEG-RBV groups respectively SVR12 was achieved in 67%, 100% and 50% of patients ***(Hezode et al., 2012).*** The COMMAND-4 study enrolled only patients with GT4 10 and randomized patients to either daclatasvir+PEG-RBV (n=82) or placebo+PEG-RBV (n=42) for 24 weeks. Median age was 49 years, 77% were white, 19% were black/African-American, 4% were Hispanic or Latino, 10% percent had compensated cirrhosis, and 75% of patients had IL-28B CT or TT genotypes. SVR12 was achieved in 82% vs 42% of patients on daclatasvir+PEG-RBV vs placebo+PEG-RBV ***(BMS, 2014).*** Special populations Several of the above clinical trials have included subjects with advanced fibrosis or cirrhosis. A pooled anlaysis of these studies revealed that the efficacy of all-oral daclatasvir-containing regimens did not vary by fibrosis stage. In phase 3 studies of daclatasvir+asunaprevir, SVR12 was achieved by 84-91% of cirrhotic patients (N=228) and by 84-85% of non-cirrhotic patients (N=637). Studies of the combination of sofosbuvir+daclatasvir ± RBV, SVR on or after posttreatment Week 12 was achieved by 100% of patients with advanced fibrosis (F3/F4–F4; N=32) and by 98% of patients with F0-F3 (N=179) ***(Jensen et al., 2014).*** Several studies are either recently completed or on-going to evaluate the efficacy of daclatasvir in HIV-HCV co-infection, including NCT01866930, a phase 3 study to evaluate daclatasvir+PEG-RBV in HIV/HCV coinfected patients with GT 1, 2, 3 or 4; NCT02124044, a phase 2 study to assess safety and efficacy of daclatasvir+asunepravir in HIV/HCV co-infected patients with GT1b; NCT01471574, a phase 3 study to evaluate daclatasvir+PEG-RBV in HIV/HCV co-infected patients with GT 1; NCT02032888, a phase 3 study to evaluate daclatasvir+sofosbuvir in HIV/HCV co-infected patients with GT 1, 2, 3, 4, 5, 6; and NCT01725542, a phase 2 study to evaluate the safety and efficacy of daclatasvir+asunaprevir+PEG-RBV in HIV/HCV co-infected patients with HCV genotype 1 or 4 and prior null response to PEG-RBV. At this point, no data on patients less than 18 years available**.**

Preferred combinations

Although daclatasvir has shown a favorable efficacy profile with a number of different combinations, including interferon-sparing (PEG-RBV+DCV), DCV+asunaprevir, DCV+asunaprevir+ BMS-791325 and DCV+SOF+/-RBV, we suggest the consideration of DCV+SOF as a potential preferred combination. Ideal HCV combinations will have high efficacy, an acceptable safety profile, have pan-genotypic potential and will be all-oral. DCV+SOF appears to meet all these criteria based on the available data. Furhter, BMS has chosen to discontinue further development of asunaprevir.

Direct acting anti HCV drugs

Direct acting anti HCV drugs are named with the following suffix according to its mode of action:

….previrs = protease inhibitors

….asvirs = NS5A inhibitors

….busvirs = polymerase inhibitors (nucs and non nucs)

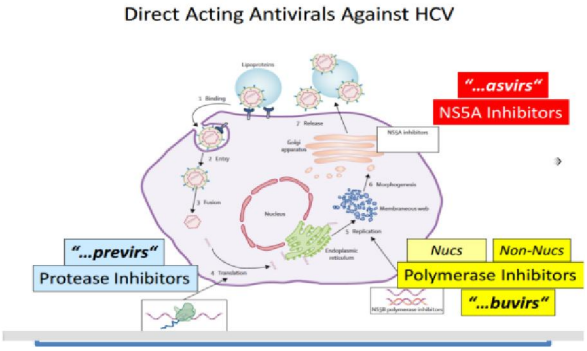

Fig. (11): shows mode of action of direct acting antiviral drugs.

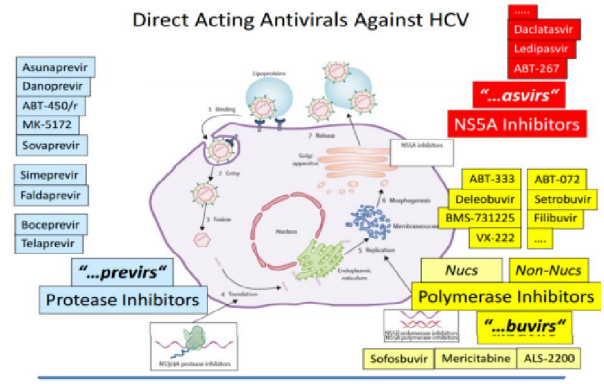

Fig. (12): shows different types and mode of action of direct acting antiviral drugs

Optimal therapy for patients with hepatitis C virus (HCV) genotype 4

HCV-4 infection is changing rapidly, and the possibility of a total cure is near. Since the introduction of sofosbuvir (SOF), simeprevir (SIM), and daclatasvir (DCV), the duration of treatment has been significantly shortened and response rates have increased.

The approval of sofosbuvir (SOF) ***(Lawitz E et al., 2013)*** simeprevir (SIM) ***(EPAR 2014)*** and daclatasvir (DC) ***(BMS Press Release. 2014)*** for the treatment of HCV-4 led to a significantly improved response to therapy.

DAAs: a rapidly changing scenario

The HCV replication complex has several drug target sites, and current DAAs include NS3/NS4 PIs, NS5A inhibitors and NS5B polymerase inhibitors. Although first generation PIs, BOC and TVR were not effective in HCV-4, they were precursors to the development of more effective drugs.

Grazoprevir

Grazoprevir (**MK-5172**) is an experimental drug for the treatment of hepatitis C. It is being developed by Merck and is currently in Phase III trials, following promising results in Phase II when used in combination with the NS5A replication complex inhibitor elbasvir, either with or without ribavirin***) Lawitz E et al., 2015).***

Grazoprevir is a second generation hepatitis C virus protease inhibitor acting at the NS3/4a protease targets.(Harper S et al., 2012) It has good activity against a range of HCV genotype variants, including some that are resistant to most currently used antiviral medications***. (Gentile I et al., 2014).***

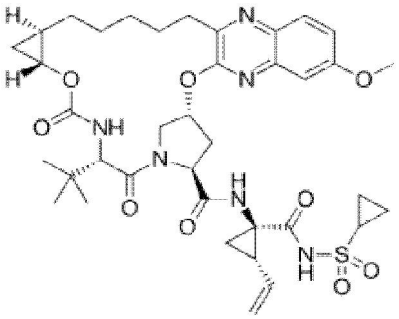

Fig. (17): Grazoprevir chemical structure.

Guidelines for treatment of HCV Genotype 4

According to *the* ***American Association for the Study of Liver Diseases and the Infectious Diseases Society of America*** **guidelines July, 2015**

Three options with similar efficacy in general are recommended for treatment-naive patients with HCV genotype 4 infections (listed in alphabetic order).

Daily fixed-dose combination of ledipasvir (90 mg)/sofosbuvir (400 mg) for 12 weeks is recommended for treatment-naive patients with HCV genotype 4 infection.

Rating: Class IIb, Level B

Daily fixed-dose combination of paritaprevir (150 mg)/ritonavir (100 mg)/ombitasvir (25 mg) and weight-based RBV (1000 mg [<75 kg] to 1200 mg [>75 kg]) for 12 weeks is recommended for treatment-naive patients with HCV genotype 4 infection.

Rating: Class I, Level B

Daily sofosbuvir (400 mg) and weight-based RBV (1000 mg [<75 kg] to 1200 mg [>75 kg]) for 24 weeks is recommended for treatment-naive patients with HCV genotype 4 infection.

Rating: Class IIa, Level B

The SYNERGY trial was an open-label study evaluating 12 weeks of ledipasvir/sofosbuvir in 21 HCV genotype 4–infected patients, of whom 60% were treatment naive and 43% had advanced fibrosis (Metavir stage F3 or F4). ***(Kohli, 2015)*** One patient took the first dose and then withdrew consent. All of the 20 patients who completed treatment achieved an SVR12; thus, the SVR12 rate was 95% in the intention-to-treat analysis and 100% in the per-protocol analysis. Abergel and colleagues reported data from an open-label single-arm study including 22 HCV genotype 4–infected, treatment-naive patients (only 1 with cirrhosis) with an SVR12 rate of 95% (21/22). ***(Abergel, 2015)*** These 2 pilot studies support the use of this regimen in patients with HCV genotype 4 infection.

PEARL-I was an open-label phase IIb study that included a cohort of 86 treatment-naive patients with HCV genotype 4 infection with or without cirrhosis who received 12 weeks of the daily fixed-dose combination of paritaprevir/ritonavir/ombitasvir (PrO) with or without weight-based RBV. SVR12 rates were 100% (42/42) in the group receiving RBV and 90.9% (40/44) in the group not receiving RBV. Adverse effects were generally mild, with headache, asthenia, fatigue, and nausea most commonly reported. There were no discontinuations owing to adverse events. ***(Hezode, 2015)***

Alternative regimen for treatment-naive patients with HCV genotype 4 infection.

Daily sofosbuvir (400 mg) and weight-based RBV plus weekly PEG-IFN for 12 weeks is an acceptable regimen for treatment-naive patients with HCV genotype 4 infection.

Rating: **Class II, Level B**

In the phase III NEUTRINO trial, ***(Lawitz, 2013a)*** 28 treatment-naive patients with HCV genotype 4 infection were treated with sofosbuvir (400 mg daily) plus PEG-IFN 2a (180 µg weekly) and weight-based RBV for 12 weeks. Of the 28 patients with HCV genotype 4 infection, 27 (96%) achieved SVR12. The single patient who did not achieve SVR had cirrhosis and had a relapse after therapy. The adverse event profile was similar to that associated with PEG-IFN and RBV therapy. There are limited clinical data to date to support the use of the combination of 12 weeks of daily sofosbuvir (400 mg) plus simeprevir (150 mg) with or without weight-based RBV (1000 mg [<75kg] to 1200 mg [≥75mg]) in HCV genotype 4–infected patients, although studies are planned. Given the demonstrated activity of simeprevir in vitro and in vivo against HCV genotype 4, this combination may be considered as an

alternative regimen. The open-label phase III RESTORE trial assessed the efficacy of simeprevir in combination with PEG-IFN and RBV in 107 patients with HCV genotype 4 infection, including 35 treatment-naive patients. In these treatment-naive patients, daily simeprevir (150 mg) for 12 weeks in combination with PEG-IFN and RBV for 24 weeks to 48 weeks (by response-guided therapy) produced an SVR in 83% (29 of 35). ***(Moreno, 2013a)*** These results are comparable to SVR rates observed with similar regimens in patients with HCV genotype 1 infection, suggesting that efficacy of sofosbuvir plus simeprevir for HCV genotype 4 infection may be roughly in line with the SVR rates of patients with HCV genotype 1 infection shown in the COSMOS trial. This combination has been approved in Europe for patients with HCV genotype 4 infection but is not FDA-approved for use in the United States.

The following regimens are NOT recommended for treatment-naive patients with HCV genotype 4 infections.

- *PEG-IFN and RBV with or without simeprevir for 24 weeks to 48 weeks*
- *Rating:* Class IIb, Level A
- *Monotherapy with PEG-IFN, RBV, or a direct-acting antiviral*
- *Rating:* Class III, Level A
- *Telaprevir- or boceprevir-based regimens*

Rating: Class III, Level A

HCV Genome and Life Cycle

Hepatitis C virus is a small (55–65 nm in size), enveloped, positive-sense single-stranded RNA virus of the family Flaviviridae. Hepatitis C virus is the cause of hepatitis C in humans.

The hepatitis C virus belongs to the genus Hepacivirus a member of the family Flaviviridae.***(Op De Beeck A et al., 2003)***.

Structure:

The hepatitis C virus particle consists of a core of genetic material (RNA), surrounded by a shell of protein, and further encased in a lipid (fatty) envelope of cellular origin. Two viral envelope glycoproteins, E1 and E2, are embedded in the lipid envelope.***(Op De Beeck A et al., 2003)***

Genome:

Hepatitis C virus has a positive sense single-stranded RNA genome. The genome consists of a single open reading frame that is 9600 nucleotide bases long.***(Kato N (2000))*** This single open reading frame is translated to produce a single protein product, which is then further processed to produce smaller active proteins.

At the 5' and 3' ends of the RNA are the UTR, that are not translated into proteins but are important to translation and replication of the viral RNA. The 5' UTR has a ribosome binding site ***(Jubin R (2001)*** (IRES - Internal ribosome entry site) that starts the translation of a very long protein containing about 3,000 amino acids. The core domain of the hepatitis C virus (HCV) IRES contains a four-way helical junction that is integrated within a predicted pseudoknot. ***(Berry KE et al., 2011)*** The conformation of this core domain constrains the open reading frame's orientation for positioning on the 40S ribosomal subunit. The large pre-protein is later cut by cellular and viral proteases into the 10 smaller proteins that allow viral replication within the host cell, or assemble into the mature viral particles***. (Dubuisson J (2007)***

Structural proteins made by the hepatitis C virus include Core protein, E1 and E2; nonstructural proteins include NS2, NS3, NS4A, NS4B, NS5A, and NS5B.

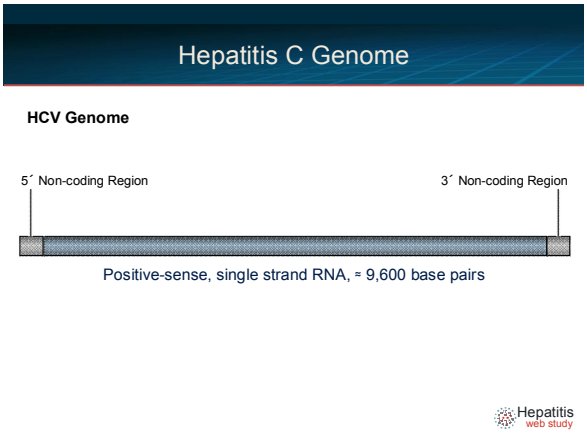

Fig. (3): HCV genome

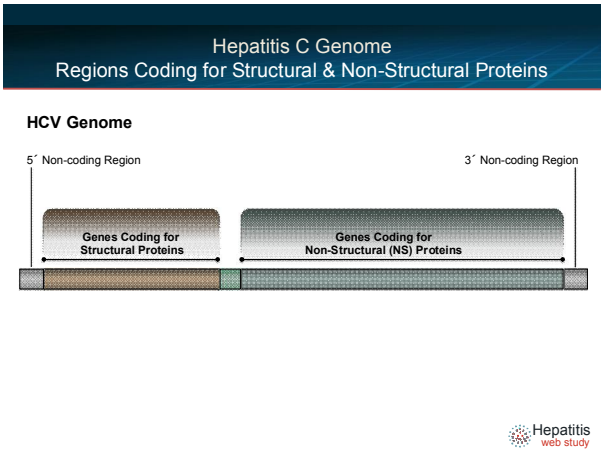

Fig. (4): HCV genome, regions coding for structural and non structural proteins

Molecular biology

The proteins of this virus are arranged along the genome in the following order: N terminal-core-envelope (E1)–E2–p7-nonstructural protein 2 (NS2)–

NS3–NS4A–NS4B–NS5A–NS5B–C terminal. The mature nonstructural proteins (NS2 to NS5B) generation relies on the activity of viral proteinases. ***(De Francesco R (1999)***

The core protein has 191 amino acids and can be divided into three domains on the basis of hydrophobicity:

Domain 1 (residues 1–117) contains mainly basic residues with two short hydrophobic regions;

Domain 2 (resides 118–174) is less basic and more hydrophobic and its C-terminus is at the end of p21;

Domain 3 (residues 175–191) is highly hydrophobic and acts as a signal sequence for E1 envelope protein.

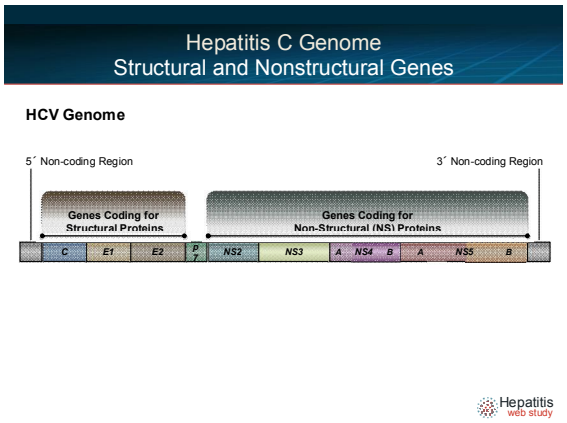

Fig. (5): HCV genome, structural and non structural Genes

Both envelope proteins (E1 and E2) are highly glycosylated and important in cell entry. E1 serves as the fusogenic subunit and E2 acts as the receptor binding protein. E1 has 4–5 N-linked glycans and E2 has 11 N-glycosylation sites.

The p7 protein is dispensable for viral genome replication but plays a critical role in virus morphogenesis. This protein is a 63 amino acid membrane spanning protein which locates itself in the endoplasmic reticulum. Cleavage of p7 is mediated by the endoplasmic reticulum's signal peptidases. Two transmembrane domains of p7 are connected by a cytoplasmic loop and are oriented towards the endoplasmic reticulum's lumen.

NS2 protein is a 21–23 kiloDalton (kDa) transmembrane protein with protease activity.

NS3 is 67 kDa protein whose N-terminal has serine protease activity and whose C-terminal has NTPase/helicase activity. It is located within the endoplasmic reticulum and forms a heterodimeric complex with NS4A—a 54 amino acid membrane protein that acts as a cofactor of the proteinase.

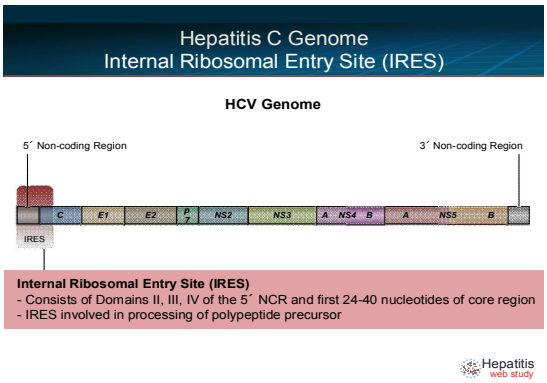

Fig. (6): HCV genome Ribosomal entery site (IRES)

NS4B is a small (27 kDa) hydrophobic integral membrane protein with 4 transmembrane domains. It is located within the endoplasmic reticulum and plays an important role for recruitment of other viral proteins. It induces morphological changes to the endoplasmic reticulum forming a structure termed the membranous web.

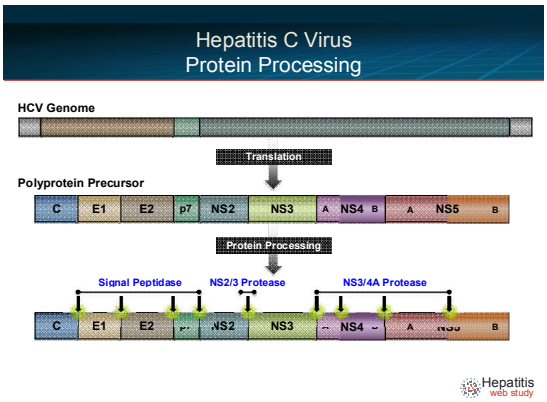

Fig. (7): HCV protein processing

NS5A is a hydrophilic phosphoprotein which plays an important role in viral replication, modulation of cell signaling pathways and the interferon response. It is known to bind to endoplasmic reticulum anchored human VAP proteins.***(Gupta Get al., 2012).***

The NS5B protein (65 kDa) is the viral RNA dependent RNA polymerase. NS5B has the key function of replicating the HCV's viral RNA by using the viral positive RNA strand as its template and catalyzes the polymerization of ribonucleoside triphosphates (rNTP) during RNA replication**.** ***(Moradpour D et al., 2007)*** Several crystal structures of NS5B polymerase in several crystalline forms have been determined based on the same consensus sequence BK (HCV-BK, genotype 1). ***(Biswal BK et al., 2005)***.

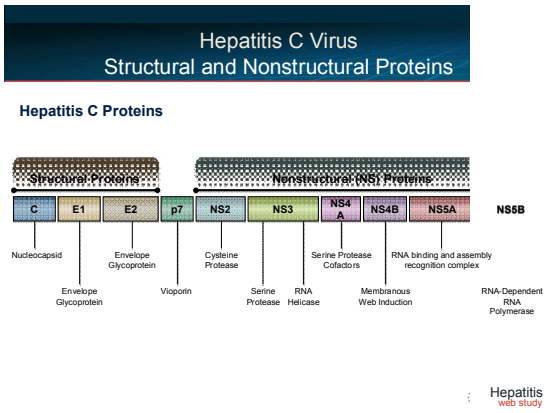

Fig. (8): HCV genome, structural and non structural proteins.

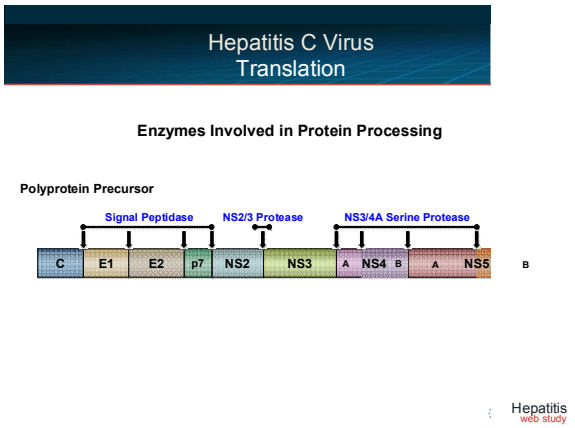

Fig. (9): HCV translation

Replication

Replication of HCV involves several steps. The virus replicates mainly in the hepatocytes of the liver, where it is estimated that daily each infected cell produces approximately fifty virions (virus particles) with a calculated total of one trillion virions generated. The virus may also replicate in peripheral blood mononuclear cells, potentially accounting for the high levels of immunological disorders found in chronically infected HCV patients. HCV has a wide variety of genotypes and mutates rapidly due to a high error rate on the part of the virus' RNA-dependent RNA polymerase. The mutation rate produces so many variants of the virus it is considered a quasispecies rather than a conventional virus species. ***(Bartenschlager R et al., 2000)*** Entry into host cells occur through complex interactions between virions and cell-surface molecules CD81, LDL receptor, SR-BI, DC-SIGN, Claudin-1, and Occludin. ***(Zeisel, M et al., 2009)***

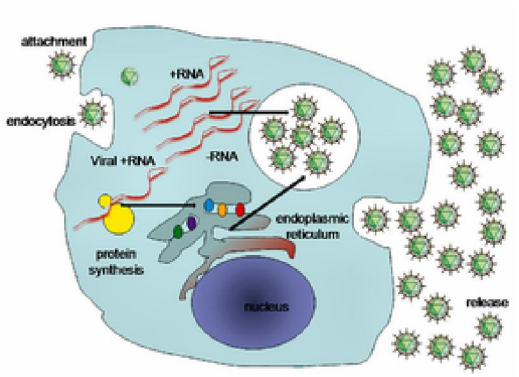

Fig. (10): A simplified diagram of the HCV replication cycle.

Once inside the hepatocyte, HCV takes over portions of the intracellular machinery to replicate. ***(Lindenbach B et al., 2005)*** The HCV genome is translated to produce a single protein of around 3011 amino acids. The polyprotein is then proteolytically processed by viral and cellular proteases to produce three structural (virion-associated) and seven nonstructural (NS) proteins. Alternatively, a frame shift may occur in the Core region to produce an Alternate Reading Frame Protein (ARFP). ***(Branch, A. D et al., 2005)*** HCV encodes two proteases, the NS2 cysteine auto protease and the NS3-4A serine protease.

The virus replicates on intracellular lipid membranes. ***(Dubuisson J et al., 2002)*** The endoplasmic reticulum in particular are deformed into uniquely shaped membrane structures termed 'membranous webs'. These structures can be induced by sole expression of the viral protein NS4B. ***(Egger D et al., 2002)*** The core protein associates with lipid droplets and utilises microtubules and dyneins to alter their location to a perinuclear distribution. ***(Boulant S et al., 2008)***

Release from the hepatocyte may involve the very low density lipoproteinsecretory pathway. ***(Syed GH et al., 2010)***

Genotypes

Based on genetic differences between HCV isolates, the hepatitis C virus species is classified into seven genotypes (1–7) with several subtypes within each genotype (represented by lower-cased letters). ***(Nakano et al., 2011)*** Subtypes are further broken down into quasispecies based on their genetic diversity. Genotypes differ by 30–35% of the nucleotide sites over the complete genome. ***(Ohno O et al., 2007)*** The difference in genomic composition of subtypes of a genotype is usually 20–25%. Subtypes 1a and 1b are found worldwide and cause 60% of all cases.

Clinical importance

Genotype is clinically important in determining potential response to interferon-based therapy and the required duration of such therapy. Genotypes 1 and 4 are less responsive to interferon-based treatment than are the other genotypes (2, 3, 5 and 6***). (Simmonds P et al., 2005)*** Duration of standard interferon-based therapy for genotypes 1 and 4 is 48 weeks, whereas treatment for genotypes 2 and 3 is completed in 24 weeks. Sustained virological responses occur in 70% of genotype 1 cases, ~90% of genotypes 2 and 3, ~65% of genotype 4 and ~80% of genotype 6 ***(Yu ML et al., 2009)***

Epidemiology

Hepatitis C virus is predominantly a blood-borne virus, with very low risk of sexual or vertical transmission. ***(Shepard et al., 2005)*** Because of this mode of spread the key groups at risk are injecting drug users (IDUs), people who are transfused blood, recipients of blood products and sometimes patients on haemodialysis. Common setting for transmission of HCV is also intra-hospital (nosocomial) transmission, when practices of hygiene and sterilization are not correctly followed in the clinic.***(Alter et al., 2011)***

HCV manifestations

Hepatitis C is an infectious disease affecting primarily the liver, caused by the hepatitis C virus (HCV). ***(Ryan KJ et al., 2004)*** The infection is often asymptomatic, but chronic infection can lead to scarring of the liver and ultimately to cirrhosis, which is generally apparent after many years. In some cases, those with cirrhosis will go on to develop liver failure, liver cancer, or life-threatening esophageal and gastric varices.***(Ryan KJ et al., 2004)***

The virus persists in the liver in about 85% of those infected. This chronic infection can be treated with medication.

Signs and symptoms

Acute infection

Hepatitis C infection causes acute symptoms in 15% of cases.***(Maheshwari et al., 2008)*** Symptoms are generally mild and vague, including a decreased appetite, fatigue, nausea, muscle or joint pains, and weight loss. ***(Wilkins et al., 2010)*** and rarely does acute liver failure result. Most cases of acute infection are not associated with jaundice ***(Springer Verlag. 2011)*** The infection resolves spontaneously in 10–50% of cases, which occurs more frequently in individuals who are young and female***. (Springer Verlag. 2011)***

Chronic infection

About 80% of those exposed to the virus develop a chronic infection.***(Nelson et al., 2011)*** This is defined as the presence of detectable viral replication for at least six months. Most experience minimal or no symptoms during the initial few decades of the infection. Chronic hepatitis C can be associated with fatigue and mild cognitive problems.***(Forton et al., 2005)*** Chronic infection after several years may cause cirrhosis or liver cancer. The liver enzymes are normal in 7–53%. Late relapses after apparent cure have been reported, but these can be difficult to distinguish from reinfection. ***(Nicot et al., 2004).***

Fatty changes to the liver occur in about half of those infected and are usually present before cirrhosis develops. Usually (80% of the time) this change affects less than a third of the liver. ***(El-Zayadi et al., 2008)*** Worldwide hepatitis C is the cause of 27% of cirrhosis cases and 25% of hepatocellular carcinoma. About 10–30% of those infected develop cirrhosis over 30 years. ***(Wilkins et al., 2010)*** Cirrhosis is more common in those also infected with hepatitis B, schistosoma, or HIV, in alcoholics and in those of male gender. ***(Wilkins et al., 2010)*** In those with hepatitis C, excess alcohol increases the risk of developing cirrhosis 100-fold.***(Mueller et al., 2009)*** Those who develop cirrhosis have a 20-fold greater risk of hepatocellular carcinoma. This transformation occurs at a rate of 1–3% per year. ***(Wilkins et al., 2010)*** Being infected with hepatitis B in addition to hepatitis C increases this risk further.***(Fattovich et al., 2004)***

Liver cirrhosis may lead to portal hypertension, ascites (accumulation of fluid in the abdomen), easy bruising or bleeding, varices (enlarged veins, especially in the stomach and esophagus), jaundice, and a syndrome of cognitive impairment known as hepatic encephalopathy. Ascites occurs at some stage in more than half of those who have a chronic infection.***(Zaltron et al., 2012).***

Extrahepatic complications

The most common problem due to hepatitis C but not involving the liver is mixed cryoglobulinemia (usually the type II form) -an inflammation of small and medium-sized blood vessels. ***(Iannuzzella et al., 2010)*** Hepatitis C is also associated with the autoimmune disorder Sjögren's syndrome, a low platelet count, lichen planus, porphyria cutanea tarda, necrolytic acral erythema, insulin resistance, diabetes mellitus, diabetic nephropathy, autoimmune thyroiditis, and B-cell lymphoproliferative disorders***. (Ko, HM; Hernandez-Prera et al., 2012)*** Thrombocytopenia is estimated to occur in 0.16% to 45.4% of people with chronic hepatitis C***. (Louie et al., 2011)*** 20–30% of people infected have rheumatoid factor- a type of antibody. Possible associations include Hyde's prurigo nodularis and membranoproliferative glomerulonephritis. ***(Ray et al., 2009)*** Cardiomyopathy with associated abnormal heart rhythms has also been reported. A variety of central nervous system disorders has been reported. Chronic infection seems to be associated with an increased risk of pancreatic cancer.***(Xu, et al., 2013)***

Occult infection

Persons who have been infected with hepatitis C may appear to clear the virus but remain infected. ***(Sugden et al., 2012)*** The virus is not detectable with conventional testing but can be found with ultra-sensitive tests.***(Carreño García et al., 2011)***

Several clinical pictures have been associated with this type of infection, it may be found in people with anti-hepatitis-C antibodies but with normal serum levels of liver enzymes; in antibody-negative people with ongoing elevated liver enzymes of unknown cause; in healthy populations without evidence of liver disease; and in groups at risk for HCV infection including those on hemodialysis or family members of people with occult HCV. The clinical relevance of this form of infection is under investigation.***(Carreño et al., 2008)*** The consequences of occult infection appear to be less severe than with chronic infection but can vary from minimal to hepatocellular carcinoma. ***(Carreño et al., 2008)***.

Diagnosis of acute and chronic hepatitis C

The diagnosis of acute and chronic HCV infection is based on the detection of HCV RNA by a sensitive molecular method (lower limit of detection <15 international units [IU]/ml). Anti-HCV antibodies are detectable by enzyme immunoassay (EIA) in the vast majority of patients with HCV infection, but EIA results may be negative in early acute hepatitis C and in profoundly immunosuppressed patients. Following spontaneous or treatment-induced viral clearance, anti-HCV antibodies persist in the absence of HCV RNA but may decline and finally disappear in some individuals ***(Robinson et al., 2008)***.

The diagnosis of acute hepatitis C can be confidently made only if seroconversion to anti-HCV antibodies can be documented, since there is no serological marker which proves that HCV infection is in the de novo acquired acute phase. Not all patients with acute hepatitis C will be anti-HCV-positive at diagnosis. In these cases, acute hepatitis C can be suspected if the clinical signs and symptoms are compatible with acute hepatitis C (alanine aminotransferase [ALT] >10 times the upper limit of normal, jaundice) in the absence of a history of chronic liver disease or other causes of acute hepatitis, and/or if a likely recent source of transmission is identifiable. In all cases, HCV RNA can be detected during the acute phase although brief interludes of undetectable HCV RNA may occur ***(Sugden et al., 2012).***

Ledipasvir

Ledipasvir: The IUPAC name for ledipasvir is Methyl [(2S)-1-{(6S)-6-[5-(9,9-difluoro - 7- { 2- [(1R, 3 S, 4S) - 2 - {(2S) - 2- [(methoxycarbonyl) amino] -3 – methylbutanoyl } - 2- azabicyclo [2. 2. 1] hept- 3 - yl] -1H-benzimidazol-6-yl}-9H-fluoren-2-yl)-1H-imidazol-2-yl]-5-azaspiro[2.4]hept-5-yl}-3-methyl-1-oxobutan-2-yl]carbamate***.(lawitz et al., 2012).***

It has a molecular formula of C49H54F2N8O6 and a molecular weight of 889.00. It has the following structural formula:

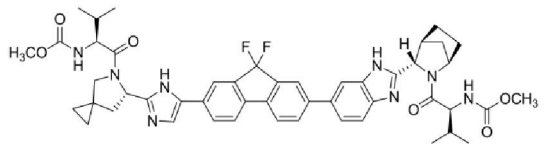

Fig. (15): ledipasvir chemical structure.

Distribution

Ledipasvir is >99.8% bound to human plasma proteins. After a single 90 mg dose of 14C]-ledipasvir in healthy subjects, the blood to plasma ratio of 14 C-radioactivity ranged between 0.51 and 0.66.

Metabolism

In vitro, no detectable metabolism of ledipasvir was observed by human CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4.

Evidence of slow oxidative metabolism via an unknown mechanism has been observed. Following a single dose of 90 mg [14C]-ledipasvir, systemic exposure was almost exclusively to the parent drug (>98%). Unchanged ledipasvir is the major species present in feces***(lawitz et al., 2012).***

Elimination

Following a single 90 mg oral dose of [14C]-ledipasvir, mean total recovery of the [14C]-radioactivity in feces and urine was approximately 87%, with most of the radioactive dose recovered from feces (approximately 86%). Unchanged ledipasvir excreted in feces accounted for a mean of 70% of the administered dose and the oxidative metabolite M19 accounted for 2.2% of the dose. These data indicate that biliary excretion of unchanged ledipasvir is a major route of elimination, with renal excretion being a minor pathway (approximately 1%). The median terminal half-life of ledipasvir following administration was 47 hours.***(lawitz et al., 2012).***

Specific Populations

Patients with Renal Impairment: The pharmacokinetics of ledipasvir were studied with a single dose of 90 mg ledipasvir in HCV negative subjects with severe renal impairment (eGFR <30 mL/min by Cockcroft-Gault). No clinically relevant differences in ledipasvir pharmacokinetics were observed between healthy subjects and subjects with severe renal impairment.

Mechanism of Action

Ledipasvir is an inhibitor of the HCV NS5A protein, which is required for viral replication.

Resistance selection in cell culture and cross-resistance studies indicate ledipasvir targets NS5A as its mode of action.

Ledipasvir is not used alone, instead it is used as drug combination with sofosbuvir as a trade name drug called HARVONI.

HARVONI is a two-drug fixed-dose combination product that contains 90 mg of ledipasvir and 400 mg of sofosbuvir in a single tablet. The recommended dosage of HARVONI is one tablet taken orally once daily with or without food.

Adverse Reactions:

The most common adverse reactions (≥10%) were fatigue and headache in subjects treated with 8, 12, or 24 weeks of HARVONI.

The proportion of subjects who permanently discontinued treatment due to adverse events was 0%, <1%, and 1% for subjects receiving HARVONI for 8, 12, and 24 weeks, respectively.

Laboratory Abnormalities:

Bilirubin Elevations: Bilirubin elevations of greater than 1.5xULN were observed in 3%, <1%, and 2% of subjects treated with HARVONI for 8, 12, and 24 weeks, respectively.

Lipase Elevations: Transient, asymptomatic lipase elevations of greater than 3xULN were observed in <1%, 2%, and 3% of subjects treated with HARVONI for 8, 12, and 24 weeks, respectively.

Creatine Kinase: Creatine kinase was not assessed in Phase 3 trials of HARVONI. Isolated, asymptomatic creatine kinase elevations (Grade 3 or 4) have been previously reported in subjects treated with sofosbuvir in combination with ribavirin or peginterferon/ribavirin in other clinical trials.

Cardiac Disorders

Serious symptomatic bradycardia has been reported in patients taking **amiodarone** who initiate treatment with HARVONI***(lawitz et al., 2012).***

- Anti-HCV antibodies are the first-line diagnostic test for HCV infection **(A1)**
- In the case of suspected acute hepatitis C or in immunocompromised patients, HCV RNA testing should be part of the initial evaluation **(A1)**
- If anti-HCV antibodies are detected, HCV RNA should be determined by a sensitive molecular method **(A1)**
- Anti-HCV-positive, HCV RNA negative individuals should be retested for HCV RNA three months later to confirm true convalescence **(A1)**

Ombitasvir, Paritaprevir, and Ritonavir

Ombitasvir, Paritaprevir, and Ritonavir, Dasabuvir Tablets (Viekira™ Pak).

The FDA has approved the fourth new product for the treatment of chronic hepatitis C (HCV) infection since November 2013. The latest is a four fixed-drug combination (3-DAA) that includes paritaprevir, a nonstructure 3/4A protease inhibitor, ombitasvir, an inhibitor of the NS5A replication complex, and copackaged with dasabuvir, a nonnucleoside NS5B polymerase inhibitor. The plasma levels of paritaprevir is boosted with ritonavir, a CYP3A inhibitor to permit once-daily dosing. The combination is marketed by AbbVie, Inc. as Viekira Pak.

Indications

3-DAA is indicated for the treatment of chronic HCV genotype 1 infections, including patients with compensated cirrhosis. 1 Co-administration with ribavirin may be required based on the genotype of the infection and presence of cirrhosis.

Dosage

The recommended dose is two tablets (ombitasvir, paritaprevir, and ritonavir) once daily in the morning and one tablet of dasabuvir twice daily in the morning and evening. The duration of treatment is 12 weeks for genotype 1a without cirrhosis and genotype 1b with or without cirrhosis. The duration is 24 weeks in patients with genotype 1a with cirrhosis. Co-administration with ribavirin is recommended for genotype 1a patients with and without cirrhosis and genotype 1b patients with cirrhosis. The tablets may be taken without regard to meals.

3-DAA is available as ombitasvir 12.5 mg, paritaprevir 75 mg, and ritonavir 50 mg in one tablet and dasabuvir 250 mg in a separate tablet.

Potential Advantages

Provides a new effective treatment option for treating HCV.

Potential Disadvantages

The presence of ritonavir increases the risks of drug-drug interactions with CYP3A isoenzyme-metabolized drugs. Drugs that are contraindicated with ritonavir include anticonvulsants (carbamazepine), statins (simvastatin), and antimycobacterials (rifampin). Other drugs may be affected to a lesser degree, and may require dose adjustments. The pill burden is slightly higher compared to sofosbuvir/ledipasvir (one tablet daily). The new combination requires ribavirin for genotype 1a and genotype 1b with cirrhosis. The most frequently reported adverse events were fatigue, nausea, pruritus, other skin reactions, insomnia, and asthenia***.(Viekira Pak 2014)***

Comment

3-DAA was evaluated in six clinical trials.2-6 These include treatment-naïve genotypes 1a infections and 1b without cirrhosis, genotypes 1a and 1b treatment-experienced without cirrhosis, and treatment-experienced and treatment-naïve genotype 1a and 1b with cirrhosis. The primary efficacy endpoint was a sustained virologic response (HCV RNA level < 25 IU/mL) at post-treatment week 12

sustained virological response (SVR12). 3-DAA with ribavirin achieved SVR12 of 96% in treatment-experienced (peginterferon/ribavirin) and treatment-naïve subjects without cirrhosis.2,3 In treatment-naïve subjects, SVR12 was similar with or without ribavirin for genotype 1b (99%) and slightly more effective with ribavirin for 1a (97% vs. 90%).5 In subjects with cirrhosis, 3-DAA with ribavirin achieved SVR12 of 92% for 12 weeks and 93% for 24 weeks of treatment for type genotype 1a in treatment-naïve subjects and 100% for genotype 1b for both durations.6 For those previously treated with peginterferon/ribavirin, SVR12 were 86% and 94% for genotype 1a, and 98% and 100%, respectively. Previous null responders with genotype 1a showed a lower response: 80% for 12 weeks compared to 93% for 24 weeks. For treatment-experienced genotype 1b without cirrhosis, SVR12 without ribavirin was noninferior to treatment with ribavirin. 4 In two other studies, 3-DAA achieved SVR12 of 92% in subjects co-infected with HIV-1 and 97% in selected liver transplant subjects**.** ***(Viekira Pak 2014)***

Simeprevir

Name of the Medicine

The chemical name of simeprevir is (2*R*,3a*R*,10*Z*,11a*S*,12a*R*,14a*R*)-*N*-(cyclopropylsulfonyl)-2-[[2-(4-isopropyl-1,3-thiazol-2-yl)-7-methoxy-8-methyl-4- quinolinyl]oxy]-5-methyl-4, 14-dioxo-2, 3, 3a, 4, 5, 6, 7, 8, 9, 11a, 12, 13, 14, 14a-tetradecahydrocyclopenta[*c*]cyclopropa[*g*][1,6]diazacyclotetradecine-12a(1*H*)-carboxamide.

Simeprevir has the following chemical structure:

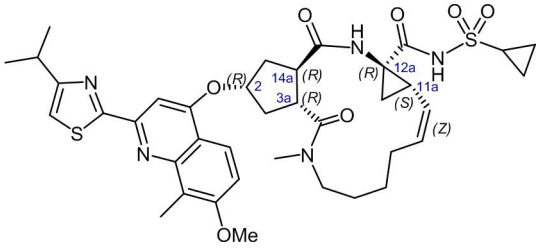

Fig. (14): Simeprevir chemical structure

Molecular formula: $C_{38}H_{47}N_5O_7S_2$

Molecular weight: 749.94

Pharmacology

Pharmacodynamics

Pharmacotherapeutic group: Direct-acting antiviral

Mechanism of action

Simeprevir is an inhibitor of the Hepatitis C Virus (HCV) NS3/4A protease which is essential for viral replication. ***(Jacobsen IM et al 2014).*** In a biochemical assay, simeprevir inhibited the proteolytic activity of recombinant genotype 1a and 1b HCV NS3/4A proteases**.**

Class and Mechanism: Simeprevir is a NS3/4A hepatitis C virus (HCV) protease inhibitor.

FDA Status: Simeprevir was approved by the U.S. FDA on November 22, 2013 for the treatment of individuals with genotype 1 chronic hepatitis C as a component of combination therapy with peginterferon-alfa and ribavirin.***(FDA 2013)*** On November 5, 2014, the U.S. FDA approved the use of simeprevir in combination with sofosbuvir for patients with genotype 1 chronic hepatitis C.

Indications: Simeprevir is indicated as a component of combination antiviral treatment for patients with chronic hepatitis C genotype 1 infection. Simeprevir is approved for use with (a) peginterferon-alfa and ribavirin or (b) in combination with sofosbuvir.

Several limitation exist for the use of simeprevir:

- Simeprevir should not be used as monotherapy.
- Simeprevir is not recommended for use in patients who have previously failed treatment for hepatitis C with a regimen that included simeprevir or another HCV protease inhibitor.
- Simeprevir is not recommended for use in patients with severe hepatic impairment (Child-Pugh Class C) because of substantial increases in simeprevir levels in this setting.

Simeprevir in Combination with Peginterferon-alfa and Ribavirin:

- Treatment-naive and prior relapsers: 12 weeks of simeprevir in combination with peginterferon-alfa and ribavirin followed by an additional 12 weeks of peginterferon-alfa and ribavirin
- Prior non-responders (including partial and null responders): 12 weeks of simeprevir in combination with peginterferon-alfa and ribavirin followed by an additional 36 weeks of peginterferon-alfa and ribavirin

Simeprevir in Combination with Sofosbuvir:

- Treatment-naive or treatment experienced patients without cirrhosis: 12 weeks of simeprevir and sofosbuvir
- Treatment-naive or treatment experienced patients with cirrhosis: 24 weeks of simeprevir and sofosbuvir

Simeprevir in Patients with Renal or Hepatic Impairment:

- The dose of simeprevir does not need adjusting in patients with mild, moderate, or severe renal insufficiency.
- Since simeprevir is highly protein-bound, dialysis is unlikely to significantly impact simeprevir levels.

- The dose of simeprevir does not need adjusting in patients with mild hepatic impairment (Child-Pugh Class A).

- Simeprevir is metabolized primarily in the liver and patients with moderate or severe hepatic impairment (Child-Pugh Class B or C) would be expected to have higher levels of simeprevir. No dosing recommendation for simeprevir is given for patient with moderate to severe hepatic impairment.

Clinical Use: Simeprevir is FDA-approved only for patients with genotype 1 infection. The duration of therapy with simeprevir plus peginterferon and ribavirin depends on prior treatment experience and prior response to treatment ***(Jacobsen IM et al 2014).*** Duration of therapy is not based on response guided therapy, but continuation of therapy assumes the patient does not meet stopping rules:

Adverse Effects: The most common adverse effects attributable to simeprevir are rash (including a potentially serious photosensitivity reaction), pruritus, and nausea. ***(Forns X et al., 2014)***

- The photosensitivity reaction that can occur with simeprevir most often has an onset during the first 4 weeks of therapy, but can develop at any time on treatment

- Patients taking simprevir should limit sun exposure, use protective sun exposure measures, and avoid use of any tanning device.

- If a photosensitivy rash does occur while taking simeprevir, discontinuation of simeprevir should be considered and the patient should have close monitoring until the rash has resolved.

- Rash not related to photosensivity can also occur and similar to the photosensitivity rash most often develops during the first 4 weeks of therapy.

- Simeprevir contains a sulfonamide moiety, but insufficient data exist to know the risk of taking simeprevir in persons with a prior "sulfa allergy".

- Simeprevir is pregnancy category C.

Clinical study examining QT interval

The effect of simeprevir 150 mg once daily and 350 mg once daily for 7 days on the QT interval was evaluated in a randomized, double-blind, placebo- and positive-controlled (moxifloxacin 400 mg once daily), 4-way cross-over study in 60 healthy participants. No meaningful changes in QTc interval were observed with either the recommended dose of 150 mg once daily or the supratherapeutic dose of 350 mg once daily ***(Moreno C et al., 2013).***

Metabolism

Simeprevir is metabolized in the liver. *In vitro* experiments with human liver microsomes indicated that simeprevir primarily undergoes oxidative metabolism by the hepatic CYP3A system. Involvement of CYP2C8 and CYP2C19 cannot be excluded.

For information on the effects of CYP inhibitors or inducers on the pharmacokinetics of simeprevir and information on the inhibition potential of simeprevir on CYP enzymes.

Following a single oral administration of 200 mg ^{14}C-simeprevir to healthy participants, the majority of the radioactivity in plasma (up to 98%) was accounted for by unchanged drug and a small part of the radioactivity in plasma was related to metabolites (none being major metabolites). Metabolites identified in faeces were formed via oxidation at the macrocyclic moiety or aromatic moiety or both and by *O*-demethylation followed by oxidation.

Elimination

Elimination of simeprevir occurs via biliary excretion. Renal clearance plays an insignificant role in its elimination. Following a single oral administration of 200 mg ^{14}C-simeprevir to healthy participants, on average 91% of the total radioactivity was recovered in faeces. Less than 1% of the administered dose was recovered in urine. Unchanged simeprevir in faeces accounted for on average 31% of the administered dose.

The terminal elimination half-life of simeprevir was 10 to 13 hours in healthy participants and 41 hours in HCV-infected patients receiving 200 mg simeprevir.

Additional information on special populations

Paediatrics (below 18 years of age)

Studies characterising the pharmacokinetics of simeprevir in paediatric patients have not been performed.

Elderly (above 65 years of age)

There is limited data on the use of simeprevir in patients older than 65 years. Age (18-73 years) had no clinically meaningful effect on the pharmacokinetics of simeprevir based on a population pharmacokinetic analysis of HCV-infected patients treated with simeprevir. No dose adjustment of simeprevir is required in elderly patients.

Renal impairment

Renal elimination of simeprevir is negligible. Compared to healthy participants with normal renal function (classified using the Modification of Diet in Renal Disease [MDRD] eGFR formula; eGFR $\geq$ 80 mL/min), the mean steady-state AUC of simeprevir was 62% higher in participants with severe renal impairment (eGFR below 30 mL/min).

Specific Drug Interactions:

Digoxin	Increased digoxin levels expected, monitor digoxin levels during concurrent therapy
Antiarrhythmics such as amiodarone, disopyramide, flecainide, mexilitine, propafenone, quinidine	Mild increases in antiarrhythmic concentrations expected. Use with caution, monitor levels if available
Anticonvulsants such as carbamazepine, oxcarbazepine, phenobarbital, phenytoin	Reduced levels of simeprevir expected; do not co-administer
Erythromycin, clarithromycin, telithromycin	Increased macrolide concentrations expected. Do not co--administer
Antifungals such as itraconazole, Fluconazole, ketoconazole, posaconazole, voriconazole	Increased simeprevir concentrations expected. Do not co-administer
Antimycobacterials such as rifampin, rifabutin, rifapentine	Reduced simeprevir concentrations expected. Do not co-administer.
Calcium Channel Blockers such as amlodipine, diltiazem, felodipine, nicardipine, nifedipine, nisoldipine, verapamil	Increased calcium channel blocker concentrations expected. Caution when used together, monitor closely.
Corticosteroid, dexamethasone	Decreased simeprevir concentrations expected. Do not co- - administer.
Milk Thistle	Increased simeprevir concentrations expected. Do not co-administer.
St. John's Wort	Decreased simeprevir concentrations expected. Do not co-administer.
Cobicistat (contained in Stribild)	Increased simeprevir concentrations expected. Do not co-administer.
Efavirenz, etravirine, nevirapine	Decreased simeprevir concentrations expected. Do not co-administer.
Ritonavir boosted and un-- - boosted protease inhibitors	Increased simeprevir concentrations expected. Do not co- - administer with any HIV protease inhibitor, whether boosted with ritonavir or unboosted.
Atorvastatin	Use lowest dose of atorvastatin, but do not to exceed 40mg daily.
Lovastatin, pitavastatin, pravastatin	Use lowest dose of lovastatin, pitavastatin, pravastatin; titrate carefully.
Rosuvastatin	Initiate rosuvastatin with 5mg daily, titrate, but do not exceed 10mg daily.
Simvastatin	Use lowest dose of simvastatin, titrate carefully.
Immunosuppressants such as cyclosporine, tacrolimus, sirolimus	Routine monitoring of immunosuppressant drug levels recommended.
PDE-- - 5 inhibitors	No dosage adjustment recommended when used for erectile dysfunction, however when used for pulmonary arterial hypertension, consider lowest dose and titrate as needed.
Midazolam (oral administration)	Increased midazolam concentrations expected, caution when used together, monitor for excess sedation
Triazolam (oral administration)	Increased triazolam concentrations expected, caution when used together, monitor for excess sedation

In a population pharmacokinetic analysis of mild or moderate renally impaired HCV-infected patients treated with simeprevir 150 mg once daily, creatinine clearance was not found to influence the pharmacokinetic parameters of simeprevir. It is therefore not expected that renal impairment will have a clinically relevant effect on the exposure to simeprevir, and no dose adjustment of simeprevir is needed in patients with mild, moderate or severe renal impairment. The safety and efficacy of simeprevir have not been studied in HCV-infected patients with severe renal impairment or end-stage renal disease, including patients requiring dialysis.***(Moreno C et al., 2013).***

As simeprevir is highly bound to plasma proteins, it is unlikely that it will be significantly removed by dialysis

Hepatic impairment

Simeprevir is primarily metabolized by the liver.

Compared to healthy participants with normal hepatic function, the mean steady-state AUC of simeprevir was 2.4-fold higher in participants with moderate hepatic impairment (Child-Pugh Class B) and 5.2-fold higher in participants with severe hepatic impairment (Child-Pugh Class C). In clinical trials, higher simeprevir exposures have been associated with a higher incidence of adverse reactions, including rash (any type), pruritus and increased bilirubin. In addition, the safety and efficacy of simeprevir have not been studied in HCV-infected patients with moderate or severe hepatic impairment (Child-Pugh Class B or C).

Major Drug Interactions: Simeprevir is primarily metabolized via CYP3A enzymes and thus administering simeprevir with medications that have moderate or strong induction of CYP3A may significantly reduce levels of simeprevir (examples include rifampin, St. John's Wort, and most anticonvulsants Among patients on a triple therapy, simeprevir-containing regimen who failed to achieve a sustained virologic response at 12 weeks post-treatment (SVR12), more than 90% developed emergent NS3/4 protease mutations.

Clinical trials in genotype 4 chronic HCV infection:
RESTORE:

The RESTORE trial is a open-label phase 3 trial that addresses the efficacy of simeprevir (150 mg once daily) with peginterferon and ribavirin for treatment-naive and treatment experienced patients with genotype 4 chronic HCV infection. All patients received 12 weeks of simeprevir and all received 24 or 48 weeks of peginterferon plus ribavirin ***(Moreno C et al., 2013).*** Patients who were treatment-naive or who failed prior therapy with relapse were eligible to receive the shorter (24 week course of peginterferon and ribavirin) if they met response guided therapy criteria. The interim results for this trial is available for 61 of the 106 enrolled patients. Overall, 85% of these patients with genotype 4 infected achieved an SRV12. The SVR12 rates were excellent with treatment naive (88%) and experienced with prior relapse (91%). Very small numbers of patients with prior partial response (n = 3) or prior null response (n = 5) had completed the study***(Moreno C et al., 2013).***.

The proportion of patients who discontinued all treatment due to an adverse event was 3%. The proportion of patients who discontinued simeprevir due to an adverse event was 1%.

Clinical Implications

The new 3-DAA adds a second interferon-free, 12-week, all oral regimen for the treatment of HCV genotype 1. The SVR12s are very similar to Gilead's sofosbuvir/ledipasvir (Harvoni) which was approved in October 2014. The current The American Association for the Study of Liver Diseases (AASLD) and the Infectious Diseases Society of America (IDSA) recommendations lists 3-DAA as alternative to sofosbuvir/ledipasvir for genotypes 1a and 1b (treatment-naïve and peginterferon/ribavirin failures), and genotype 4. This sets up a potential competition between AbbVie and Gilead for this lucrative market. Currently the wholesale cost for 3-DAA is $27,773 for 28 days compared to $31,500 or sofosbuvir/ledipasvir. However with the exception of genotype 1b without cirrhosis, ribavirin is required for 3-DAA at an additional cost of approximately $200 for 28 days. In addition, genotype 1a with cirrhosis requires 24 weeks of treatment compared to 12 weeks for sofosbuvir/ledipasvir.

Sofosbuvir

Sofosbuvir, a NS5B polymerase inhibitor in the treatment of hepatitis C: a review of its clinical potential.

Sofosbuvir (GS-7977) is a HCV NS5B nucleotide polymerase inhibitor that has now been evaluated extensively in phase II and III interferon-free clinical trials. Sofosbuvir has a pan-genotypic effect on HCV, although viral genotype-specific differences in sustained virological response (SVR) have emerged in phase III clinical trials. Sofosbuvir has been studied both as dual therapy with ribavirin and also as triple therapy with either NS5A inhibitors or a protease inhibitor. High rates of SVR have been reported with these interferon-free combinations, particularly with genotypes 1 and 2, and the safety profile has been very favourable in both cirrhotic and noncirrhotic patients, without issues of viral resistance. Interferon-free, once-daily treatment of HCV is now becoming a reality.

Sofosbuvir, also known as GS-7977 (and previously known as PSI-7977), is a nucleotide inhibitor of NS5B and this review will consider its clinical potential as a promising drug for the treatment of HCV infection.

Pharmacology of sofosubuvir:

Sofosbuvir is a prodrug of 2'-deoxy-2'-fluoro-2'-C-methyluridine monophosphate that is converted within hepatocytes to its active uridine triphosphate form, causing chain termination during replication of the viral genome **(Murakami et al. 2010).** *In vitro*, the active triphosphate inhibits recombinant NS5B polymerases from HCV genotypes 1-4 with similar half maximum inhibitory concentration values for each genotype, indicating broad activity across HCV genotypes **(Lam et al., 2012).**

The chemistry of sofosbuvir:

2-((5-(2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-4-fluoro-3-hydroxy-4- methyltetrahydrofuran-2-ylmethoxy)phenoxyphosphorylamino) propionic acid isopropyl ester It has a molecular formula of C22H29FN3O9P and a molecular weight of 529.45. It has the following structural formula: **(Herbst and Reddy, 2013)**

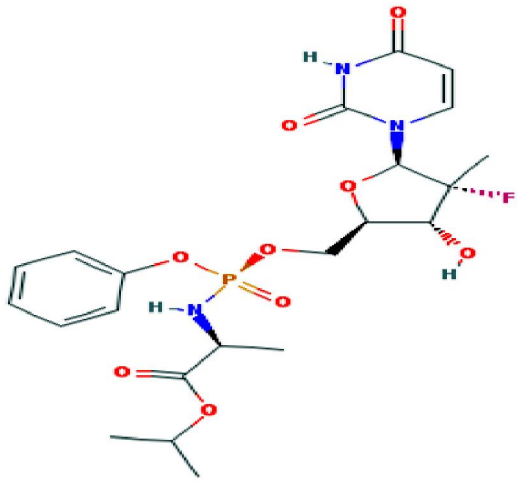

Fig. (13): Sofosbuvir chemical structure.

Sofosbuvir is primarily eliminated from the body via the kidney as GS-331007 (formerly called PSI-6206), an inactive nucleoside metabolite. Single-dose pharmacokinetics of sofosbuvir were studied in

subjects with normal renal function (estimated glomerular filtration rate (eGFR) > 80 ml/min), mild (eGFR 50–80 ml/min), moderate (eGFR 30–49 ml/min) and severe (eGFR < 30 ml/min) renal impairment. The area under the curve (AUC) of GS-331007 and, to a lesser extent, sofosbuvir increased with decreased renal status. There was a linear relationship between GS-331007 renal clearance and creatinine clearance.

In a study of hepatic impairment, HCV-infected subjects with moderate hepatic impairment were administered sofosbuvir 400 mg QD for 7 days; sofosbuvir was generally well tolerated and resulted in similar systemic exposure to GS-331007 as noncirrhotic subjects. Significant declines in HCV RNA were observed in all subjects over 7 days of dosing **(Lawitz et al. 2012).** Therefore, dose modifications are not required in hepatic impairment.

There is no clinically significant interaction of sofosbuvir with food, or with co administration of methadone, cyclosporine or tacrolimus **(Denning et al. 2011; Mathias et al. 2012)**

Clinical trial data

Proton

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 peginterferon and ribavirin as 12-week triple therapy, followed by additional peginterferon and ribavirin in the genotype 1 patients, with SVR12 results greater than 90% in all sofosbuvir-containing arms of the study **(Lawitz et al. 2013c)**.

Phase II studies of sofosbuvir:

Atomic

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 peginterferon 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 **(Kowdley et al. 2013).** A total of 11 patients with genotype 4 and 5 patients with genotype 6 were also included in cohort B of the study. 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 **(Kowdley et al. 2013).** The findings also suggested that there was no additional benefit from extending sofosbuvir triple therapy beyond 12 weeks.

Electron

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. In the initial cohort, 40 treatment-naïve patients with genotype 2 or 3 HCV were randomized to four groups, all containing sofosbuvir 400 mg/day and weight-based ribavirin. Three of the groups also received peginterferon for 4, 8 or 12 weeks. All 40 patients had a SVR at week 24, irrespective of whether or not they received peginterferon **(Gane et al. 2013)**. Two additional cohorts of patients with genotype 2 or 3 infection received either sofosbuvir monotherapy for 12 weeks, resulting in 60% SVR24, or sofosbuvir-based triple therapy for 8 weeks, with 100% SVR24.

Later cohorts of ELECTRON explored sofosbuvir/ribavirin in treatment-experienced patients with genotypes 2 and 3 and also cohorts with shorter durations of treatment and lower doses of ribavirin **(Clinical Trials.gov identifier: NCT01260350)**, although these results have only been partially published in abstract form **(Gane et al. 2012),** and weight-based ribavirin dosing has been used for ongoing clinical development with sofosbuvir-dual therapy. Patients with genotype 1 HCV were also treated with triple therapy combinations of sofosbuvir/ribavirin and additional DAA drugs including the NS5A inhibitor ledipasvir (LDV; GS-5885) in a once-daily fixed dose combination, or the HCV NS5B nonnucleoside inhibitor GS9669. With both of these triple therapy combinations, excellent SVR rates have been reported in both treatment-naïve patients and also null responders who are traditionally resistant to other forms of DAA triple therapy with first-generation protease inhibitors **(Gane et al. 2013a; Gane et al. 2013b)**.

Lonestar

The LONESTAR study continued to evaluate different lengths of therapy (8 or 12 weeks) with the fixed-dose combination of sofosbuvir and ledipasvir with or without ribavirin in patients with genotype 1 HCV. Cohorts included both treatment-naïve GT1 patients, and also 40 patients who had previously failed therapy with an HCV-specific protease inhibitor-based regimen. Half of these patients had compensated cirrhosis. SVR4 rates of at least 95% in all cohorts have been reported in a recent Gilead press release **(Gilead Sciences, 2013),** and phase III trials are planned with the fixed-dose combination of sofosbuvir and ledipasvir.

Sofosbuvir in genotype 4:

Ruaneet al. 2014:

Drug regimen: Sofosbuvir (400 mg) +weight-based RBV (1000–1200mg) for 12 or 24 weeks in

populations Born in Egypt, with both maternal and paternal Egyptian ancestry

Naeive: n=28

Experienced n=32

Results:

Treatment-naeive:

12weeks: 79%

24weeks: 100%

Treatment-experienced:

12weeks: 59%

24 weeks: 87%

Esmat et al.,(2014) Sofosbuvir (400 mg) +weight-based RBV (1000–1200mg)

12or 24 N=103

Naeive: 48%

Cirrhosis: 17%

Treatment-naeive:

12weeks: 84%

24weeks: 92%

Treatment-experienced:

12weeks: 70%

24weeks: 89%

Cirrhotics:

24weeks: 63%

24weeks: 78%

Non-cirrhotics:

24weeks: 80%

24 weeks: 93%

Everson et al.,(2014) Sofosbuvir 400 mg +GS-5816 (25 or 100 mg)12Naeive, No cirrhosis.

n=7:7

87 %for GS-5816 25 mg

for GS-5816 100 mg 100%

Daclatasvir and sofosbuvir

Two clinical trials have evaluated sofosbuvir in combination with the NS5A inhibitor daclatasvir. The first trial reported was of treatment-naïve patients with HCV genotypes 1, 2 and 3 who were randomized to daclatasvir plus sofosbuvir with or without ribavirin for a total treatment duration of 24 weeks although some arms had a 1-week sofosbuvir lead-in phase before the daclatasvir was added. SVR12 was achieved in 100% of genotype 1 patients and 86–100% of patients in the genotype 2/3 cohort **(Sulkowski et al., 2012).**

The combination of daclatasvir and sofosbuvir has also been evaluated in 41 HCV genotype 1 patients who had previously failed protease-inhibitor-based triple therapy with either telaprevir or boceprevir and were treated with 24 weeks of sofosbuvir plus daclatasvir, with or without ribavirin **(Sulkowski et al., 2013).**Impressively, SVR12 rates of 95–100% were obtained, whether or not the combination included ribavirin, demonstrating that this combination of sofosbuvir with an NS5A inhibitor is an effective therapy even in people with NS3A protease inhibitor resistance.

Cosmos

The COSMOS study is evaluating a once-daily regimen of the NS3/4A protease inhibitor simeprevir (TMC435) plus sofosbuvir with or without ribavirin for 12 or 24 weeks in HCV genotype 1 patients. Cohort 1 consists of patients with prior null response to peginterferon with mild to moderate fibrosis, and preliminary results from the 12-week cohort have been recently presented showing SVR4 rates of 96% and 93% for patients with and without ribavirin, respectively **(Lawitz et al., 2013b).**Cohort 2 includes both peginterferon null responders and treatment-naïve patients with advanced fibrosis and a recent press release reports SVR4 results of 96–100% in the 12-week group **(Medivir, 2013).**

Phase III trials of sofosbuvir

Four phase III trials of sofosbuvir have been published to date, all evaluating sofosbuvir 400 mg plus ribavirin (weight-based dosing) for at least 12 weeks in patients with chronic HCV

In the **FISSION** study, 499 treatment-naïve patients with genotype 2 or 3 HCV were randomized to sofosbuvir 400 mg plus ribavirin for 12 weeks or peginterferon plus ribavirin for 24 weeks in a noninferiority trial. In this trial, despite a marked difference in the RVR rates (99% *versus* 67%, respectively), the SVR12 rates were identical at 67% **(Lawitz et al., 2013d)**. Notably, in this study there was a marked difference in response rates between patients with genotype 2 (97% SVR 12) and genotype 3 (56% SVR12) HCV, and between cirrhotic (47% SVR12) and noncirrhotic (72% SVR12) patients.

The **NEUTRINO** study was a 12-week open label study of sofosbuvir plus ribavirin in treatment naive patients with HCV genotype 1, 4, 5 or 6 (of whom 98% had genotype 1 or 4). SVR12 rates of 90% were observed overall, with 81% response rates in genotype 1 with cirrhosis. These cirrhotic patients also had extremely low rates of treatment discontinuation of only 2%, suggesting that this combination is safe and extremely well tolerated even in cirrhotic patients **(Lawitz et al., 2013d).**

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 **(Fontana et al., 2013).** This is promising, and results of future trials of sofosbuvir in these types of patient groups are awaited with interest.

Adverse events

Treatment discontinuations because of adverse events have been uncommon in these sofosbuvir-based interferon-free treatment regimens. In phase III trials, treatment discontinuation rates of 1–2% were seen in the sofosbuvir plus ribavirin cohorts, as compared with 11% among patients receiving peginterferon–ribavirin for 24 weeks. Adverse events associated with ribavirin therapy (fatigue, insomnia and anaemia) were commonly reported, and headache was also frequently reported. Haematologic abnormalities were more common among patients who received interferon than among patients who did not in FISSION and ELECTRON **(Gane et al. 2013c; Lawitz et al., 2013d).** Neutropenia and thrombocytopenia were not generally seen in groups who did not receive interferon. 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. Achieving SVR on sofosbuvir plus ribavirin was also associated with improvement in health-related quality of life **(Younossi et al., 2013).**

Viral resistance

Viral mutations in the HCV NS3 and NS5B regions have been associated with resistance to protease and nonnucleoside inhibitors, and can be present even in previously untreated patients with HCV infection. The S282T mutation has been identified as the common mutation selected in replicon studies with sofosbuvir, and this change can confer resistance to sofosbuvir **(Lam et al., 2012)**; however, this mutation is not generally present in untreated patients with HCV, and has very poor replicative fitness **(Kuntzen et al., 2008).**In the initial large phase II and III studies involving sofosbuvir the S282T mutation was not detected on deep-sequencing assays in any patient receiving sofosbuvir and is in marked contrast with the rapid emergence of viral resistance that has been observed with other classes of DAA in patients who had breakthrough during treatment or relapse after completion of therapy **(Gane et al., 2013c; Jacobson et al., 2013; Lawitz et al., 2013d)**.

Conclusions and future directions

Although sofosbuvir is by no means one of the first DAA drugs to reach phase III clinical trials, the collective trial data for sofosbuvir do represent a significant paradigm shift in the management of HCV infection. With sofosbuvir-based regimens, successful interferon-free treatment of HCV is now achievable across multiple genotypes **(Jacobson et al., 2013; Lawitz et al., 2013d).**

Sovaprevir

Sovaprevir (ACH-1625) is an experimental drug designed to treat the hepatitis C virus. It is under development by Achillion Pharmaceuticals.

Sovaprevir received Fast Track status from the U.S. Food and Drug Administration (FDA) in 2012.

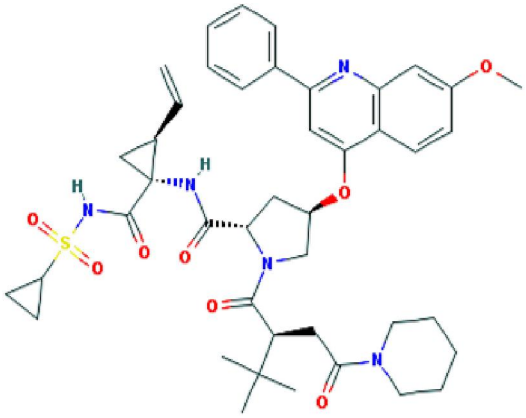

Fig. (18): Sovaprevir chemical structure

Sovaprevir (formerly ACH-1625) is a potent, linear, non-covalent inhibitor of HCV NS3 protease that is in phase 2 studies in combination with ACH-3102, an HCV NS5A inhibitor. In vitro, sovaprevir binds to NS3 protease slowly and tightly with a steady state Ki of ≤ 0.2 nM against GT-1a and GT- 1b enzymes. In phase 1b studies, administration of sovaprevir in GT-1 HCV patients at doses of 200 - 600 mg BID or 400 – 600 mg QD for 5 days resulted in 3.40 - 4.25 log10 IU/mL mean maximal reductions in plasma HCV RNA (Table 1). In phase 2a studies, co-administration of sovaprevir (200 mg, 400 mg or 800 mg, QD), Peg-IFN and ribavirin (RBV) to treatment-naïve GT-1 HCV patients for 12 weeks resulted in reductions of HCV RNA to below LLOQ (TND, target not detected or TD, target detected) from Week 8-12 in 100% of patients; no viral breakthrough was seen in any dose groups over this same period Sovaprevir is administered once daily without "boosting". Boosting is not required as sovaprevir distributes rapidly and selectively to the liver (site of infection) ***(Fabrycki J et al., 2015)*** Sovaprevir has shown low potential for drug-drug interactions. In vitro studies have demonstrated that sovaprevir does not inhibit or induce CYP enzymes. No clinically significant change was observed in the PK of either sovaprevir or ACH-3102 when co-administered in either the fed or the fasted state ***(Fabrycki J et al., 2015).***

Traditional treatment of HCV

The combination of pegylated interferon alpha and ribavirin has improved treatment success rates in patients with hepatitis C with sustained response rates of just over 50% overall and more than 70% for those with genotypes 2 and 3. Acute infection with hepatitis C is followed by chronic infection in 50-80% of those infected. ***(Somsouk M et al., 2003)*** Individuals with chronic hepatitis C without treatment rarely spontaneously clear virus unless there is a change in their immune status. ***(Somsouk M et al., 2003)***

The current standard of care for hepatitis C is pegylated-interferon (PEG-IFN) alpha (given once weekly) in combination with oral ribavirin (given daily). ***(Kaplan M 3t al., 2005)***

Any evidence of hepatic decompensation precludes treatment with IFN alpha combined with ribavirin (unless in rare circumstances just prior to a liver transplant) ***(Forns X et al 2003)***.

Ribavirin therapy is complicated by varying degrees of haemolysis in at least one-third of individuals undergoing combination therapy, ***(Fried MW et al., 2002)*** which may induce a sudden fall in haemoglobin. As it is a bone marrow suppressant IFN monotherapy is also associated with a more gradual fall in haemoglobin. Thus, individuals who are anaemic at baseline need correction of their anaemia prior to the initiation of therapy. The risk incurred by a sudden fall in haemoglobin upon introduction of ribavirin means that individuals with ischaemic vascular disease (cardiac in particular) are unwise to undergo therapy for their chronic hepatitis C, if it involves the use of ribavirin.

Ribavirin is excreted via the kidney, and so any renal impairment will promote high blood levels (particularly within erythrocytes) and as the usual half life of ribavirin is 120 days, the presence of renal failure promotes severe ongoing haemolysis, and thus its use is contraindicated in renal failure. Very low dose ribavirin has been utilized in end stage renal disease in a few studies. ***(Bruchfeld A et al., 2003)***. PEG-IFN, because its molecular size is much larger than that of standard IFN, has less renal excretion and may be better tolerated in patients with end stage renal failure. Pharmacokinetic studies conducted in patients with varying degrees of impairment indicate that when the creatinine clearance is <30 mL/min blood levels of IFN when given as PEG IFN alfa 2b become markedly elevated, ***(Gupta SK et al., 2002)***. Not so with PEG IFN alpha 2a (the larger molecular weight form of PEG IFN alpha).

Ribavirin is teratogenic whether the ribavirin is taken by the male or the female in a partnership and thus it is essential that all treated patients and their sexual partners perform safer sex, not only during the entire course of therapy, but for at least 6 months after the cessation of therapy because of the prolonged half life of ribavirin.

Interferon is a bone marrow depressant, and it causes a dose related neutropenia, thrombocytopenia, and anaemia. ***(Bruchfeld A et al., 2003)*** Adjunctive therapy to support blood components and high dose IFN therapy, is efficacious in individuals with either a low absolute neutrophil count and/or haemoglobin at baseline. ***(Dietrich DT et al., 2003)***.

Interferon, a cytokine, causes flu like symptoms, malaise and mood changes, and frank depression ***(Horikawa N et al., 2003)***. Whereas epilepsy may be exacerbated by the use of IFN therapy, those individuals whose seizure disorder is well controlled with drug therapy, can safely be treated for their hepatitis C if this is felt necessary.

Recent data indicates that both the degree of fat within hepatocytes and overall body weight, better still, body mass index appear to influence treatment response ***(Bressler B et al., 2003)***.

It remains unclear whether all PEG-IFNs should be weight based. Weight based dosing is recommended for the 12 kDa PEG-IFN alpha 2b, but because of the much smaller volume of distribution of the larger molecule (limited to the vascular space), the 40 kDa PEG-IFN alpha 2a, this agent should theoretically not have to be weight based ***(Lamb MW et al., 2002)***.

Real-life data of PEG-RBV therapy: 40–55%

Sustained virological response:

In the PROPHESYS worldwide real-life cohort of 7163 treatment-naaive patients with HCV, who were treated with PEG-IFN/RBV, only 41% of HCV-4 infected patients achieved a sustained virological response (SVR).

The Egyptian national programme for the treatment of hepatitis C treated approximately 350000 patients between 2007 and 2014 ***(Doss W et al., 2014).*** Although the programme excluded difficult to cure patients (those with F4 fibrosis, previous treatment failures, high BMI and age over 60), their published real-life data showed an SVR rate of 45–55% ***(Esmat Get al., 2014).***

Treatment of hcv – 4 infected persons whom perior therapy had failed

Data are limited to help guide decision making for patients infected with HCV genotype 4. Nonetheless, for patients in whom treatment is required, the following recommendations can be made.

Four options with similar efficacy in general are recommended for patients with HCV genotype 4 infection, in whom prior PEG-IFN and RBV treatment has failed (listed in alphabetic order; see text).

Daily fixed-dose combination of ledipasvir (90 mg) /sofosbuvir (400 mg) for 12 weeks is an acceptable

regimen for patients with HCV genotype 4 infection, in whom prior PEG-IFN and RBV treatment has failed.
Rating: Class IIa, Level B
Daily fixed-dose combination of paritaprevir (150 mg)/ritonavir (100 mg) /ombitasvir (25 mg) and weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg]) for 12 weeks is recommended for patients with HCV genotype 4 infection, in whom prior PEG-IFN and RBV treatment has failed.
Rating: Class IIa, Level B
Daily sofosbuvir (400 mg) for 12 weeks and daily weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg]) plus weekly PEG-IFN for 12 weeks is recommended for retreatment of IFN-eligible patients with HCV genotype 4 infection, in whom prior PEG-IFN and RBV treatment has failed.
Rating: Class IIa, Level B
Daily sofosbuvir (400 mg) and weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg]) for 24 weeks is recommended for retreatment of patients with HCV genotype 4 infection, in whom prior PEG-IFN and RBV treatment has failed.
Rating: Class IIa, Level B

PEARL-I was an open-label phase IIb study that included a cohort of 49 treatment-experienced patients without cirrhosis with HCV genotype 4 infection who received 12 weeks of paritaprevir/ritonavir/ombitasvir with or without weight-based RBV (1000 mg [<75 kg] to 1200 mg [≥75 kg]). In intention-to-treat analysis, SVR12 was achieved in 100% (41/41) of patients. This regimen was well tolerated with no serious adverse events reported. ***(Pol, 2014)***

Sofosbuvir-based regimens have also shown efficacy in patients infected with HCV genotype 4. Sofosbuvir administered with PEG-IFN and RBV for 12 weeks was investigated in the phase III NEUTRINO trial. ***(Lawitz, 2013a)*** Of the 28 treatment-naive patients with HCV genotype 4 infection, 27 (96%) achieved SVR12. In a pilot study of treatment-experienced patients of Egyptian ancestry with HCV genotype 4 infection, patients were randomized to receive sofosbuvir and RBV for 12 weeks or 24 weeks. SVR12 rate was numerically higher in the 24-week arm (89% [24/27] in the 24-week arm vs 70% [19/27] in the 12-week arm), supporting the recommendation for longer treatment duration with a sofosbuvir and RBV regimen. ***(Esmat, 2014)*** In the SYNERGY trial, 20 patients with HCV genotype 4 were treated with ledipasvir/sofosbuvir for 12 weeks. Of these patients, 40% were treatment experienced and 40% had advanced fibrosis. Preliminary data support efficacy, with 95% achieving SVR12 in an intention-to-treat analysis. ***(Kapoor, 2014).***

The following regimens are NOT recommended for patients with HCV genotype 4 infection in whom prior PEG-IFN and RBV treatment has failed.
- ***PEG-IFN and RBV with or without telaprevir or boceprevir***

Rating: Class IIb, Level A
- ***Monotherapy with PEG-IFN, RBV, or a direct-acting antiviral***

Rating: Class III, Level A

PEG-IFN and RBV for 48 weeks was previously recommended for patients with HCV genotype 4 infection. ***(AASLD/IDSA/IAS-USA, 2014)*** Adding sofosbuvir (400 mg daily) to PEG-IFN and RBV increases response rates and markedly shortens therapy with no apparent additional adverse effects. The addition of simeprevir to PEG-IFN and RBV increases response rates but has inferior SVR rates to the other available regimens and requires a longer duration of PEG-IFN and RBV, which increases the risk of adverse events and thus is no longer recommended. ***(Moreno, 2013b)***

Because of their limited activity against HCV genotype 4 in vitro and in vivo, boceprevir and telaprevir should not be used as therapy for patients with HCV genotype 4 infection.

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