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„Therapeutical Aspects of Hepatitis C infections“

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Aim of work

- To review the literatures to study the efficacy of anti HCV drugs in the treatment of chronic hepatitis C infiction.
- To evaluate the sftety and efficacy of new anti HCV drugs and their combination therapies for the treatment of HCV infection.

Abstract

Viral hepatitis is currently one of the worldwide health problems which infected aproximately more than 500 million people particularly hepatitis B virus and hepatitis C virus which are at high risk of developing chronic liver disease, cirrhosis and hepatocellular carcinoma. Notwithstanding many common features in the pathogenesis of hepatitis B virus and hepatitis C virus related liver disease; these viruses differ in their virological properties, immune escape and survival strategies.

Hepatitis A is an acute viral hepatitis caused by an enterovirus hepatitis A (HAV) that is transmitted by ingestion of infected food and water. From 7 genotype of HAV, four are able to infect humans with no specific antiviral therapy for treatment.

HBV a viral hepatitis that is either acute or chronic, caused by a DNA- HBV, and transmitted through different ways. The aims of chronic hepatitis B infection treatment are to clear virus DNA and normaly prevent the development of desiease. Currently there are five nucleos(t)ide analogues and two interferon-based therapies medications are available for the treatment of chronic hepatitis B infection.

Hepatitis C is caused by an RNA virus that often persists in the blood serum and can cause chronic liver damage. Investigations showed that about 75%-85% of HCV-infected people will progress to chronic HCV infection, extrahepatic manifestations, compensated and decompensated cirrhosis and hepatocellular carcinoma. Progression of cirrhosis is variable in HCV infected persons and depednds to several factors. An estimated 10%-15% of HCV-infected persons will advance to cirrhosis within the first 20 years.

Treatment of CHC infection aims to clear HCV RNA and prevention or reversal of progression to cirrhosis, liver cancer, liver failure and sustained normalisation of ALT levels. Currently many therapeutic substances are use for the treatment of chronic hepatitis C to be approved as efficacious and safe.

In the last few years, numerous directly acting antiviral agents (DAAs) have been implemented successfully in treatment algorithms of chronic hepatitis C virus (HCV) infection. As

combination therapy with pegylated interferon (PEGIFN) α, ribavirin, and / or other DAAs, potent DAA-based regimens result in HCV eradication in the vast majority of patients with chronic hepatitis C.

Principally four HCV structural and six non-structural proteins of HCV, HCV-specific RNA structures such as the IRES, as well as host factors on which HCV depends, are suitable targets for the direct acting antivials (DAAs).

Direct acting antivirals (DAAs) and also indirect acting antivirals (IDAAs) are in various stages of development and just few of them are clinically available. This thesis assesses recent advances in our understanding of viral hepatitis treatment and outlines areas for future studies.

Abbreviations

AIDS: Acquired Immune Deficiency Syndrome

ALT: Alanine Aminotransferase

AST: Aspartate Aminotransferase

BID: Twice a day

BOC: Boceprevir

cccDNA: Covalently Closed Circular DNA

cEVR: Complete Early Viral Response

CHC: Chronic Hepatitis C

DAA: Direct Acting Anti-viral

DNA: Deoxyribonucleic Acid

E1 and E2: Envelope Glycoproteins 1 & 2

EHM: Extrahepatic Manifestation

ELISA: Enzyme-Linked Immunosorbent Assay

EOT: End of Treatment

EPO: Erythropoietin Epoetin alfa

ER: Endoplasmic Reticulum

eRVR: Extended Rapid Viral Response

ESLD: End-Stage Liver Disease

EVR: Early Virological Response

GT: Genotype

HAV- Hep A: Hepatitis A virus

HBeAg: Hepatitis B virus antigen

HBsAg: hepatitis B surface antigen

HBV- Hep B: Hepatitis B virus

HCC: Hepato Cellular Carcinoma

HCV- Hep C: Hepatitis C virus

HDV- Hep D: Hepatitis D virus

HEV- Hep E: Hepatitis E virus

HIV: Human Immunodeficiency Virus

HLA – DQB: Major histocompatibility complex, class II, DQ beta 1

HLA: Human Leukocyte Antigen

HLA-DRB1: HLA class II histocompatibility antigen, DRB1-9 beta chain

HVL: High Viral Load (≥800,000 IU)

IDAAs: Indirect Acting Antivirals

IFN, Inf: Interferon

IFN-alpha 2a/2b: Interferon (Alpha 2a/2b)

IL-28: Interleukin-28

IMPDH: Inosinmonophosphat-Dehydrogenase

IRES: Internal ribosome entry site

IU: International Unit

LdT: Telbivudine

LFT: Liver Function Test

LVL: Low Viral Load (<800,000 IU)

P/R Tx: Pegelated Interferon / Ribaviron

PCR: Polymerase Chain Reaction

PEG: Poly Ethylene Glycol

Peg-IFN: Pegylated Interferon

Peg-Inf: Pegelated Interferon

PI: Protease Inhibitor

QD: Once a day

QW: Once a week

RBV, Riba/Rbv: Ribavirin

RGT: Response-Guided Therapy

RNA: Ribonucleic Acid

RVR: Rapid Virological Response

SMV: Simeprevir

SOT: Start of Treatment

SPRINT-2: Serine Protease Inhibitor Therapy 2

SVR: Sustained Virological Response

TDF: Tenofovir Disoproxil Fumarate

TID: Three Times a Day

INTRODUCTION

Hepatitis (pl: hepatitides) is the inflammation of the liver that characterized by the presence of inflammatory hepatocytes in the liver. Or hepatitis is Inflammation of the liver, caused by infectious or toxic agents and characterized by jaundice, fever, yellow discoloration of the skin, mucus membranes, conjunctivae, liver enlargement, and abdominal pain, or it may occur with no symptoms. Hepatitis can be acute and chronic. The acute one will last less than six months but chronic normaly persists longer. Hepatits can be a self-limiting condition which healing on its own or can progress to fibrosis and cirrhosis [1,2].

Liver inflammation can be caused by viral infections of the liver, or excessive alcohol intake, toxic chemicals, incorrect diet, fatty liver, adverse reactions to some prescribed drugs or medications, autoimmune diseases and some diseases of the biliary system (the bile ducts). Many viruses are attacking, damaging and causing inflammation of the liver cells, but the most common are known hepatitis A, B, C, D, E, F and G viruses [2].

Now more than 250 million people are globally suffering from hepatitis C and more than 300 million people Hepatitis B carriers. Hepatitis A is caused by consuming contaminated food or water and Hepatitis B is a sexually transmitted disease. Hepatitis C is commonly spread via direct contact with the blood of an infected person. Hepatitis D virus can not infect a person without interference of HBV. Hepatitis E virus is transmitted through contaminated drinking water. Hepatitis G is another type of hepatitis caused by hepatitis G virus and hepatitis that cannot be attributed to one of the viral forms of the disease is called hepatitis X.

Viral hepatitis basically is caused in response to the body immune system which targets the infected liver cells. Hepatitis can be severe which can happen by HAV and HBV, or it can be a long period desiease which can happen with HBV and HCV.

Currently several classes of drugs are used for the treatment of viral hepatitis, among them a growing number of agents are becoming available to manage and potentially cure hepatitis C.

In case HCV has a high genetic variability than variants with lower susceptibility to DAAs can occur naturally prior to even starting therapy, thus giving DAAs a low barrier for resistance when given as monotherapy. Regardless of the reason current guidelines recommend the use of DAAs in combination with p-IFN and RBV that helps to prevent resistance and virologic breakthrough.

FDA approved in May 2011 telaprevir and boceprevir which are a highly potent first-generation of NS3/4A protease inhibitors, for treatment-naïve and treatment-experienced HCV-1patients. Currently several NS5A inhibitors, including daclatasvir which has demonstrated potent inhibition of NS5A and HCV replication, are undergoing clinical trials. Recent studies showed that the second-generation of NS5A inhibitors are more potent HCV-1 and HCV-2 inhibitors with higher barriers for resistance and better safety profiles than first generation. NS5B polymerase inhibitors like sofosbuvir and BI 207127, filibuvir and VX-2220 have a broader genotypic coverage with a higher genetic barrier to resistance. In addition to DAAs, cyclophilin (Cyp) inhibitors are playing important role for the treatment of HCV infection. Cyp-inhibitors inhibits HCV polyprotein processing and prevents viral replication also interferes with NS5B polymerase activity and neutralizes HCV activity. Oral based Cyp-inhibitors including alisporivir, NIM-811 and SCY-635, target the host protein and provide support when treatment resistance or virologic breakthrough occurs.

In this monograph it is focused on the treatment and medications viral hepatitis C. But first, some basics information regarding viral hepatitis is useful.

FIRST PART

GENERAL BACKGROUND OF HEPATITIS VIRUSES

What is a virus?

Viruses are tiny organisms that have a central core of genes surrounded by a coat made from protein with at least 50%, and in some cases up to 90%, of their mass being protein and/or fat. Viruses range in size from around 15 nm to 250 nm. Thus viruses are very tiny and are much smaller than our own body's cells. The genetic material of the hepatitis viruses can be either ribonucleic acid (RNA) or deoxyribonucleic acid (DNA).Viruses cannot support themselves and to survive they must infect a human or animal cell where the virus lives off the cell's resources. Thus the virus becomes a parasite and damages the cell. Understandably the important functions that the cell was originally designed for (such as making energy, proteins or hormones etc) become second place to replicating the virus. No wonder chronic viral infections make us extremely tired, especially if the amount of virus in our body (the viral load) is high. The virus hides itself in the genetic material situated in the nucleus of your body's cells and takes over control of these cells. The virus then directs your body's cells to make many more copies of it, so that the cells become factories for making more viruses. In essence, your cells are taken hostage and turned into virus factories [2,3,4].

Viruses love to replicate and change their genes (mutation), and by continually changing their identity, they escape detection and destruction by the body's immune system. Thus viruses are hard to destroy by any means. Because viruses live deep inside the center of the cells, any drugs used to kill the virus will also often damage our cells as well. Viruses cause a range of different diseases and are classified from different ways like on the basis of disease, host organism, virus particle morphology, viral nucleic acids and on the basis of taxonomy. A virus that has genetic material made of RNA is known as an RNA virus or retrovirus, like Hepatitis C Virus (HCV), the influenza virus, the SARS virus and the Human Immunodeficiency Virus (HIV), or it could has a genetic material made of DNA is known as a DNA virus, like the Hepatitis B Virus (HBV) [2,3,4].

FIRST CHAPTER

TYPES OF HEPATITIS VIRUSES

Many viruses like the virus of mononucleosis and the cytomegalovirus but mostly A, B, C, D, E, F (not confirmed), and G viruses can inflame the liver. They are responsible for about half of all human hepatitis. The most common hepatitis viruses are types A, B, and C. Hepatitis viruses replicate (multiply) primarily in the liver cells. This can cause the liver to be unable to perform its functions. For better understanding regarding viral hepatitis treatment, it would useful to know basis of each hepatitis virus.

HEPATITIS A

Hepatitis A is an acute viral hepatitis caused by an enterovirus hepatitis A (HAV) that is transmitted by ingestion of infected food and water and that has a shorter incubation period and causes milder symptoms than hepatitis B. HAV is a single-stranded 27 nm non-enveloped, icosahedral RNA virus which belongs to the hepadnavirus genus of the Picornaviridae. HAV uses host cell exosome membranes as an envelope which leads to protection from antibody mediated neutralization [3,5].

Four of seven different HAV genotypes are able to infect humans. The positive sense single stranded HAV RNA has a length of 7.5 kb. It consist a 5’ non-coding region of 740 nucleotides and a coding region of 2225 nucleotides and a 3’ non-coding region of approximately 60 nucleotides [3,6].

Acute hepatitis A is associated with a limited type I interferon response, which may be explained by cleavage of essential adaptor proteins by an HAV protease-polymerase precursor [3,7]. A dominant role of CD4+ T cells to terminate HAV infection has been established in HAV infected chimpanzees [3,8]. However, in humans strong HAV- specific CD8 T cells have also been described, potentially contributing to resolution of infection [3,9]. A failure to maintain these HAV-specific T cell responses could increase the risk for relapsing hepatitis A.

HAV is usually transmitted fecal -orally either by person - to -person contact or ingestion of contaminated food or water. The incubation time ranges between 15 and 49 days with a mean of approximately 30 days [3,10]. Initial symptoms of HAV infection are usually weakness, nausea, vomiting, anorexia, fever, abdominal discomfort and right upper quadrant pain [3,11]. As the disease progresses, some patients develop jaundice, darkened urine, uncoloured stool and pruritus. The prodromal symptoms usually diminish when jaundice appears [3].

TREATMENT AND PROGNOSIS: There is no specific antiviral therapy for treatment of hepatitis A. Recently studies and researches are going on the use of post-exposure HAV vaccination or prophylaxis with immunglobulins in patients with household contact with HAV. HAV has usually mild to moderate course of disease with no requires of hospitalization but just symptomatic therapy in case of fulminant cases. Prolonged or biphasic courses should be monitored closely because HAV may persist in the liver even for some time when HAV RNA becomes negative in blood and stool. This condition is especially necessary to be kept in mind for immunocompromised individuals [3,12].

HEPATITIS B

The Hepatitis B Virus consists of an outer fatty envelope and a core made of protein. The protein core encloses the genetic material (double stranded DNA) of the virus. The HBV is one of the smallest enveloped animal viruses with a diameter of 42 nm [2].

A viral hepatitis that is either acute or chronic, caused by a DNA hepatitis B virus (HBV), and usually transmitted by infected blood products, contaminated needles, sexual, percutaneous, perinatal (Transmission from an HBeAg-positive mother to her infant may occur in utero, at the time of birth or after birth), horizontal, nosocomial (Transmission from patient to patient or to health care worker and vice versa), and organ transplantation. An estimated 350-400 million people are surface HBV antigen. Thus, HBV infection is one of the most important infectious diseases worldwide which can cause chronic liver damage and cancer [3,13,14].

ACUTE HEPATITIS B: HBV has an incubation period from one to four months. A prodromal phase may be with serum sickness-like syndrome (fever, skin rash, arthralgia and arthritis) will appear before acute hepatitis develops. The most prominent clinical symptoms

of hepatitis are right upper quadrant discomfort, nausea, jaundice and other unspecific constitutional symptoms. The symptoms including jaundice generally disappear after one to three months. In the acute phase the concentration of alanine and aspartate aminotransferase levels (ALT and AST) may raise to 1000-2000 IU/L. Typically the ALT concentration is higher than AST. Bilirubin concentration may be normal in a substantial portion of patients. The rate of progression from acute to chronic hepatitis B is primarily determined by the age at infection. The chronicity rate is 5% or less in adult-acquired infection but it is higher if acquired at younger ages [3,15].

HBV DNA may persist lifelong in the form of covalently closed circular DNA (cccDNA) in patients with positive anti-HBs and/or anti-HBc and this latent infection maintains the T cell response that keeps the virus under control. This is an important finding, as immunosuppression can lead to reactivation of the virus, for example in the case of organ transplantation or during chemotherapy. Fulminant hepatitis B is believed to be due to massive immune-mediated lysis of infected hepatocytes [3,16,17,18,19,20].

HEPATITIS B TREATMENT: Antiviral treatment of patients with acute hepatitis B usually is not recommended. Treatment HBV infection with polymerase inhibitors can be considered in certain subsets of patients, eg, patients with a severe or prolonged course of hepatitis B or coinfected with other hepatitis viruses also immunosuppression patients, or patients with fulminant liver failure undergoing liver transplantation [3,21,22].

CHRONIC HEPATITIS B: as it is mentioned above**,** the chronicity rate of HBV is about 5% or less in adult-acquired infection. Chronic infection occurs in 90% of those infected perinatally but is less frequent in those infected in Childhood (e.g. 20 to 50% in children between one and five years of age) [3,4].

Most of the patients have no history of acute hepatitis B or in chronic phase have no clinically symptoms. While some patients have nonspecific symptoms such as fatigue which will develop by progression of liver disease to decompensated cirrhosis. Clinical signs of chronic liver disease such as splenomegaly, spider angioma, caput medusae, palmar erythema, testicular atrophy, gynecomastia, etc may be rise in advanced liver disease. In patients with

decompensated cirrhosis, jaundice, ascites, peripheral edema, and encephalopathy may be present [3].

In most patients laboratory testing shows mild to moderate elevation in serum concentration of AST and ALT, but rarely normal transaminases will occur. During exacerbation the serum ALT concentration may be raised about 50 than the normal state. Alpha-fetoprotein concentrations correlate with disease activity. The consequence of chronic HBV infection usually depends on the severity of liver disease at the time HBV replication. Liver fibrosis is potentially reversible once HBV replication is controlled. There are two distinguishable states in chronic HBV infection: (1) A highly replicative state with active liver disease and elevated serum ALT concentration, also presence of HBV DNA and HBeAg. (2) A low or non-replicative state, where the serum ALT may in normal level, HBeAg disappears, and anti-HBe antibodies appear. The first highly replicative state may switch into the non-replicative state either spontaneously or by antiviral therapy. Conversely, the non-replicative phase may reactivate to the highly replicative phase either spontaneously or with immunosuppression (eg, in HIV infection or chemotherapy) [3].

There are three different states in perinatally acquired chronic HBV infection: (1) Immune tolerance phase with characterization of high levels of HBV replication, presence of HBeAg and high levels of HBV DNA in serum, which usually lasts 10 - 30 years. Spontaneous HBeAg clearance is in a very low rate, only 15% after 20 years of infection, during this phase. (2) Immune clearance phase, during the second to third decade, the immune tolerant phase may convert to immune clearance. In this phase the spontaneous HBeAg clearance rate will increases to about 10 to 20% annually. (3) Non-replicative phase, which usually characterized by the absence of HBV DNA and normalization of serum ALT, like in adult chronic HBV [3].

CHRONIC HEPATITIS B TREATMENT: Currently two classes of antivial drugs are available for the treatment of chronic HBV infection: alpha interferons (standard or pegylated) as well as nucleoside or nucleotide analogs, which act as reverse transcriptase inhibitors of the HBV polymerase. The nucleoside analogs lamivudine, telbivudine, entecavir and the acyclic nucleotide analogs adefovir dipivoxil and tenofovir disoproxil fumarate are now available. Usually early diagnosis of chronic hepatitis B by HBsAg screening plays a crucial role in the

management of HBV infection in high-risk group patients and patients with elevated transaminases [3].

Also hepatitis B vaccine which contains HBsAg adsorbed onto $Al(OH)_3$ adjuvant, is available for prophylaxis. It is prepared from yeast cells using recombinant DNA technology. Fendrix®, for patients with renal insufficiency, is adjuvanted by monophosophoryl lipid A, and adsorbed onto $AlPO_4$.

A combined vaccine containing purified inactivated HAV and purified recombinant HBsAg, separately adsorbed onto $Al(OH)_3$ and $AlPO_4$, is also available where protection against both hepatitis A and hepatitis B infections is required [42].

HEPATITIS C

Hepatitis C is caused by an RNA virus that often persists in the blood serum and can cause chronic liver damage [1]. Hepatitis C is a very common infection and worldwide it is estimated that over 200 million people are infected with the HCV, this gives an incidence of 3.3% of the world's population [2].

The HCV contains genetic material known as RNA and there are 6 different genetic types (genotypes 1 through 6) Genotypes 1a and 1b are associated with more severe liver disease and genotypes 2 to 6 are thought to have a better outlook. The HCV is 10 times more infectious than the Human Immunodeficiency Virus (HIV), which is the virus that causes AIDS. In the 1980s and 1990s, AIDS was the major public health challenge for community based doctors, but since the year 2000, hepatitis C has acquired this dubious honor. There are approximately 4 times as many people infected with the HCV than HIV. The hepatitis C virus is a very tiny RNA virus, which contains genetic material surrounded by a core protein. The HCV invades the human cells and takes over the cell's manufacturing structures to replicate itself. The human cell is thus converted into a factory for replicating hepatitis C viruses, which spread gradually throughout the body to the blood, lymphatic system, liver and the fluid around the brain and spinal cord [2]. HCV is usually a blood transmitted virus infection which will transfer from a infected person through drug injection, blood transfusion, sexual intercourse, religious scarification, having been struck or cut with a bloody object, pierced

ears or body parts and Immunoglobulin injection and also having been in jail more than three days [3].

ACUTE HEPATITIS C: HCV has a variable incubation period after inoculation. The HCV RNA will be detectable in blood or liver by PCR within several days to eight weeks. The level of serum aminotransferases will elevated approximately 6-12 weeks after inoculation (range 1-26 weeks) and they are varies considerably among individuals, but tends to be more than 10 - 30 times higher than the normal state (usually around 800 U/l) [3].

CHRONIC HEPATITIS C: 75-100% of patients remain HCV RNA positive after acute hepatitis C; therefore the risk of chronic HCV infection is high with persistently elevated liver enzymes among these patients. Hepatitis C is regarded to be chronic after persistence of more than six months. There is a very low rate of spontaneous clearance from chronic infection phase. So far it is not clear why infection with HCV results in chronic infection in most cases. May be genetic diversity of the virus and its tendency toward rapid mutation allow HCV to escape immune recognition [3,23,24].

Host factors like HCV-specific CD4 T cell responses, high titers of neutralizing antibodies against HCV structural proteins, IL28B gene polymorphisms and specific HLA-DRB1 and HLA-DQB1 alleles may also be involved in the spontaneous clearance of virus. Infection with HCV during childhood appears to be associated with a lower risk of chronic infection, approximately 50 -60%. Most of the chronic infected patients are asymptomatic or have only mild nonspecific symptoms as long as cirrhosis is not present. The most frequent complaint is fatigue and the less common manifestations are nausea, weakness, myalgia, arthralgia, and weight loss. Hepatitis C is rarely incapacitating [3,25,26,27,28].

HEPATITIS D

Hepatitis delta is the most severe form of viral hepatitis in humans. The HDV is a defective RNA virus which requires the surface antigen of HBV named HBsAg for complete of themselves replication and transmission but so far the fully HBV helper function is exactly not clear [3,29,30]. Therefore, hepatitis D occurs only in HBsAg-positive individuals either as acute coinfection or as superinfection in patients with chronic hepatitis B [3,31,32]. Many

studies have shown that chronic HDV infection leads to more severe liver disease than chronic HBV monoinfection, with an accelerated course of fibrosis progression, possibly a slightly increased risk of hepatocellular carcinoma and early decompensation in the setting of established cirrhosis [3,33,34].

Data on the use of pegylated interferon confirm earlier findings, leading to prolonged virological off-treatment responses in about 25% of patients but long-term HDV RNA relapses may occur [3,35].

TREATMENT OF HEPATITIS D: Nucleoside and nucleotide analogs: Famciclovir, Lamivudine, Adefovir monotherapy for 12 months, Entecavir and Ribavirin alone or in combination with interferon had no significant antiviral activity against HDV also did not lead to increased rates of HDV RNA clearance. ***Tenofovir*** therapy regimen may be considered for interferon intolerant hepatitis D patients in the absence of alternative treatment options. ***Clevudine***, a nucleoside analog no longer in development for the treatment of hepatitis B, was shown to inhibit delta virus viremia in woodchucks [3,36].

HEPATITIS E

Hepatitis E is an inflammatory liver disease caused by non-enveloped, single - stranded RNA hepatitis E virus (HEV), from the family of Hepeviridae and its own genus Hepevirus [3,37]. There are 5 known genotypes HEV. The genome of HEV contains three open reading frames (ORFs). ORF1 encodes the nonstructural proteins, ORF2 encodes the capsid protein, and ORF3 encodes a small multifunctional protein. The ORF2 and ORF3 proteins are translated from a single, bicistronic mRNA. The coding sequences for these two ORFs overlap each other, but neither overlaps with ORF1. Genetic information of ORFs containing codons of various proteins those are necessary for capsid formation, virus replication and infectivity of HEV. Various HEV isolates have been differentiated via phylogenetic analysis based on a hypervariable region within ORF [3,38].

Four out five genotypes of HEV are able to infect humans, whereas genotype 5 “avian HEV” has only been detected in birds, genotype 3 can be found in humans and animals and genotype 4 has been detected in humans and swine. Genotype 3 is a zoonotic and foodborne transmission or viacontact with infected animals. This genotype has been identified in swine, shellfish, deer, oysters, cats, rats and various rodents [3,37]. Foodborne transmission can be avoided by cooking meat at above 70°C, which inactivates the virus [3,39].

TREATMENT OF HEPATITIS E: PEG-IFN-α or Ribavirin can be used for the treatment of chronic hepatitis E between 3 and 12 months. HEV infection treatment also include the reduction of immunosuppression. First for effective treatment of chronic HEV infection it should be evaluated to reduce the immunosuppressive medication as much as possible [3,37,40,41].

HE-Vaccination: A group from China reported data from a very large successful Phase III vaccine trial, the vaccine efficacy after three doses was 100%. This vaccine was approved in China in early 2012 [3]. Among the hepatitis viruses, HCV is a more serious and progressive liver infection virus which cause more than 75% chronic hepatitis infection. Currently the standard therapy of HCV is PEG-IFN-α and ribavirin with a restricted usage due to sever side effects. Also no HCV vaccine is currently available in clinics. Regardless of this reason it has focused on the treatment of HCV in this monograph.

SECOND CHAPTER

Nature of Hepatitis C Virus particle (HCV)

Hepatitis C virus is an enveloped RNA virus was found as the causative agent of the Non-A, Non-B Hepatitis in 1989. HCV could be classified into six genotypes. The human hepatocyte is a major host cell supporting HCV replication. HCV is primarily a blood transmitted infection which exists in host blood as quasispecies and a population of dynamic strains closely related to each other.

THE HCV PARTICLE

HCV is a small, about 50 nm, consists of a core of genetic material (single-stranded positive-sense RNA molecule of approximately 9.6kb), surrounded by an icosahedral protective shell of protein, and further encased in a lipid (fatty) envelope of cellular origin. The translated protein of the HCV genome is a polyprotein with nearly 3000 amino acids long. This protein then cleaved by viral and cellular enzymes into three structural (core, E1, E2) and six non-structural (P7, NS2, NS3, NS4A, NS4B, NS5A and NS5B) proteins.

The p7 is a small hydrophobic protein which consist 63 amino acids. Following its release from the HCV polyprotein, p7 oligomerizes on the Endoplasmic Reticulum (ER) membrane to form calcium ion-conductive pores. Thus deletion of p7 does not appear to affect HCV entry and replication; it significantly abrogates infectious virion assembly and release of HCV. The NS2-3 cysteine protease consists of the C-terminal domain of NS2 in conjunction with the N-terminal region of NS3. It is responsible for the autocatalytic cleavage of the NS2-NS3 junction in HCV polypeptides. NS3 is a multifunctional protein with N-terminal domain of serine protease activity, as well as a C-terminus domain of RNA helicase and Nucleotide Triphosphatase (NTPase) activity. The NS3 N-terminal serine protease domain, which engages the NS4A polypeptide as a cofactor, is responsible for the cleavage of all remaining junctions in the NS portions of the HCV polyprotein. NS4A is required for efficient processing of the HCV polyprotein by functioning as a viral protease cofactor and provides stability to NS3. NS4B is an integral membrane protein which recently has been identified to be responsible for the formation of a novel intracellular membrane structure, termed the

"membranous web", which appears to be the platform upon which HCV replication occurs. Furthermore, NS4B is implicated in modulation of the RNA-dependent-RNA polymerase (RdRp) activity of NS5B. The NS5A phosphoprotein has the ability to modulate the Interferon response of host cells; therefore it generated wide interest in HCV research. NS5A harbors a potential Interferon Sensitivity Determining Region (ISDR) and sequence variations within this cluster may contribute to the resistance to IFN-α therapy which is often observed in patients with HCV. Because of no known enzymatic function of NS5A, it is still believed to be an essential component of HCV replication. Furthermore, NS5A has been identified to be an RNA-binding protein and a major determinant of viral assembly. Being the RdRp for HCV, NS5B is critical for RNA replication in the host cells and is membrane-associated. These non-structural and structural proteins are important targets of specific antiviral agents for the treatment of HCV infection. In addition to the HCV polyprotein, an Alternate Reading Frame protein or Frameshift Protein is also produced in host cells [3,43,44,45,46].

HCV GENOTYPES

The HCV Isolated genotypes are classified into six (or seven) major genotypes and more than 100 subtypes. The genotype 7 is often classified as part of genotype 6, which explains the difference between HCV genotyping methods. The main genotypes of HCV are identified as genotypes 1 through 6 (or 7), with a more than 30% sequence divergence. Each genotype is further divided into subtypes which are differing from one another by 10-30% sequence divergence. These subtypes are further clustered into quasispecies based on their genetic diversity, generated by the low-fidelity level of HCV RdRp and are designated by lowercase letters. Genotypes are clinically important in that they are among the principal determinants of potential response to IFN-based therapy and duration of such therapy. For example, genotype 1 and 4 infected patients are less responsive to IFN-based therapy than other HCV genotypes infected persons. Also duration of IFN-based therapy for genotypes 1 and 4 infections is 48 weeks, while for genotypes 2 or 3 it can be reduced to 24 weeks. Besides PEG-IFN-α and Ribavirin standard HCV therapy, HCV genotype also affects the clinical usage of Direct Acting Antivirals, eg, Boceprevir and Telaprevir the first DAAs, which are novel selective inhibitors of the HCV protease and in combination with the standard HCV therapy nearly double the cure rates of HCV infection [3,43].

HCV LIFECYCLE AND TARGETS FOR DRUG DEVELOPMENT

The HCV life cycle in human body begins after attachment of virion to its specific receptor. Thus the key steps in HCV life cycle include entry into the host cell, uncoating of the viral genome, translation of viral proteins, viral genome replication, and the assembly and release of virions. All these events occur outside the nucleus of the host cell.

CD81 is the main factor that identified as a necessary receptor for HCV entry into the host cell. This receptor expresse on many cell types and cannot explain HCV's liver tropism, HCV entry is strongly reduced in the presence of antibodies to CD81, or when CD81 expression is downregulated in hepatoma cells. The virus genome undergoes an uncoating process into the cytoplasm of host-cell to expose the viral genome to host-cell machinery. The genome then translated in preparation for viral replication.

After translation of the viral proteins which are necessary for establishment of viral-replication machinery, viral RNA replication begins. The HCV RNA genome serves as a template for viral replication and as a viral messenger RNA for viral production. Then the viral assembly and release occurs. Each step of the viral cycle is a target for drug development and HCV successful treatment (Fig 1 & 2) [47].

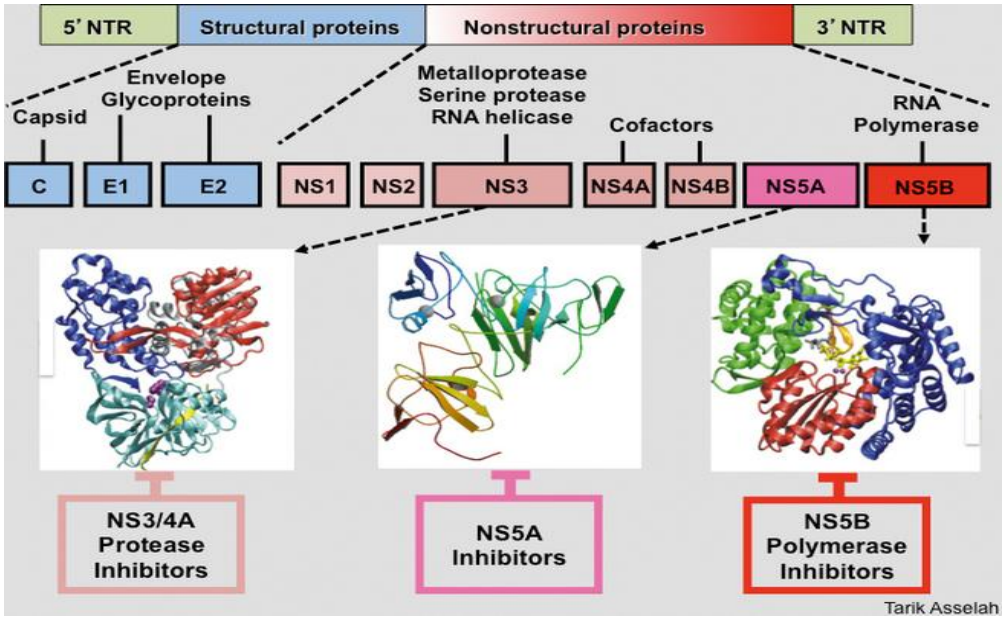


Figure (1): HCV genome and potential drug discovery targets. The HCV RNA translated into a polyprotein which than cleaved by proteases into NS2-3 and NS3-4A proteases, NS3 helicase, NS5A and NS5B RdRp enzymes, that are essential for HCV replication and also drug discovery targets[47].

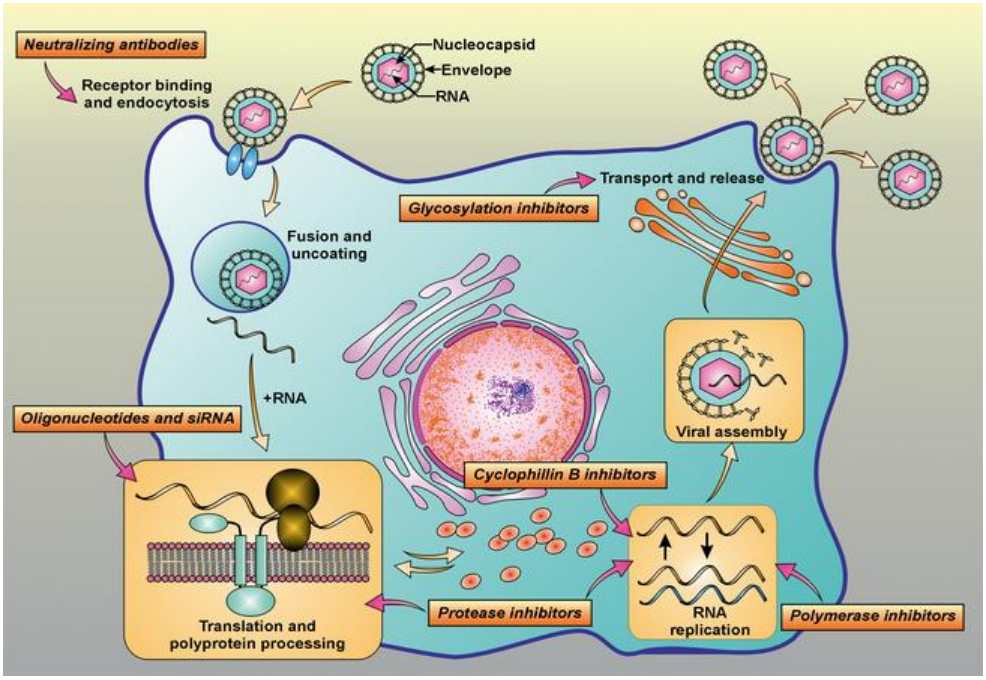


Figure (2): HCV viral cycle. The HCV lifecycle in human body starts after this virus attacht to its specific receptor. As it is shown the viral life cycle include into host cell entry, genome uncoating, proteins translation, replication, assembly and release of virions.

Promoting Factors of Chronic Hepatitis C (CHC) Progression

Different factors, including host factors (race, age, gender, HIV or HBV co-infection, obesity, alcohol, Human Leukocyte Antigen and Interleukin-28 genotyping, etc), viral factors (genotype and viral mutations) and the transmission route of HCV can affect progression of chronic Hepatitis C. Host factors playing important roles in CHC progression. For instance, the clearance of HCV genome is lesser in black HCV infected pateints. Also young patients in compare to older patients have higher rate of disease progression. Inasmuch as the average time from HCV infection to cirrhosis development and hepatocellular carcinoma is more than twice longer in patients with medium age of 29 year than those with medium age of 58 years. As well as women eliminate HCV more rapidly, have a lower rate of disease progression and mortality than men [43].

HCV Persistence in occult

HCV infection recovered patients are clinically having no Hepatitis symptoms and showing negative HCV RNA in serum tests by standard viral assays. But may be HCV genomes at very low levels remain in their serum and cells. Many studies have recently shown that patients continue to harbor very low levels of HCV in their serum. Such persistent replication of residual HCV after spontaneous or therapy induced recovery from Hepatitis C is referred to as occult HCV persistence. The replicative intermediates of HCV were detected in Peripheral Blood Mononuclear Cells, monocyte derived Dendritic Cells, and hepatic tissues after eight years resolution of Hepatitis C. The correlation of occult HCV infection with disease progression has not been documented [43].

SECOND PART

STANDARDIZATION OF THE HCV INFECTION THERAPY

The antiviral therapy goal of hepatitis C infection is to cure it through a sustained elimination of the virus. HCV sustained elimination is achieved when six months after end of the treatment the HCV RNA remains negative (sustained virological response, SVR). Follow-up studies have shown that more than 99% of SVR achieved patients remain 4-5 years after the end of treatment HCV RNA negative and have been no signs of hepatitis documented. Long-term SVR are important for the reduction of HCV-related hepatocellular carcinoma and overall mortality. The FDA in 2011 accepted SVR-12 as endpoint for future trials because HCV relapse usually occurs within the first 12 weeks after the end of treatment. Thus the SVR-12 shows the HCV RNA negativity at 12 weeks after end of treatment. Antiviral treatment may improve symptoms even if an SVR is not achieved [48,49,50,51,52].

Recently investigations have revolutionized the treatment of hepatitis C virus (HCV) infection. Antiviral drugs base on their acting, can be *direct acting antivirals (DAAs)* which are novel small molecules that target specific viral proteins of the HCV life cycle and/or *indirect acting antivirals (IDAAs)* or ‘non DAAs’or indirect inhibitors which are not focused on one site of the life cycle. Many of these drugs are in pre-clinical and clinical phase I, II and III trials or various stages of development just some of them are yet available [53,54].

FIRST CHAPTER

BASIC THERAPEUTIC CONCEPTS AND MEDICATION

Until recently, a combination of PEG-IFN-α and ribavirin is a standard therapy regimen for HCV infection treatment for 24 to 48 weeks which depends on the viral genotype.

INTERFERONS (IFNs)

GENERALITIES: Interferons (IFNs) possess antiviral, immunomodulating, and antiproliferative activities. Interferons are a group of signaling proteins of cytokines that make communication between cells to trigger the protective defenses of the immune system that help eradicate pathogens. They are synthesized by host cells in response to various inducers and stimulate an antiviral state in cells. Interferons are named for their ability to "interfere" with viral replication by protecting cells from virus infections. There are three major classes of human interferons with antiviral activity: *α, β,* and γ. clinically used recombinant *α*-IFNs are nonglycosylated proteins of about 19,500 Da. Approximately all cells produce IFN-*α* and IFN-*β* in response to viral infection and/or other stimuli, like double-stranded RNA and certain cytokines for instance interleukin 1 & 2, and tumor necrosis factor *α* (TNF- *α*). IFN-γ production is restricted to T-lymphocytes and natural killer cells in response to antigenic materials, mitogens and specific cytokines. IFN-*a* and IFN-*b* have antiviral and antiproliferative actions; stimulate the cytotoxic activity of lymphocytes, natural killer cells, and macrophages; and up-regulate class I major histocompatibility complex (MHC) antigens. IFN-γ has less antiviral activity but more potent immunoregulatory effects. Most animal viruses are inhibited by IFNs, although DNA viruses are relatively insensitive [55].

FUNCTION OF IFN-α AND β

Part of the innate, nonspecific immune response: Virus-infected cells, after stimulation, produce IFN-α and β. Natural killer cells (NK-cells) killing the infected cells through releasing the apoptosis induction factors. IF-α and β activates the release of these factors. However, the uninfected neighboring cells also produce IFN-α and β after binding to IFN-receptors, and also JAK/STAT pathway antiviral proteins, which are inhibit virus replications and by this mechanism the cells get resistant against the virus. Also the uninfected cells, after stimulation with IFN-α and β, express the MHC-molecules which recognized by killer cell inhibitory receptors (KIR). The activated NK-cell will inhibited when KIR bind to the MHC-molecules and through this mechanism, the uninfected cells are saving, but binding of KIR to MHC-molecules of infected cells, activating the apoptosis ability of NK-cells [56].

Part of the specific adaptive immune response: The host adaptive antiviral immune response is initiated by virus-specific T-cell responses, including $CD4^+$ helper (Th) and $CD8^+$ cytotoxic T lymphocytes (CTL). The antigen presenting cells presenting cell surface receptors of MHC I and MHC II which intracellularly binding to peptides of pathogens (degradated or intercellularly synthesized proteins of these pathogens) and then presenting them to immune cells, particularly $CD4^+$ and $CD8^+$. $CD4^+$ T-lymphocytes recognizes epitops of MHC II and $CD8^+$ recognizes the epitops of MHC I. Activated $CD4^+$ produces cytokines, including IFN-γ and TNF-α in response to viral pathogens, which are activating macrophages. $CD8^+$ cells are cytotoxic T-killer cells, which recognize the MHC-I of infected cells. The $CD8^+$ will stimulate through antigen recognition and releasing perforins and granzymes. Perforins forming pores at the cell membrane of target cells, through these pores protease granzymes inserts into the cells and kills virus-infected cells by promoting apoptosis [56,57,58].

RECOMBINANT α-INTERFERONS

Thiese interferons are produced by recombinant DNA technology. In this technology a genetically engineered E-coli bacterium containing DNA codes for the human protein is used. The produced proteins of this method are not glykosylated and having an extra Methionin at the N-terminus [57].

6) Interferon alfa-2a (*Roferon A*®)
7) Interferon alfa-2b (*Intron A*®)
8) Interferon alfacon-1 (*Inferax*®)
9) Peginterferon alfa-2a (*Pegasys*®)
10) Peginterferon alfa-2b (*PegIntron*®)

1) *INTERFERON alfa-2a (Roferon A®)*

DRUG DESCRIPTION: Roferon-A (Interferon alfa-2a, recombinant) is a sterile protein which is produced by recombinant DNA technology. Interferon alfa-2a, recombinant is a highly purified protein with 165 amino acids and approximately 19,000 daltons molecular weight. Roferon-A is supplied in prefilled syringes which contain: 3 million IU (11.1 mcg), 6 million IU and/or 9 million IU Interferon alfa-2a, recombinant, 3.605 mg NaCl, 0.1 mg polysorbate-80, 5 mg benzyl alcohol as a preservative and 0.385 mg ammonium acetate ($C_2H_7NO_2$) in 0.5mL colorless solution. The route of administration is by subcutaneous injection [59].

THERAPEUTIC USES: For the treatment of chronic hepatitis C, hairy cell leukemia, AIDS-related Kaposi's sarcoma, and chronic myelogenous leukemia, also oral warts arising from HIV infection [60].

PHARMACODYNAMICS: interferon-alpha upregulates the MHC-I proteins expression, which allowing increased presentation of peptides derived from viral antigens. This increase the activation of $CD8^+$ T cells that are the precursors for cytotoxic T lymphocytes and make the macrophage a better target for CTL-mediated killing. Interferon alpha also induce the synthesis of several key antiviral mediators, including 2'-5' oligoadenylate synthetase (2'-5' A synthetase) and protein kinase R [60].

PHARMACOKINETICS: Roferon-A has greater than 80% absorption rate when administered intramuscularly or subcutaneously and totally filtered through the glomeruli and undergo rapid proteolytic degradation during tubular reabsorption. Interferon alfa-2a has an intramuscular and intravenous infusion half-life of about 6 to 8hr and 3.7 to 8.5hr (mean 5.1hr) respectively with clearance of 2.14 to 3.62 mL/min/kg [60].

ADVERSE EFFECTS: Interferon alfa-2 may cause serious adverse effects such as anemia; autoimmune diseases (including vasculitis, arthritis, hemolytic anemia, and erythematosus syndrome) hyperthyroidism or hypothyroidism, cardiotoxicity, hepatotoxicity, leucopenia, transient ischemic attacks, neurotoxicity, peripheral neuropathy and thrombocytopenia. Also some lesser side effects that may not need medical attention are blurred vision, change in taste or metallic taste, cold sores or stomatitis, diarrhea, dizziness, dry mouth, dry skin or itching, flu-like syndrome (Fever, chills, generalized aches and pains, headache, poor appetite, fatigue), increased sweating, leg cramps, appetite and weight loss also partial hair loss or alopecia, nausea or vomiting, skin rash and unusual tiredness. Also interferon-alpha will cause high or low blood pressure, low blood counts, low calcium, high glucose and triglyceride levels, injection site reaction (redness and pain), cough, depression, constipation, sore throat, insomnia, excessive sleepiness, memory loss, edema and anxiety [60].

CONTRAINDICATIONS: sever depression, having thoughts of suicide, disease in the retina of eye, angina pectoris, chronic heart failure, inadequate blood flow disease to the heart muscle, inflammation & obstruction of smallest breathing passages, pneumonia, interstitial pneumonitis, large intestine inflammation with bleeding, liver & kidney failure, autoimmune hepatitis, organ transplantation, overactive or underactive thyroid gland, diabetes, high blood level of triglyceride, sarcoidosis, autoimmu diseases, bone marrow decreased function, and decreased blood platelets & neutrophils [61].

2) INTERFERON alfa 2b (Intron A®):

DRUG DESCRIPTION: Intron A is a purified sterile recombinant interferon product. Prepared like IFN-alpha-2a. Intron A Powder for Injection is a white to cream colored which prior to administration do not require reconstitution. Intron A Solution for Injection is a clear, colorless solution. Intron A can be injected intramuscularly, subcutaneously, intralesional, or intravenously [62].

POWDER FOR INJECTION: containing approximately 2.6 x 10^8 IU/mg intron A, 20 mg glycine, 2.3 mg sodium phosphate dibasic (Na_2HPO_4), 0.55 mg sodium phosphate monobasic (NaH_2PO_4), and 1.0 mg human albumin [62].

SOLUTION VIALS FOR INJECTION

3 million international units/0.5-ml vial, 5 million international units/0.5-ml vial, 10 million international units/1-ml vial; 18 million international units/3.2-ml vial, 25 million international units/3.2-ml vial [62].

18 million IU: This is a multidose vial which contains a total of 22.8 million IU of interferon alfa-2b, recombinant per 3.8 mL in order to provide the delivery of six 0.5-mL doses, each containing 3 million IU of intron A for a label strength of 18 million IU. This vial containing about 2.6 x 10^8 IU/mg protein, 7.5 mg NaCl, 1.8mg Na_2HPO_4, 1.3mg NaH_2PO_4, 0.1mg disodium edetate, 0.1mg polysorbate 80, and 1.5mg mcresol as a preservative [62].

25 million IU: This is a multidose vial which contains a total of 32.0 million IU of interferon alfa-2b, recombinant per 3.2 mL in order to provide the delivery of five 0.5-mL doses of 5 million IU of intron A for a label strength of 25 million IU [62].

THERAPEUTIC USES: This drug is approved for the treatment of chronic hepatitis C & B, hairy cell leukemia, chronic myelogenous leukemia, multiple myeloma, follicular lymphoma, carcinoid tumor, and malignant melanoma [62].

Pharmacodynamics, function, Pharmacokinetics, Adverse effects and Contraindication are like Roferon A.

3) INTERFERON ALFACON-1 (Inferax®) Inferax injection has a 9µg protein (30µg/ml), plus 100 mmol NaCl, 25 mmol and sodium phosphate. Interferon alfacon-1 has better *in vitro* activity (antiviral, antiproliferative, immunomodulatory) than natural alpha-Interferons [56].

PEGINTERFERON alfa-2a (*Pegasys*®) once a week
PEGINTERFERON alfa-2b (*PegIntron*®)

A pegylated form of IFN-alpha-2a was developed in order to improve its pharmacological properties. This 40 kDa PEG-IFN-alpha-2a is obtained by the covalent binding of a 40 kDa branched PEG-polymer to a lysine side-chain of IFN-alpha-2a. Surface plasmon resonance

technique showed that the pegylation does not abolish the binding to the receptor, but significantly reduces the affinity mainly due to a change of the association rate [63].

PEGASYS® is a prescription medication for the treatment of CHC that is used asmonotherapy in cases that the patients can not tolerat other anti hepatitis C medicines or in combination with other anti hepatitis C agents, often ribavirin, in adults and children 5 years and older. It is also used to treat CHB virus in adult patients with progressive viral liver damaging. It is contraindicated as in patients who have had solid organ transplantation. It is not known if pegasys is safe and will work in children under 5 years of age.

ADVERSE EFFECTS: pegasys in combination with Ribavirin may cause birth defects or death of unborn baby. Regardless of this reason, before, during and 6 months after pegasys+ribavirin combination therapy, females must have pregnancy tests. Also it causes mental problems like depression and aggressivity.

RIBAVIRIN AND RIBAVIRIN DERIVATIVES

RIBAVIRIN (Copegus, Rebetol, Ribasphere, Vilona, and Virazole)

CHEMICAL STRUCTURE & DESCRIPTION: Ribavirin is a purine nucleoside analog with a modified base and D-ribose sugar [64].

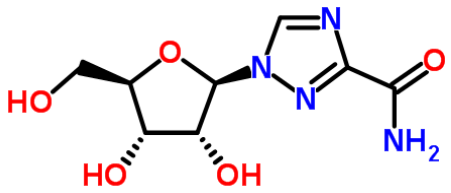


Figure (3): structure of ribavirin

Ribavirin inhibits the replication of a wide range of RNA and DNA viruses, including bunyaviruses, orthomyxoviruses, arenaviruses, paramyxoviruses and flaviviruses. Therapeutic concentrations reversibly inhibit macromolecular synthesis and proliferation of uninfected cells, suppress lymphocyte responses, and alter cytokine profiles [64].

MECHANISMS OF ACTION: Ribavirin alters cellular nucleotide pools and inhibits viral mRNA synthesis. Host cell enzymes mediate the intracellular phosphorylation of ribavirin to the mono-, di-, and triphosphate derivatives. The triphosphate rebavirin in both uninfected and RSV-infected cells is the predominant derivative, which has an intracellular half life of less then 2hr and may competitively inhibits the Guanosintriphosphat (GTP)-dependent 5′ capping of viral messenger RNA and specifically the influenza virus transcriptase activity. Ribavirin monophosphate competitively inhibits cellular inosine-5′-phosphate dehydrogenase and interferes with the synthesis of GTP and thus nucleic acid. Ribavirin also may enhance viral mutagenesis such that some viruses may be inhibited in effective replication, so-called lethal mutagenesis [64].

Indirect antiviral effects of ribavirin: Ribavirin may elicit indirect antiviral effects by (1) promoting T-cell mediated immunity against viral infection via the induction of antiviral type 1 cytokines such as IFN-γ, tumour necrosis factor-α (TNF-α) and interleukin-2 (IL-2), and the suppression of proviral type 2 cytokines such as IL-4 and IL-10 or (2) by affecting the intracellular GTP concentration via inhibition of host inosine monophosphate dehydrogenase (IMPDH), an effect which presumably inhibits viral RNA or DNA synthesis in proliferating lymphocytes. The host adaptive antiviral immune response is initiated by virus-specific T-cell responses, including CD4 helper (Th) and CD8 cytotoxic T lymphocytes (CTL). In addition to MHC II-restricted CD4 Th cells (providing virus-specific T-cell help to CD8 CTL and B cells) and MHC I-restricted CD8 CTL (providing cytotoxic killing of virus infected cells), both T-cell subsets also secrete type 1 cytokines, including IFN-γ and TNF-α in response to viral pathogens [65].

PHARMACOKINETICS: Ribavirin has an average oral bioavailability of about 50% after actively taken up by nucleoside transporters in the proximal small bowel. Ribavirin has an extensive plasma accumulation with about 4 weeks of steady state occurrence. Food increases plasma levels substantially. The average peak plasma concentrations of RBV following single or multiple oral doses of 600 mg is approximately 0.8 and 3.7 μg/ml respectively. Whereas an intravenous dose of 500 mg and 1000 mg has plasma concentrations average approximately 24 and 17 μg/ml, respectively. Aerosol dosage form or ribavirin may increase plasma levels with the duration of exposure and range from 0.2 to 1.0 μg/ml after 5 days. The apparent

volume of distribution for ribavirin, owing to its cellular uptake, is large and approximately 10 L/kg. Ribavirin has a poor plasma protein binding and a complex elimination procedure. At steady state the plasma *t* increases to approximately 8 to 12 days. Erythrocytes concentrate ribavirin triphosphate; the drug exits red cells gradually, with a *t* of approximately 40 days. Hepatic metabolism involves deribosylation and hydrolysis to yield a triazole carboxamide. Hepatic metabolism and renal excretion of ribavirin and its metabolites are the principal routes of elimination. Ribavirin clearance decreases threefold in in patients with advanced renal insufficiency [64,66].

THERAPEUTIC USES: Oral ribavirin in combination with pegIFN alfa-2a or 2b is standard treatment for chronic HCV infection. In about 30% of patients Ribavirin monotherapy for 24 to 48 weeks reversibly decreases aminotransferases level to normal state, but does not affect HCV RNA levels. Ribavirin with a oral dose of 500 mg or 600 mg twice daily in combination therapy with pegIFN alfa-2a for 24–48 weeks may increases the likelihood of sustained responses to about 60%. The combination has better SVR efficacy than IFN or pegIFN monotherapy. Also in a minority of HCV/HIV-coinfected patients the combined ribavirin and pegIFN alfa-2a or 2b is effective in achieving sustained viral responses. Combined therapy has been used in the management of recurrent HCV infection after liver transplantation [64]. Aerosolized ribavirin for treatment of RSV bronchiolitis and pneumonia in hospitalized children with a usual dose of 20 mg/mL as the starting solution in the drug reservoir of the small particle aerosol generator unit for 18 hr' exposure per day for 3–7 days) may reduce some illness measures, but it generally is not recommended. Intravenous and/or aerosol ribavirin has been used occasionally treatment for a variety of viral hemorrhagic fevers, including Lassa fever, Venezuelan hemorrhagic fever, Crimean-Congo hemorrhagic fever and Hantavirus infection [67,68,69,70]. It is noted by the USAMRIID (United States Army Medical Research Institute of Infectious Diseases) that ribavirin has poor in vitro and in vivo activity against the filoviruses (Ebola and Marburg) and the flaviviruses (dengue, yellow fever, Omsk hemorrhagic fever, and Kyasanur forest disease) [71,72].

COMBINATION THERAPY

RIBAVIRIN + INTERFERON-α: this combination is a standard regimen for the treatment of HCV which was approved by FDA- in 2002. Between 2002 and 2010, there were no significant improvements in antiviral therapy for HCV [73].

Triple therapy: ribavirin can combine with many DAAs for the treatment of HCV.

Table (1): Use of ribavirin in interferon-sparing regimens for the treatment of HCV [73]

Small molecule antivirals	**Company**
ABT-450/r +ABT-333 +ABT-267 ± RBV	Abbott
BI 207127 +BI 201335 ± RBV	Boehringer Ingelheim
BMS-790052 +BMS-650032 ± RBV	Bristol–Myers Squibb
GS-5885 +GS-9451 +GS-9190 ± RBV	Gilead Sciences
GS-7977 +RBV	Gilead Sciences
GS-7977 +TMC435 ± RBV	Tibotec
RO5190591/r +RO5024048 ± RBV	Hoffmann–La Roche
DEB025 ± RBV	Novartis Pharmaceuticals

UNWANTED EFFECTS: The main toxicity observed with ribavirin is haemolytic anaemia. Ribavirin is actively transported into erythrocytes and subsequently phosphorylated to its triphosphate form by intracellular kinases. In the case of the erythrocyte, these cells lack the phosphatase that converts ribavirin back to its unphosphorylated form. Consequently, high levels of ribavirin triphosphate accumulate in the erythrocyte due to the fact that the triphosphate form is not actively exported from the cell. Intracellular erythrocyte concentrations can reach approximately 100 × the concentration found in the serum. Consequently, extremely high intracellular concentrations of the nucleotide deplete intracellular ATP concentrations and lead to oxidative stress, ultimately resulting in damage to the cell membrane and lyses of the erythrocyte. Since the major dose-limiting toxicity of ribavirin is haemolytic anaemia, it can result in worsening of cardiac disease and can lead to fatal and non-fatal myocardial infarctions. As in pharmacokinetics mentioned that ribavirin has a renal clearance, thus the drug should be used with extreme caution in patients with chronic kidney disease. Other side effects associated with ribavirin include mild itching, rash, cough and nasal stuffiness, lymphopaenia and hyperuricaemia. Furthermore significant

teratogenic and/or embryocidal effects, also more than 10%: fatigue, headache, myalgia, nausea, rigors, fever, insomnia, decreased Hgb, depression, hyperbilirubinemia, arthralgia, alopecia, irritability, musculoskeletal pain, anorexia, dizziness, pruritus, flu-like syndrome, dyspnea, dyspepsia, impaired concentration, thrombocytopenia, sinusitis, vomiting, emotional lability, decreased WBC, weakness, chest pain, taste perversion, nervousness [73].

CONTRAINDICATIONS: hypersensitivity, pregnancy or planning pregnancy including men whose female partners are pregnant/planning to get pregnant, adults, non-severe RSV infections, Pancreatitis, Hemoglobinopathies (eg, thalassemia major, sickle cell anemia), in combination with Zidovudine because of the increased risk of anemia [74]; also because of an increased risk of mitochondrial toxicity, it should not combind with Didanosine. *In combination with alpha interferons:* Autoimmune hepatitis and Hepatic decompensation in patients with cirrhosis [75].

RIBAVIRIN DERIVATIVES

Two natural products with imidazole riboside structure were already known. Substitution at the 5' carbon with OH results in pyrazomycin/pyrazofurin with antiviral properties but high toxicity, and replacement with an amino group results in the natural purine synthetic precursor 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside, which has only modest antiviral properties.

TARIBAVIRIN (rINN; ICN 3142, *viramidine and ribamidine):*

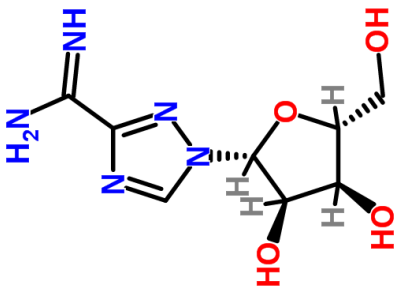


Figure (4): structure of taribavirin

INDICATION: Viramidine is in phase III human trials for the treatment of HCV infection. In case use of Ribavirin for the treatment of chronic HCV infection is limited because of ribavirin-induced hemolytic anemia and other substantial side effects. Taribavirin which is a prodrug of ribavirin, designed to concentrate more within the liver and target HCV-infected hepatocytes with lower distribution rate within red blood cells (RBCs) and the subsequent development of hemolytic anemia. The studies have shown that taribavirin at 25 mg/kg dose demonstrates lower rates of hemolytic anemia with comparable rates of SVR and is the optimum dose for further studies comparing the efficacy of taribavirin with weight-based dosing of ribavirin [76].

PHARMACOKINETICS: Viramidine has very different pharmacokinetics properties in compare to its parent compound. Presumably viramidine does not transport into RBCs efficiently because of its positively charged 3-carboxamidine group. Studies on animal suggest that viramidine in compared with ribavirin, yields three times more in the liver but only half of the drug level in RBCs. It has also indicated that viramidine has a better liver targeting property than ribavirin and the liver is thought to be the main conversion site of the prodrug. Because of this preferential liver targeting property and the concomitant reduction in the haemolytic potential, viramidine appears to be a safe alternative to ribavirin, and it may provide many clinical benefits to HCV patients [77].

SECOND CHAPTER

HCV INFECTION THERAPIES WITH SPECIFIC TARGETED ANTI-VIRALS

It has been expected that new therapies of HCV infection may increase the efficiency of antiviral activity. HCV can easily develop mutations to resist therapies because it is a highly mutable RNA virus. Enzyme inhibitors targeting viral polymerase, protease and helicase are emerging as new therapies. In 2011, boceprevir and telaprevir, the first DAAs protease inhibitors, in combination with peg-IFN and ribavirin for treatment of genotype-1 HCV infection were approved in the United States. Until now many DAAs have approved or currently are in late-stage clinical trials. These durgs including NS3/NS4A serine PIs, NS5A inhibitors, NS5B polymerase inhibitors (nucleoside and non-nucleoside) and cyclophilin inhibitors, in combination with or without peg-IFN and RBV, are promising for the treatment of HCV infection. DAA therapy regimens have many advantages while they specifically target HCV viral replication and thus appear to have less host characteristics dependency, very high rates of SVR accompanied by fewer side effects and lower pill burdens [78].

Currently there are four classes of DAAs, which are defined by their mechanism of action and therapeutic target. The characteristics of different classes of DAAs are indicated and summarized in the following table [47].

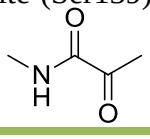
Table (2): Characteristics of different classes of DAAs.

Classes of DAAs	*Efficacy*	*Genotype dependency*	*Barrier to resistance*
Nonstructural 3/4A (NS3/4A) protease inhibitors (PIs)	+++	+++	++
NS5A inhibitors	+++	+++	++
NS5B nucleoside polymerase inhibitors (NPIs)	+++	+++	+++
NS5B non-nucleoside polymerase inhibitors (NNPIs)	++	+	+

I

HCV NS3/4A PROTEASE INHIBITORS

HCV NS3/4A protease inhibitors by binding to HCV NS3/4A protease interfere with HCV replication. These viral enzyme inhibitors according to the nature of the active site binding groups are now divided into two classes [79].

A. *Reversible covalent inhibitors*, form a covalent bond with a residue of the catalytic triad or active site (Ser139), such as linear α-ketoamide 

B. *Non-covalent inhibitors*, bind more strongly to active site than substrate through noncovalent interactions. Depend on the *C*-terminal carboxylate, which is mostly a macrocyclic compound.

1. Boceprevir (SCH503034)
2. Telaprevir (VX950)
3. Narlaprevir (SCH900518)
4. Paritaprevir (ABT-450)
5. Asunaprevir (BMS-650032)
6. Danoprevir (RG7227; ITMN-191):

1. Simeprevir (TMC435)
2. Vaniprevir (MK7009)
3. Faldaprevir (BI201335)

According to the above classification, this class of direct acting antiviral drugs inhibits HCV replication in two mechanisms. For instance, Telaprevir and boceprevir have in their molecular structure an α-ketoamide, that forms a reversibly covalent bond with the serine of the HCV NS3/4A protease catalytic triad. Whereas faldaprevir and may other member of this class protease inhibitors, contain functional groups, that form ionic interactions with residues of the catalytic triad and thus exclusively non-covalent interactions with the HCV NS3/4A protease [79]. Also their relatively high molecular weight and poor chemical stability lead to a problematic ADME (absorption, distribution, metabolism and excretion) profile. In addition, various mutation sites were reported to confer resistance to these protease inhibitors, such as the broad cross-resistant mutation sites, R155K and A156T/S, for all protease inhibitors and the D168A/V mutation, usually associated with macrocyclic inhibitors [80].

A. REVERSIBLE COVALENT HCV NS3/4A INHIBITORS

MECHANISM OF ACTION OF α-KETOAMIDES: These are typically peptides containing an electrophile that form reversible covalent bonds with serine139 in the active site.

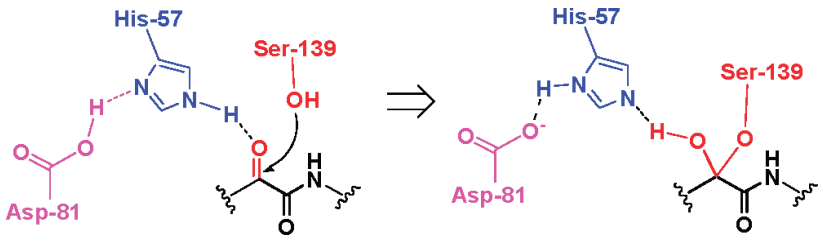


Figure (5): mechanism of action of α-ketoamides

1) **BOCEPREVIR** (INN, trade name **Victrelis**)

CHEMICAL STRUCTURE & DESCRIPTION

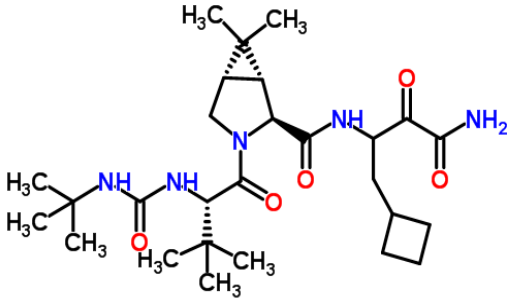


Figure (6): structure of boceprevir

As it mentioned above, Boceprevir is a linear ketoamide serine protease inhibitor that binds reversibly to the serine of HCV NS3/4A protease catalytic triad [81]. Boceprevir monotherapy cuase a significant decline of HCV RNA however it meanwhile leads to a rapid emergence of viral resistance. Thus, at this stage combination with PEG-IFN/RBV is still necessary. In the SPRINT-2 study (serine protease inhibitor therapy 2) Poordad *et al*. showed that the addition of boceprevir to PEG-IFN/RBV significantly improved the sustained response rate in previously untreated patients with HCV genotype 1. Furthermore, boceprevir was tested in treatment-experienced patients in the RESPOND-2 study by Bacon *et al*. and its superiority to SOC alone was confirmed [79].

PHARMACOKINETICS: Following oral administration, Boceprevir is absorbed with a median Tmax of 2hr. Steady state AUC, Cmax, and Cmin increased in a less than dose proportional manner and individual exposures overlapped substantially at 800 mg and 1200 mg, suggesting diminished absorption at higher doses. It has a minimal accumulation rate of 0.8 to 1.5-fold. The pharmacokinetics steady state is achieved after approximately 24hr with three daily dosing. Studies have shown that food enhanced the exposure of boceprevir by up to 65% at the 800 mg three daily doses, relative to the fasting state. Thus it recommended that Boceprevir should be administered with food. Boceprevir has in healthy subjects a mean apparent volume of distribution of approximately 772 L at steady state and it binds approximately 75% to human plasma protein following a single dose of 800 mg. Boceprevir has a mean total body clearance of about 161 L/hr. Boceprevir is distributed into rats milk but yet it is not known whether it can into human milk distribute. The data indicate that boceprevir is eliminated primarily by the liver. Thus following a single 800 mg oral dose, it excreted approximately 79% in feces and 9% in urine [82].

INDICATION: Victrelis (boceprevir) must not be used as monotherapy. So it always indicates in combination with PEG-TFN-α and ribavirin for the treatment of adult CHC genotype-1 infected patients with compensated liver disease and/or cirrhosis, previously untreated patients or who have failed previous interferon and ribavirin therapy, including prior null or partial responders, and relapsers. and should only be used in combination with peginterferon alfa and ribavirin [83].

DOSING: The Victrelis dose is 800 mg (four 200-mg capsules) three times daily with food in combination with PEG-TFN-α and ribavirin. Duration of treatment depends on patient's HCV-RNA levels and will run for 8, 12 and 24 weeks [83].

ADVERSE EFFECTS: Fatigue, anemia, nausea, headache and dysgeusia are the most common adverse effects, greater than 35%, of victrelis in combination with PEG-TFN-α and ribavirin which has reported in clinical trials in adult subjects. Anaemia is the most important adverse event thus Erythropoetin is recommended as a co-administration on a triple therapy. Other less common adverse effects of this combination are neutropenia grade 3, pancytopenia, hypersensitivity, rash and flu-like symptoms [83].

CONTRAINDICATIONS: Patients with history of hypersensitivity to boceprevir also in case victrelis must always combine with peg-TFN-α and ribavirin, thus the contraindications of theise two drugs are also apply to victrelis. For instance, this therapy regimen is avoided for pregnant women and men whose female partners are pregnant because of the high risks of defects birth and fetal death associated with ribavirin. In addition, victrelis in co-administration with drugs that their clearance are highly dependent on CYP3A4/5 enzyme. Coadministration with potent CYP3A4/5 inducers, where significantly reduced boceprevir plasma concentrations may be associated with reduced efficacy [83].

DRUG INTERACTIONS: The potential for drug-drug interactions must be considered prior to and during therapy because victrelis strongly inhibit CYP3A4/5 and is partly metabolized by this enzyme [83].

2) TELAPREVIR (**VX-950**, brand names **Incivek**, **Incivo)**

CHEMICAL STRUCTURE & DESCRIPTION

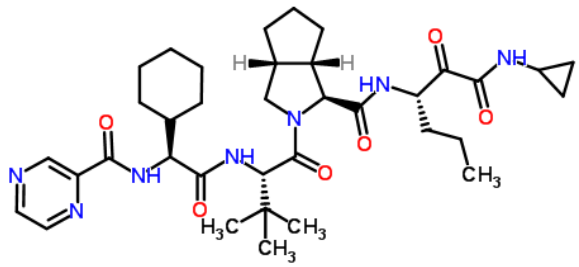


Figure (7): structure of telaprevir

Telaprevir belongs to the α-ketoamids group of NS3/4A serine protease inhibitors. It binds covalently to the serine amino acid of HCV NS3/4A serine proteas. The binding is reversibly, with a half-life of 58 min of the enzyme-inhibitor complex. The main function of NS3/4A protease is to cleave the junctions between NS3/NS4A, NS4A/NS4B, NS4B/NS5A and NS5A/NS5B. As well as, besides its essential role in protein processing, NS3 is integrated into the HCV RNA replication complex, supporting the unwinding of viral RNA by its helicase activity [84].

INDICATION: Telaprevir (Incivek) is indicated in combination therapy with peg-IFN-α and ribavirin for the treatment of hepatitis C genotype 1 viral infection [85,86]. Also this combination regimen is indicated for the treatment of CHC genotype 1 infection in adult patients with compensated liver disease, including cirrhosis, who are treatment-naive or who have previously been treated with interferon-based treatment, including prior null or partial responders, and relapsers. Incivek is supplied as purple film-coated capsule-shaped tablets containing 375 mg of telaprevir and is packaged 28-day packer [87].

PHARMACOKINETICS: Telaprevir is orally available and probably absorbed in the small intestine; there is no evidence for absorption in the colon. Steady state after multiple doses of telaprevir every 8hr in HCV-infected patients was reached after 3 to 7 days of administration. the mean accumulation index (ratio of the area under the concentration–time curve from 0–8 h [AUC0–8hr] at steady state versus AUC0–8hr after a single dose) in healthy volunteers receiving 750 mg telaprevir every 8 h was about 2.2. The liver-to-plasma ratio of telaprevir is 35 to 1. Approximately 59–76% of telaprevir was bound to human plasma proteins at concentrations ranging from 0.1–20 mM [88]. Telaprevir has: well absorption rate with fatty food, moderately protein bound about 59-76 %, approximately 252 L/kg volume of distribution. Telalrevir is primarily metabolized by cytochrome (CYP) P450, CYP 3A4 and P-glycoprotein, and is largely excreted into feces. Pharmacokinetics and pharmacodynamics parameters are well described in healthy subjects and individuals infected with HCV [89].

ADVERSE EFFECTS: The most common adverse events in patients who received telaprevir-based triple therapy compared to PEG-IFN/RBV in these phase 3 clinical trials included influenza-like symptoms, anaemia, nausea, diarrhoea, anal-rectal discomfort, alopecia, insomnia, rash and pruritus, fatigue, pyrexia [90].

CONTRAINDICATION: The contraindications of PEG-IFN/RBV also apply to Incivek combination treatment like: HCV infected pregnant women and men whose females partners are pregnant, Coadministration with drugs that their clearance are highly dependent on CYP3a, and for which elevated plasma concentrations are associated with serious and/or life-threatening events. It is also contraindicated with drugs that strongly induce CYP3a and thus

may lead to lower exposure and loss of efficacy of Incivek. For more information look the reference [91].

UPDATE: As of August 2014 Vertex announced it will discontinue selling Incivek

3) NARLAPREVIR (SCH 900518):

CHEMICAL STRUCTURE & DESCRIPTION

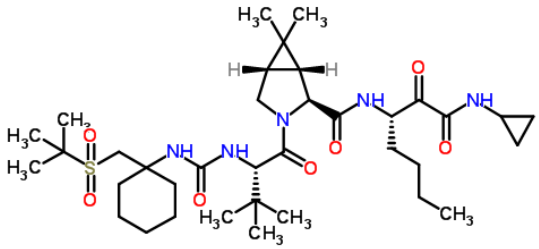


Figure (8): structure of narlaprevir

Narlaprevir is a potent and selective NS3 protease inhibitor which target HCV NS3/4A Protease and is orally bioavailable. The mechanism of inhibition involves the covalent, yet reversible, binding of narlaprevir to the NS3 protease active site serine through a ketoamide functional group. In the replicon system, the 50% and 90% maximal effective concentration for suppression of the HCV genotype 1b is approximately 20 ± 6 nM and 40 ± 10 nM (~28 ng/mL), respectively. These data indicate that narlaprevir is approximately 10-fold more potent *in vitro* than other protease inhibitors currently in phase 3 trials (telaprevir and boceprevir) [92].

PHARMACOKINETICS: The pharmacokinetics profile of narlaprevir 800 mg three times daily or 400 mg with ritonavir two times daily was characterized: Narlaprevir was eliminated more slowly when coadministered with ritonavir than when administered alone. Narlaprevir is primarily metabolized by the cytochrome P450-3A4 system. [92].

EFFICACY: narlaprevir 200mg or 400mg with ritonavir has an efficacy of approximately 84 – 90% at 12 weeks of treatment [93].

ADVERSE EFFECTS: the most common adverse effects at 12 weeks of treatment are fatigue, nausea, flu-like symptoms, headache, insomnia, anemia, arthralgia, diarrhea, pyrexia, irritability, pruritus, rash, dizziness, vomiting, chills, anxiety, myalgia, decreased appetite and depression [93].

INDICATION & DOSING: Narlaprevir has been tested in a phase II clinical trial recently. However, distinct drug-resistance of Narlaprevir has been discovered. The molecular mechanisms of drug-resistance of Narlaprevir due to the mutations V36M, R155K, V36M+R155K, T54A, and A156T of NS3/4A protease have been investigated by molecular dynamics (MD) simulations, free energy calculations, and free energy decomposition analysis. Dosing as a Monotherapy 800mg, combined with ritonavir 200mg and 400mg oral. [94,95].

4) PARITAPREVIR (ABT-450)

CHEMICAL STRUCTURE & DESCRIPTION

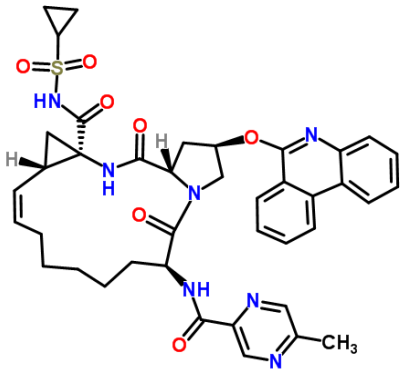


Figure (9): structure of paritaprevir

Paritaprevir is HCV nonstructural 3/4A (NS3/4A) serine protease inhibitor, which administered with ritonavir to increase paritaprevir plasma levels and half-life, permitting once-daily dosing. Paritaprevir has a potent activity against HCV genotype 1 in vitro. It is combined with ombitasvir, and dasabuvir with or without ribavirin, for 12 weeks of HCV therapy [96].

INDICATION: Combination therapy with other DAAs with different mechanisms of action, have demonstrated promising efficacy rates and favorable tolerability profiles in patients with

HCV. Paritaprevir is administered with low-dose of ritonavir (paritaprevir/ritonavir), in combination with ombitasvir, enabling once-daily dosing. Both paritaprevir and ombitasvir have potent *in vitro* antiviral activity against multiple subtypes, including 1a, 1b, 2a, 2b, 3a, 4a, and 6a [97].

Paritaprevir in combination with ritonavir and ribavirin for 12 weeks, the rate of sustained virologic response at 24 weeks after treatment has been estimated to be 95% for those with HCV genotype 1 [98]. Resistance to treatment with paritaprevir is uncommon, because it targets the binding site, but has been seen to arise due to mutations at positions 155 and 168 in NS3 [99]. Paritaprevir is a component of Viekira Pak.

The ***Viekira Pak*** is an all-oral regimen comprised of four medications: ombitasvir, paritaprevir, ritonavir, and dasabuvir. In this medication Pack, ombitasvir-paritaprevir and ritonavir are combined as a fixed-dose tablet and the dasabuvir is a separate tablet. This regimen can be used with or without ribavirin. Ritonavir was originally developed and FDA-approved as an HIV protease inhibitor; it does not have activity against HCV but it is a potent CYP3A4 enzymes inhibitor which significantly increases the peak plasma concentrations, as well as the area under the curve of paritaprevir. Thus it is used as a pharmacologic booster for paritaprevir [100].

INDICATION: Viekira Pak is approved by FDA for the treatment of chronic hepatitis C genotype-1, including patients with compensated cirrhosis.

DOSING: Viekira Pak has a dosing regimen of two tablets of the fixed-dose combination ombitasvir-paritaprevir-ritonavir (12.5/75/50 mg respectively) once daily plus one tablet twice daily of dasabuvir (250 mg). The genotype-1 subtype and presence or absence of cirrhosis are determining the duration of treatment and inclusion of ribavirin. Normaly ribavirin is recommended with all regimens except for genotype-1b without cirrhosis [100].

ADVERSE EFFECTS: Pooled safety data are available from six Phase 3 clinical trials. Inpatients without co-administration of ribavirin, the most common adverse reactions (≥5%) were nausea, pruritus and insomnia. In patients with co-administered ribavirin, most common

adverse reactions (≥10%) were fatigue, nausea, pruritus, other skin reactions, insomnia and asthenia. Laboratory abnormalities included ALT and bilirubin elevations [100].

5) ASUNAPREVIR (BMS-650032)

CHEMICAL STRUCTURE & DESCRIPTION

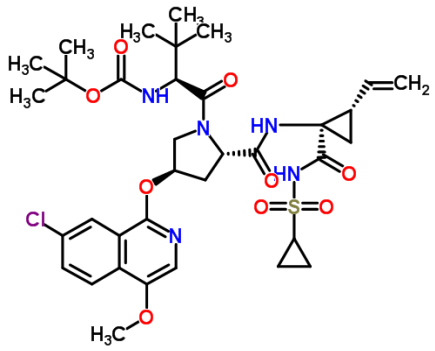


Figure (10): structure of asunaprevir

Asunaprevir targets the serine protease NS3 that divide the translated protein of the HCV into single proteins whose able to exert their enzymatic activity (eg, RNA-dependent RNA polymerase) or their structural role in viral particles (eg, core or E1). Protein NS3 also exerts ATPase/helicase activity and plays a role in inhibiting the transport of interferon signaling and therefore in HCV viral immune escape [101].

INDICATION: In vitro, asunaprevir exhibited good antiviral activity against replicons based on HCV genotypes 1a, 1b, 4, 5, and 6 and was less active against genotypes 2 and 3. Moreover, asunaprevir is a highly selective anti-HCV agent, and is not active against viruses closely related to HCV. Asunaprevir had a good in vitro cytotoxicity profile in various human cell lines and exerts an additive and/or synergistic effect when combined with other antiviral agents, like interferon and daclatasvir, and an additive effect with ribavirin. Asunaprevir exerts optimal in vitro activity particularly against HCV genotypes 1 and 4. Asunaprevir in combination with pegylated interferon and ribavirin, as well as in interferon-free regimens with other DAAs including daclatasvir is being tested. Asunaprevir plus Daclatasvir and peginterferon/ribavirin demonstrated SVR-12 rates of 93% (95% CI 90-96) in prior non-responders infected with HCV genotype 1. SVR-12 rates among genotype 4-infected patients were 98% (95% CI 93-100). The drawback of asunaprevir, and of all protease inhibitors, is its

low barrier to resistance. Consequently it is combined with other DAA drugs, specifically daclatasvir, to prevent resistance [101,102,103].

PHARMACOKINETICS: Asunaprevir revealed 2–4 hours to achieve Cmax, a mean terminal half-life of 15–20 hours, and a mean oral clearance of 302–491 L/hours. This drug is metabolized by the liver and primarily eliminated via the feces. The pharmacokinetics parameters found in healthy individuals were similar to those recorded in infected patients. No significant differences in Cmax and AUC between the normal renal function and patients on hemodialysis groups have been showed [101].

DRUG INTERACTION: Finally, a study evaluated the possible drug–drug interaction between asunaprevir and the antidepressants like escitalopram and sertraline. The results showed that neither escitalopram nor sertraline affected exposure to asunaprevir, and that asunaprevir did not affect exposure to escitalopram or sertraline [101].

ADVERSE EFFECTS: Serious adverse Grade 3/4 laboratory abnormalities included neutropenia, lymphopenia, anemia, thrombocytopenia, and ALT/AST elevations. *Asunaprevir + daclatasvir side effects:* The most common adverse events were nasopharyngitis, increased alanine aminotransferase (ALT) and aspartate aminotransferase (AST), headache, diarrhea, and pyrexia [102,103].

Japan approved daclatasvir and asunaprevir in July 2014, in order to meet the needs of Japanese patients [104]. Bristol-Myers Squibb (BMS) is withdrawing its New Drug Application (NDA) for asunaprevir in combination treatment with daclatasvir for the treatment of HCV genotype 1b patients [104].

6) DANOPREVIR (InterMune/Genentech) (RG7227; ITMN-191)

CHEMICAL STRUCTURE & DESCRIPTION

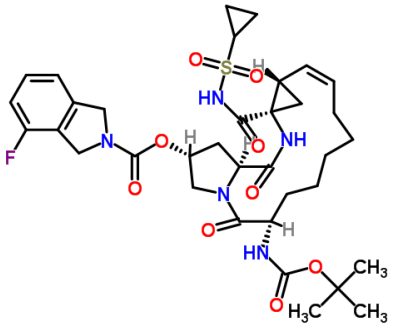


Figure (11): structure of *danoprevir*

Danoprevir is a potent, macrocyclic and highly selective HCV NS3/4A protease inhibitor; a chymotrypsin-like serine protease inhibitor, playing an essential role in the viral replication process of HCV. Danoprevir binds non-covalently to HCV NS3 protease with 50% inhibition concentration (IC_{50}) values ranging from 0.2 to 3.5 nM. [105].

INDICATION: Danoprevir is candidate for the treatment of chronic HCV infection because of its favorable potency profile across multiple HCV genotypes and key mutant strains and for its good in vitro pharmacokinetics profiles and in vivo target of liver tissue exposures across multiple animal species [105]. Danoprevir ± retonavir (danoprevir/r) plus PEG-IFNalfa-2a/RBV was well tolerated. The authors concluded that danoprevir/r plus PEG-IFNalfa-2a /RBV provide profound and robust reductions in serum HCV RNA, at substantially lower systemic exposures than with higher doses of danoprevir alone [106].

BIOAVAILABILITY: Danoprevir is an orally bioavailable of the HCV protease inhibitor, and a substrate of cytochrome P450 (CYP) 3A. It is co-administered with low dose of ritonavir (potent CYP3A inhibitor) which enhance the pharmacokinetics of danoprevir [107]. Danoprevir transport in vitro involved organic anion transporting polypeptide (OATP) 1B1, OATP1B3, P-glycoprotein, and multidrug resistance protein-2, but not breast cancer resistance protein. Ritonavir and ciclosporin inhibited transport of danoprevir by human hepatocytes. The pharmacokinetics of intravenous danoprevir 6 mg were not altered by oral ritonavir 100 mg. In contrast, exposure to oral danoprevir 100 mg increased two- to threefold when co-administered with ritonavir. Absolute bioavailability of danoprevir 100 mg was low (1.15 %), but increased more than threefold (3.86 %) when co-administered with ritonavir. Oral ciclosporin 100 mg increased exposure to intravenous danoprevir 2 mg and oral ritonavir 100 mg [108].

B. NON-COVALENT HCV NS3/4A INHIBITORS

1) SIMEPREVIR (TMC435; trade name Olysio™; Galexos™; Sovriad®)

CHEMICAL STRUCTURE & DESCRIPTION

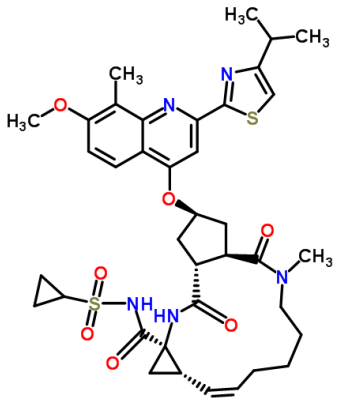


Figure (12): structure of simeprevir

Simeprevir is reversibly, macrocyclic that selectively inhibit the HCV NS3/4A serine protease. Simeprevir significantly decrease the HCV RNA but viral resistance emerges rapidly if given as monotherapy. Simeprevir has potent antiviral activity against all HCV genotypes, except genotype 3[48] &[109].

INDICATION: Simeprevir is approved by the FDA and also in Japan for the treatment of chronic hepatitis C infection genotype-1. It is administered once daily as capsule with PEG-IFN/RBV combination for the treatment of genotype 1 or genotype-4 CHC in adult patients with compensated liver disease, cirrhosis, with or without HIV-1 co-infection, naive patients or who have failed previous interferon therapy [110].

DOSING: Simeprevir with an oral dose of 150 mg once daily together with food for 12 weeks of standard therapy requires PEG-IFN/RBV combination [48]. Appropriate dosing of Simeprevir is dependent upon the HCV genotype, viral load and patient's liver & kidney function. It is not recommended for patients with moderate or severe liver impairment and patients with end-stage kidney disease while Olysio (simprevir) is not studied for use in these patients. Discontinuation of Olysio in the treatment of CHC infection might be depending on

the viral load. For instance, if the detectable viral load of the patient during the 4th week of their treatment regimen is more than 25 units/mL, it is considered an inadequate treatment and simeprevir must be discontinued [111].

PHARMACOKINETICS: Simeprevir is orally bioavailable and its absorption increases within the food. Simeprevir peak effect happens after 4 to 6 hr of administration with a half-life of 41hr in the plasma in HCV infected patients. It is mainly metabolized by CYP3A4 and may also by CYP2C8 and CYP2C19 of liver enzymes and primarily excreted about 91% into the feces [111].

ADVERSE EFFECTS: mostly mild or moderate skin rashes; rare serious photosensitivity reactions. Simeprevir is efficacious and generally well tolerated in patients with chronic HCV genotypes 1 and 4 infections, pruritis and rash [111].

CONTRAINDICATIONS: There are no specific contraindications to olysio. However, as olysio should always be administered in combination with other antiviral drugs for the treatment of CHC infection, prescribers should consult the complete prescribing information for these drugs for a description of contraindications. If olysio is administered with Peg-IFN-alfa and RBV, the contraindications for use of Peg-IFN-alfa and RBV also apply to this combination regimen. Refer to the prescribing information for Peg-IFN-alfa and RBV for a list of all contraindications. Serious Symptomatic Bradycardia When Coadministered with Sofosbuvir and Amiodarone [112].

DRUG INTERACTION: Simeprevir is a CYP3A4 substrate so its plasma concentration will significantly increase when it taken with strong CYP3A4 inhibitor drugs *like Erythromycin and Ritonavir*, and will significantly decrease if taken with strong CYP3A4 inducers for instance *Efavirenz, Rifampin, Saint John's Wart*. Simeprevir also inhibits intestinal (but not liver) CYP3A. Thus, if simeprevir coadministered with medications that metabolized by intestinal CYP3As, for example *Midazolam* which is an anticonvulsant, leads to increase their plasma level concentration and may toxicity. OATP1B1/3 and P-glycoprotein transporters are the transporters of some drugs which are pumping out the drugs out of the plasma. These drug transporters are also inhibit by simeprevir. Thus, the plasma concentration of medications that are substrates for these transporters can lead to increased with Simeprevir coadministration.

For example the plasma concentration of Calcium Channel blocker of *Diltiazem and Amlodipine* the *substrates for P-glycoprotein transporters* and *cyclosporine the substrate for OATP1B1/3* may significantly increase with Simeprevir. Therefore theis drugs are not recommended to be taken together [112].

2) VANIPREVIR (MK-7009)

CHEMICAL STRUCTURE & DESCRIPTION

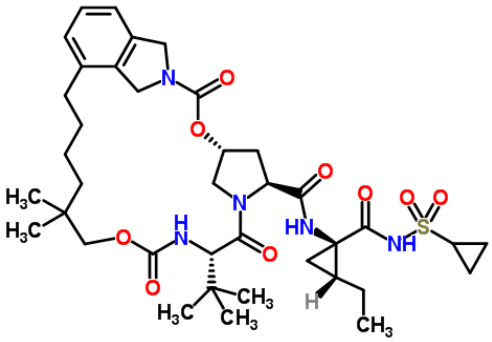


Figure (13): structure of vaniprevir

Vaniprevir is a non-covalent competitive HCV NS3/4A protease inhibitor which is active against both the genotype 1 and 2 NS3/4a protease enzymes. Vaniprevir has good plasma exposure and excellent liver exposure in multiple species [113].

INDICATION: In a controlled phase 2B trial, vaniprevir with peginterferon and ribavirin significantly increased rates of SVR among treatment-experienced patients with chronic HCV genotype 1 infection, compared with retreatment with peginterferon and ribavirin alone. Vaniprevir generally was well tolerated for up to 48 weeks in patients with compensated cirrhosis [114,115].

ADVERSE EFFECTS: The more frequently adverse effects experienced mild-moderate nausea, vomiting, diarrhea, grade 2 anemia. Resistance-associated variants were detected predominantly at positions 155, 156, and 168 in the HCV protease gene in patients under vaniprevir treatment [115].

3) FALDAPREVIR (BI 201335):

CHEMICAL STRUCTURE & DESCRIPTION

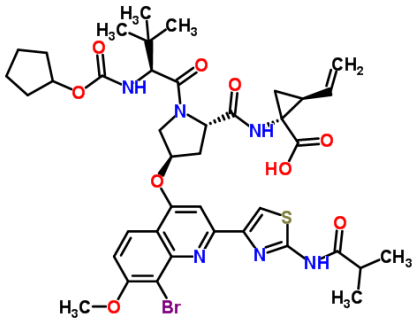


Figure (14): structure of faldaprevir

Faldaprevir is a potent and high selective HCV NS3/4A protease inhibitor. It is a peptidomimetic HCV-specific protease inhibitor with high in vitro activity against HCV subgenotypes 1a and 1b, with EC50 values of 6.5 and 3.1 nM, respectively. It has completed phase 3 clinical trials in combination with peg-IFN-α and ribavirin also phase 2 assessment with Deleobuvir (HCV NS5B polymerase inhibitor) with or without ribavirin in interferon free regimens [116].

INDICATION: Of interest, faldaprevir in combination with peg-IFN-α and ribavirin, and interferon-free treatment with Deleobuvir plus ribavirin provides high sustained virological response rates for HCV genotype 1 infection and may hold promise for interferon-ineligible and interferon-intolerant patients. Faldaprevir in combination with peg-IFN-α and ribavirin treatment appears to be associated with fewer adverse effects than Telaprevir or Boceprevir. In virological breakthrough-patients treated with triple therapy with faldaprevir, pegylated interferon and ribavirin, HCV NS3 R155K and D168V/E were the most frequently observed resistant variants in HCV subgenotypes 1a and 1b, respectively [116].

SOME OTHER PROTEASE INHIBITORS

4) DELDEPREVIR, NECEPREVIR (ACH-2684)

CHEMICAL STRUCTURE & DESCRIPTION

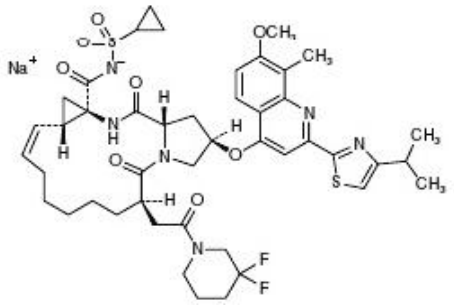


Figure (15): structure of ACH-2684

Deldeprevir is a potent pan-genotypic HCV NS3/4A protease inhibitor. The potency and virology profile of ACH-2684 demonstrates that it very effectively suppresses a broad range of natural variants of the HCV, and may be effective in prevention and treatment of emerging resistant variants. Preclinical studies demonstrate its excellent potency in the low pico-molar range, safety and tolerability, and a pharmacokinetics profile supportive of once daily dosing with excellent metabolic stability [117].

POTENCY: Deldeprevir is in the low pico-molar range 10 to 20 fold more potent than other HCV inhibitors under development [117].

VIROLOGY: Deldeprevir shows very effective suppression against a broad range of natural variants of the HCV, and may be effective in prevention and treatment of emerging resistant variants and retains potent activity against all genotypes 1 through 6 [117].

COMBINATION THERAPY: In vitro studies have been demonstrated that Deldeprevir can combine with other HCV inhibitors. Deldeprevir has a synergistic effect with NS5B nucleoside polymerase inhibitors. In in vitro preclinical studies, deldeprevir demonstrated activity against all genotypes of HCV at very low concentrations of less than 100 picomolar. Deldeprevir demonstrated high safety margins in animals with minimal dose-related side effects in both

single ascending-dose and multiple dose trials. Deldeprevir is metabolically stable and it metabolized rapidly and extensively in the liver, therefore supporting once-daily dosing in clinical development. ACH-2684 achieved Phase 1 proof-of-concept with a 3.73 log10 reduction in genotype-1 HCV RNA supporting future testing in combination with other DAAs [117].

5) SOVAPREVIR (ACH-1625)

CHEMICAL STRUCTURE & DESCRIPTION

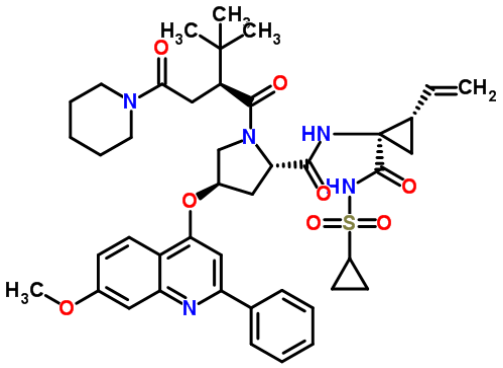


Figure (16): structure of Sovaprevir

Sovaprevir is a potent Phase 2 investigational NS3/4A protease inhibitor discovered by Achillion. Achillion has completed a Phase 2 clinical trial to evaluate the interferon-free combination of sovaprevir with ribavirin an oral therapy regimen for 12 weeks, in treatment of naïve patients infected with HCV genotype-1 [118].

POTENCY: In in vitro studies, sovaprevir has shown activity against all HCV genotypes, including equipotent activity against both genotype-1a and -1b with a IC_{50} of approximately 1nM [118].

INDICATION: sovaprevir has been clinically demonstrated to allow for once-daily-dosing. Sovaprevir has demonstrated high rates of clinical cures in combination with pegylated-interferon and RBV in a challenging, real world, patient population of genotype 1 treatment-naive patients [118].

COMBINABILITY: Sovaprevir is believed to be synergistic when combined with other classes of DAAs [118].

6) VEDROPREVIR (GS-9451)

CHEMICAL STRUCTURE & DESCRIPTION

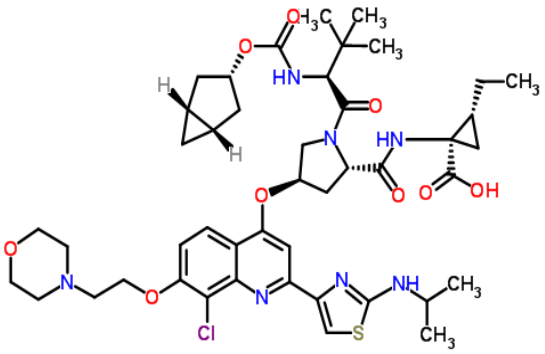


Figure (17): structure of vedroprevir

Vedroprevir is a selective HCV NS3 protease inhibitor in development for the treatment of genotype 1 HCV infection [119].

COMBINABILITY: Additive to synergistic in vitro antiviral activity was observed when Vedroprevir was combined with other agents, including alpha interferon, ribavirin, and the polymerase inhibitors GS-6620 and tegobuvir, as well as the NS5A inhibitor ledipasvir. Vedroprevir retained wild-type activity against multiple classes of NS5B and NS5A inhibitor resistance mutations [119].

7) GRAZOPREVIR (MK-5172)

CHEMICAL STRUCTURE & DESCRIPTION

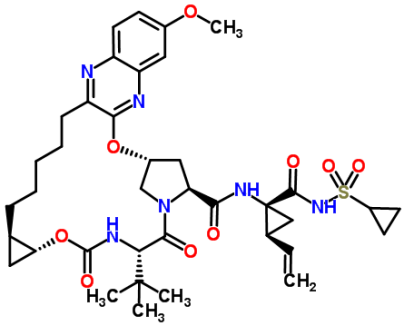


Figure (18): structure of grazoprevir

Grazoprevir is a second-generation protease inhibitor. It works by interfering with the HCV proteins NS3/4A. In laboratory experiments with cells and HCV, grazoprevir is active against HCV genotypes 1a, 1b, 2a, 2b and 3a. In initial clinical trials with this drug, grazoprevir is generally well tolerated. Side effects reported so far include fatigue, headache, nausea and diarrhea; these are usually of mild intensity and temporary [120].

INDICATION: Treatment with grazoprevir plus elbasvir, both with and without ribavirin and for both 12 and 18 weeks' treatment duration, showed high rates of efficacy in previously untreated patients with cirrhosis and previous PR-null responders with and without cirrhosis. These results support the phase 3 development of grazoprevir plus elbasvir [121].

The pharmacokinetics profile allows for once-a-day administration. Combined with pegylated interferon and ribavirin, grazoprevir results in a high rate of HCV eradication (in about 90% of cases) and a better outcome than boceprevir-based triple therapy. Also in interferon-free combinations, grazoprevir-associated eradication rates are very high (89 - 100%). Grazoprevir has a higher barrier to resistance than first-generation protease inhibitors and is active against most variants associated with resistance to first-generation protease inhibitors. Tolerability and safety profile are good. Although data are limited, grazoprevir appears to overcome most of the drawbacks of the first-generation protease inhibitors and is thus a very promising agent to be used in combination with other antivirals to eradicate HCV infection [122].

II

NON-STRUCTURAL 5B (NS5B) POLYMERASE INHIBITORS

STRUCTURE OF NS5B POLYMERASE: The viral polymerase NS5B (591 amino acids, *aa*) synthesizes a complementary negative strand RNA using as template genomic positive strand RNA. Schematically, the NS5B protein has the shape of a right hand. The catalytic domain, formed by N-terminal 530 aa, exhibits the classical *"fingers"*, *"palm"* and *"thumb"* subdomains typically seen in all RNA dependent RNA polymerases. The active site of NS5B is fully encircled by the fingers and thumb domains, which closely interact. All regular structures, studied until now, reveal a closed conformation, encircled on one side by the fingertips and on the other side by the linker and the so-called *β*-hairpin. Therefore, the active site is fully enclosed and the nucleotide molecules can bind easily with no further rearrangement of the domains. As observed in *in vitro* studies, NS5B is able to conduct a template-directed RNA synthesis on its own, requiring only divalent metals (magnesium or manganese) as cofactors. NS5B can also catalyze both *de novo* synthesis from a single-stranded template and primer extension from the subsequent RNA duplex or from a pre-annealed template/primer duplex [123].

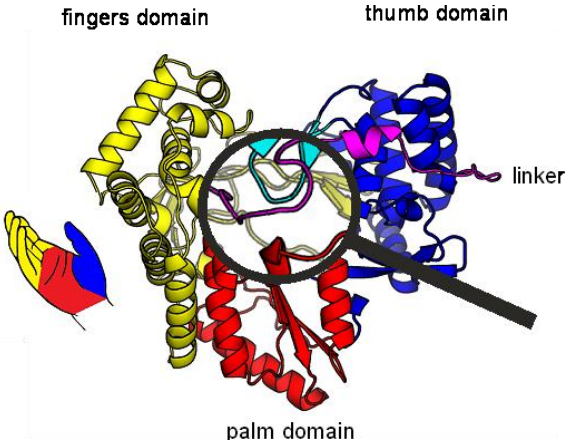


Figure (19): Schematically, the NS5B protein [124].

CLASSES OF NS5B POLYMERASE INHIBITORS

Several classes of nucleoside and non-nucleoside inhibitors, targeting the different allosteric sites, have demonstrated efficacy in clinical trials. Compared to other allosteric sites, thumb site I is a more compelling allosteric target with a significant number of inhibitors in clinical trials. Among them, indole analogues are the most important series of NS5B thumb site I inhibitors with considerable antiviral activity NS5B Inhibitors are classified in two main categories: nucleoside/nucleotide and non-nucleoside polymerase inhibitors [125,126].

A. NON-NUCLEOSIDE POLYMERASE INHIBITORS

Members of this class of DAAs bind outside the active site and target allosteric sites on the surface of the enzyme, downregulating the RdRp activity through induction of conformational changes (Fig. 20). Non-nucleoside inhibitors are so far specific for HCV genotype 1. However, the efficacy against genotype 1 subtypes may differ. The barrier to resistance of non-nucleoside inhibitors is considered to be low. Four groups of non-nucleoside inhibitors have entered clinical development: [127].

* *Thumb I inhibitors (benzimidazole site)*: Deleobuvir, MK-3281 and B ILB1941.
* *Thumb II inhibitors (thiophene site):* Filibuvir, VX-759, VX-916 and VX-222.
* *Palm I inhibitors (benzothiadiazine site)*: Dasabuvir, Setrobuvir and ABT-072
* *Palm II inhibitors (benzofuran site)*: Nesbuvir (HCV-796) and IDX-375

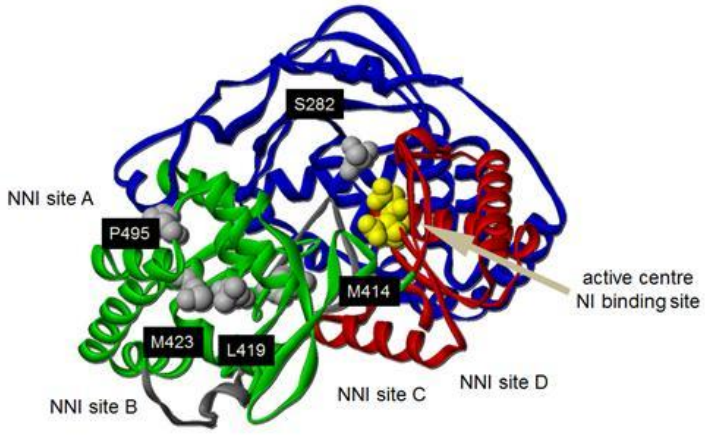


Figure (20): Structure of the HCV NS5B RNA polymerase and binding sites [128]

1. DELEOBUVIR (BI 207127)

CHEMICAL STRUCTURE & DESCRIPTION

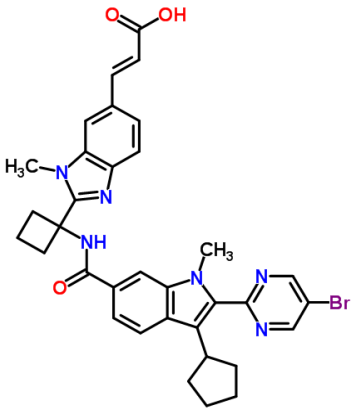


Figure (21): structure of deleobuvir

Deleobuvir was an experimental drug for the treatment of hepatitis C. It was being developed by Boehringer-Ingelheim. It is a non-nucleoside hepatitis C virus NS5B polymerase inhibitor.

INDICATION: the combination of deleobuvir, faldaprevir and ribavirin resulted in rates of a sustained virologic response of 52 to 69% 12 weeks after the completion of therapy [129]. In a study found that sustained virological response rates 12 weeks after completion of therapy were 17% and 95% in patients infected with HCV genotype-1a and genotype-1b respectively [130].

PHARMACOKINETICS: Deleobuvir had moderate to high clearance, and the half-life of about 3 hr, indicating that there were no metabolites with half-lives significantly longer than that of the parent. The low in vitro clearance was not predictive of the observed in vivo clearance, likely because major deleobuvir biotransformation occurred by non-CYP450-mediated enzymes that are not well represented in hepatocyte-based in vitro models [129].

ADVERSE EFFECTS: Adverse events were common; overall, 94% of patients had adverse events, and 9% had severe adverse events. Jaundice due to increased levels of bilirubin is more common.

CONTRAINDICATIONS: all contraindication of ribavirin.

2. FILIBUVIR (PF-868554)

CHEMICAL STRUCTURE & DESCRIPTION

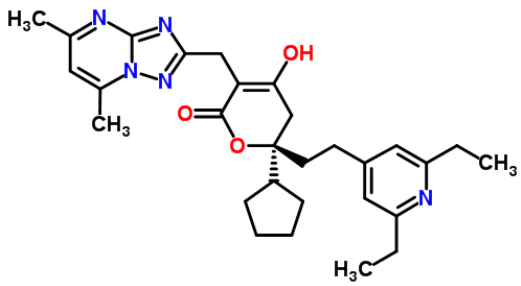


Figure (22): structure of Filibuvir

Filibuvir is a non-nucleoside HCV NS5B RNA-dependent RNA polymerase inhibitor, being developed by Pfizer for the potential treatment of chronic HCV infection. Filibuvir is a potent and specific inhibitor of the virally-encoded NS5B polymerase, and inhibited genotype 1 subgenomic HCV replication in the cell-based replicon system. Filibuvir demonstrated a good pharmacokinetics profile and oral bioavailability in preclinical animal studies, which is consistent with twice-daily dosing in humans. In phase I and IIa clinical trial in treatment-naïve patients infected with HCV genotype-1, filibuvir in monotherapy or in combination with pegylated IFNα2a/ribavirin for up to 4 weeks significantly reduced HCV RNA levels [131].

INDICATION: Filibuvir has shown great promise in phase IIb clinical trial. However, drug resistant mutations towards Filibuvir have been identified. In the present study, the drug resistance mechanism of wild-type (WT) and mutant NS5B polymerases (including V494I, V494A, M426A, and M423T) toward Filibuvir was investigated by molecular modeling methods [132]. Filibuvir plus pegIFN/ribavirin did not improve the percentage of patients achieving SVR compared with administration of pegIFN/ribavirin alone [133].

3. DASABUVIR (ABT-333, Exviera in Europe)

CHEMICAL STRUCTURE & DESCRIPTION

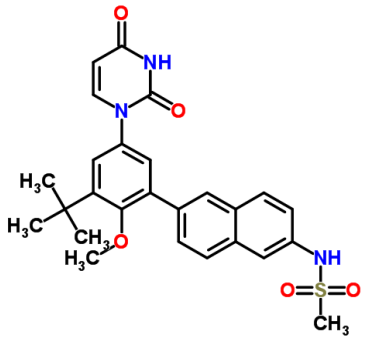


Figure (23): structure of dasabuvir

Dasabuvir is a non-nucleoside inhibitor of HCV NS5B which binds to the palm domain 1 of the enzyme. In vitro, dasabuvir has a good potency against the replicon of genotype 1a and 1b, with EC50 ranging from 2 to 7 nM. Moreover, dasabuvir showed a synergistic effect when combined for 3 days with the protease inhibitor ABT- 450 and the NS5A inhibitor ombitasvir in in vitro replicon model [134].

INDICATION: Dasabuvir is approved at the 19 of December 2014 by the FDA for use in combination with ombitasvir, paritaprevir, and ritonavir tablets co-packaged with dasabuvir tablets in the product Viekira Pak to treat chronic HCV genotype 1, including cirrhosis. Viekira Pak can be used with or without ribavirin, but it is not recommended for decompensated cirrhosis patients [134]. Results from multiple populations, including those considered difficult to treat, showed 91- 100% of participants who received Viekira Pak at the recommended dosing achieved SVR [134]. For more information, In particular dosing regimen, look paritaprevir on page 36.

PHARMACOKINETICS: An in vitro study conducted on human hepatocytes showed that dasabuvir did not exert inhibitory or inducing effects on cytochrome P450 enzymes. Its metabolite profile indicates a primary oxidative pathway which is followed by conjugation as a glucoronide. The same study evaluated the main in vivo pharmacokinetics parameters in rats

and dogs and showed that dasabuvir has a good bioavailability, a liver distribution that results in 12-hour levels significantly higher than its EC50 and finally biliary excretion [134].

Single doses of dasabuvir ranging from 10 to 1200 mg with mean half-life 5-8 hours and Cmax of approximately 3 hours post-dose. The Cmax increased proportional to the dose. It is studied that food has no impact on the main pharmacokinetics parameters (PP) of dasabuvir. The PP, evaluated in presence of ketoconazole, an inhibitor of cytochrome P-4503A, showed a modest increase in Cmax and AUC [134].

ADVERSE EFFECTS: Viekira Pak is the fourth drug product which approved by the FDA to treat chronic HCV infection. The FDA approved Olysio (simeprevir) in November 2013, Sovaldi (sofosbuvir) in December 2013 and Harvoni (ledipasvir and sofosbuvir) in October 2014. The most common side effects of Viekira Pak are feeling tired and weak or lack of energy, itching, nausea and trouble sleeping [135]

4. SETROBUVIR (ANA-598)

CHEMICAL STRUCTURE & DESCRIPTION

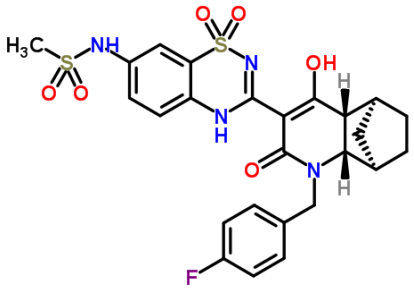


Figure (24): structure of Setrobuvir

Setrobuvir was discovered at Anadys Pharmaceuticals and is a NS5B polymerase inhibitor that binds to the palm I of this enzyme. It is currently in Phase IIb clinical trials and a candidate for the treatment of CHC genotype-1 in combination with interferon and ribavirin [136].

5. BECLABUVIR (BMS-791325)

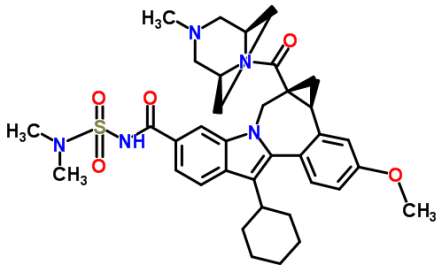


Figure (25): structure of beclabuvir

Beclabuvir is targeting the thumb site 1in HCV NS5B, an RNA-dependent RNA polymerase. Beclabuvir is currently in phase 3 clinical development with the most potent NS5B inhibitor activity. Robust viral clearance of HCV was observed in infected patients treated with beclabuvir in combination with other anti-HCV drugs in Phase 2 clinical studies [137].

Beclabuvir is an allosteric inhibitor of the HCV NS5B enzme at 50% inhibitory concentrations (IC_{50}) below 28 nM, inhibits recombinant NS5B proteins from HCV genotypes 1, 3, 4, and 5. In vitro study on cell culture has schown that, beclabuvir inhibited replication of HCV subgenomic replicons representing genotypes 1a and 1b at 50% effective concentrations (EC_{50}) of 3 nM and 6 nM, respectively, with similar (3–18 nM) values for genotypes 3a, 4a, and 5a. Potency against genotype 6a showed more variability (9–125 nM) and activity was weaker against genotype 2 (EC_{50} 87–925 nM) [138].

6. NESBUVIR (HCV-796)

CHEMICAL STRUCTURE & DESCRIPTION

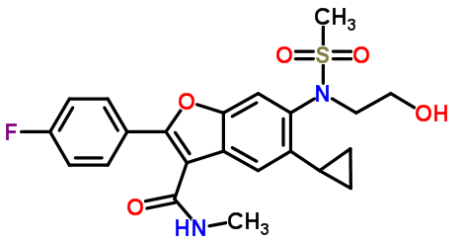


Figure (26): structure of Nesbuvir

Nesbuvir is a selective HCV NS5B RNA-dependent RNA polymerase inhibitor. Investigation on this drug has stopped [139].

B. NUCLEOSIDE POLYMERASE INHIBITORS

1) SOFOSBUVIR (GS-7977, brand names **Sovaldi**, **Resof**, **Hepcvir**, **SoviHep)**

CHEMICAL STRUCTURE & DESCRIPTION

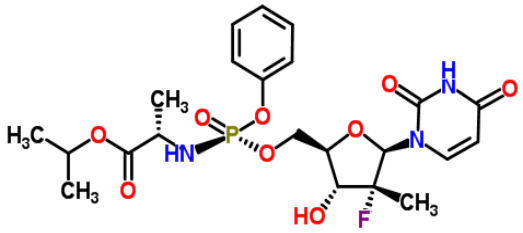


Figure (27): structure of sofosbuvir

Sofosbuvir (SOF) is a HCV NS5B RNA-dependent RNA polymerase inhibitor with a pangenotypic activity and a very high barrier to resistance. Thus SOF in monotherapy should be avoided and must only be oraly taken in combination with other antivirals [48].

Sofosbuvir is a highly conserved region not only among quasispecies but also among HCV genotypes 1a, 1b, and 2a. Sofosbuvir is an oral direct uridine nucleotide analog that phosphorylates within the host hepatocyte to the active triphosphate form and, by competing with the natural nucleotides, causes premature RNA chain termination in the viral genome [140].

PHARMACOKINETICS: After single or multiple dosing, sofosbuvir was reported to be absorbed orally within a median Tmax of one hour (range: 30 – 180 min). Elimination of sofosbuvir is rapid and well cleared within a median of about 48 - 75 min. Sofosbuvir is metabolized by the liver to GS-331007 which has a longer Tmax (median 4 hr, range: 1.5–8 hr) and a longer half-life (range: 7.27-11.80 hr). It has no different half-life between patients with and those without hepatic impairment. The Cmax and AUC of sofosbuvir was roughly 80% and 130% higher in subjects with hepatic impairment than in non-cirrhotic patients. Several

studies have been conducted using sofosbuvir either in monotherapy or combined with Peg-IFN, ribavirin, or other DAAs [140**].**

INDICATION: Sofosbuvir based regimens in compare to previous HCV infection treatment regimens provide a higher cure rate, fewer side effects, and a two to four times reduced duration of therapy [141,142,143]. Sofosbuvir allows most patients to be treated successfully without the use of peginterferon [144]. In early 2014, a recommendation for the management of hepatitis C has published by the American Association for the Study of Liver Diseases and the Infectious Diseases Society of America jointly. In this recommendation, sofosbuvir and ribavirin, with or without pegylated interferon, are parts of all first-line treatments for HCV genotypes 1 to 6, and also part of some second-line treatments [145]. This drug has been marketed since 2013.

The triple combination of Sofosbuvir with PEG-IFN and RBV for 12 weeks in naïve patients results in a SVR in more than 95%. The results of the first oral combination of Sofosbuvir and RBV for 12 weeks in genotype 3-infected patients have been rather disappointing with a slightly lower SVR around 60%, and only 30% in patients with cirrhosis, than after 24 weeks of PEG-IFN. Extending treatment from 12 to 16 weeks in treatment experienced patients doubled the SVR rate and an 80% SVR rate is expected by extending treatment to 24 weeks. The best oral combination of new DAAs is probably the combination of Sofosbuvir and a NS5A inhibitor (Daclatasvir, Ledipasvir…) for 24 weeks, which resulted in a 100% SVR rate in a limited series [146].

ADVERSE EFFECTS: sofosbuvir as monotherapy has a good safety profile with only a slight decrease in hemoglobin levels (0.54g/dl) and a reduced cumulative incidence of side effects compared to patients who received interferon-containing combinations. The most frequent adverse events are headache, insomnia, fatigue, nausea, dizziness, upper respiratory tract infections, rash, back pain, grade 1 anemia, and grade 4 lymphopenia [140].

CONTRAINDICATION: Sofosbuvir alone has been assigned a Pregnancy Category B by the FDA and currently it is not known whether sofosbuvir passes into breastmilk. Therefore, it is recommended that the mother does not breastfeed during treatment with sofosbuvir alone or in combination with ribavirin [147,148].

2) MERICITABINE (RG7128)

CHEMICAL STRUCTURE & DESCRIPTION

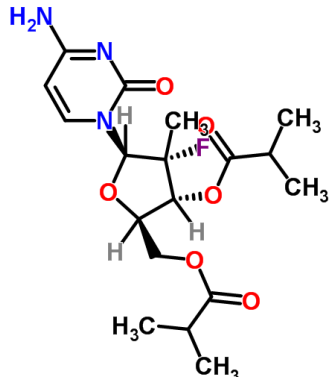


Figure (28): structure of Mericitabine

Mericitabine is a first-in class nucleoside polymerase inhibitor (NPI), which requires intracellular uptake and phosphorylation to two active triphosphates. Mericitabine is an oral cytidine nucleoside analog prodrug that exhibited strong antiviral effectiveness against the HCV polymerase across all HCV genotypes, with no evidence of resistance reported in patients treated with mericitabine monotherapy for two weeks [149].

MECHANISM OF ACTION: Mericitabine is a tri-ester prodrug of RO5855 (PSI-6130, β-D-2′-deoxy-2′-fluoro-2′-C-methylcytidine). After oral administration, mericitabine is hydrolyzed to form RO5855, which is taken up by hepatocytes and metabolized into 2 active polymerase inhibitors, RO5855-triphosphate and the corresponding uridine-triphosphate (as the result of the deamination of the monophosphorylated RO5855 which, in turn is di- and tri-phosphorylated), both compete with natural nucleoside triphosphates at the active site of the HCV RNA-dependent RNA polymerase [150].

INDICATION & DOSING: The efficacy, safety and tolerability of mericitabine in a dose of 1000mg twice daily in combination with PEG-IFN/RBV for 8 and 12 weeks on 408 chronic HCV genotype-1 and 4 infected naive patients has been investigated. The rapid viral response (RVR) rates were up to 62% in the treatment arms with mericitabine compared with 18% in the control arm with PEG-IFN/RBV alone [123].

III

HCV NON-STRUCTURAL 5A (NS5A) INHIBITORS

GENERALITIES: HCV NS5A is a RNA-binding phosphoprotein which undergoes post translation processing by NS3 viral protease. HCV NS5A mainly exists in two phosphorylated forms, namely hypophosphorylated (p. 56) and a hyperphosphorylated (p. 58). Recently have shown that the host factor, phosphatidyl-inositol-4 kinase III alpha (PIKIIIα) binds to and regulates the phosphorylation status of NS5A at the center and near the C terminus, whereas p58 is a form of hyperphosphorylated NS5A at the center of the serine-rich region. The phosphorylation state may play an important role in assigning its various roles in RNA replication and virus assembly. NS5A is anchored to the endoplasmic reticulum (ER) and ER-derived membranes through an N-terminal amphipathic α-helix. It is thought that this structure is conserved across all HCV genotypes.

NS5A is further consists of three domains and two linker regions. Each structure shows unique homodimeric conformations. Domains II and III of NS5A are essentially unstructured and highly flexible. It is this flexibility that may explain the broad spectrum of functions that have been associated with NS5A. NS5A interacts with a wide array of viral factors like, HCV RNA and NS5B enzyme, and host factors include various kinases as well as the lipid membrane of the ER that form pockets of HCV replication within "membranous web-like" structures. Domain II of NS5A interacts with the host factor cyclophilin A (CypA). Disrupting this interaction is detrimental to viral replication, and several CypA inhibitors are potent antivirals. Of note, mutations that emerge under selective pressure of CypA inhibitors are located in domain II of HCV NS5A [151]. Some HCV NS5A inhibitors are as follow:

1. OMBITASVIR (ABT-267, Ombitasvirum, Ombitasvirum hydricum)

CHEMICAL STRUCTURE & DESCRIPTION

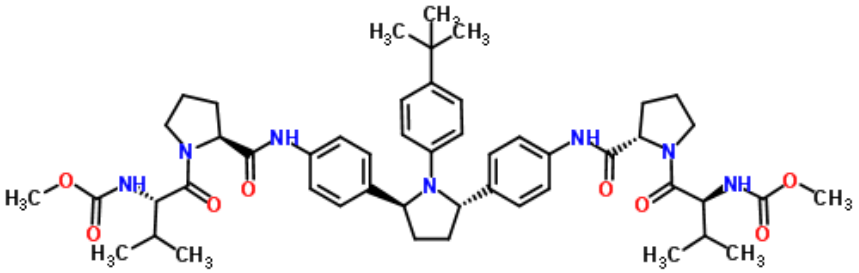


Figure (29): structure of ombitasvir

Ombitasvir is a HCV NS5A inhibitor with picomolar potency, pan-genotypic activity, and 50% effective concentrations (EC_{50}s) of 0.82 to 19.3 pM against HCV genotypes 1 to 5 and 366 pM against genotype 6a [152].

INDICATION & DOSING: Ombitasvir is a member of the Viekira Pak component which is the fourth drug product approved by the FDA to treat chronic HCV infection. The FDA approved Olysio (simeprevir) in November 2013, Sovaldi (sofosbuvir) in December 2013 and Harvoni (ledipasvir and sofosbuvir) in October 2014. A 12 weeks multitargeted regimen of paritaprevir/ritonavir, ombitasvir and dasabuvir with ribavirin was highly effective in previously untreated HCV genotype 1 infected patients with no cirrhosis. For more information regarding Viekira Pak alone or in combination with ribavirin, look the other members of this pack in above [153,154].

ADVERSE EFFECTS: lack of energy, tiredness, weakness, itching, rash, reddening of the skin, nausea and vomiting, sleeping difficulty, falling asleep or staying asleep, loss of appetite, yellowing of skin or eyes and changes in stool color are the most common side effects that reported in clinical trial participants [148].

Viekira Pak should keep and storage tightly closed in its own drug package at room temperature and away from excess heat and moisture, and out of reach of children.

2. DACLATASVIR (BMS-790052, trade name Daklinza)

CHEMICAL STRUCTURE & DESCRIPTION

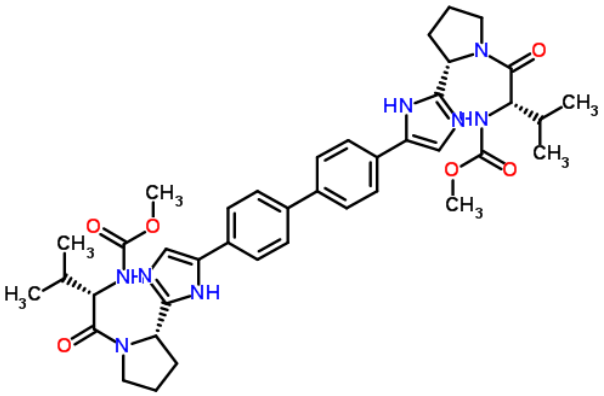


Figure (30): structure of daclatasvir

INDICATION: Daclatasvir is an HCV NS5A oral PI with a pan-genotypic activity, but a lower barrier to resistance in genotype 1a. A recent phase IIb study evaluated a triple combination comprising daclatasvir plus PegIFN/RBV including treatment-naïve patients with HCV genotype 1. Based on this preliminary data, daclatasvir at daily dose of 60mg plus PegIFN/RBV regimen has been included as an option for the treatment of genotype 1b and 4 patients. This combination regimen of three medications should be administered for 12 weeks. It is recommended that those patients do not achieve an HCV-RNA level < 25 IU/mL at week 4 and undetectable at week 10, all three drugs should be continued for an additional 12 seeks. Conversely, PegIFN/RBV should be continued alone between week 12 and 24 in those who achieve such response [155, 156].

DOSING & PHARMACOKINETICS: The efficacy of daclatasvir was confirmed in a single ascending-dose-study in which a mean 3.3$\log_{10}$ reduction in viral load 24 hr after drug administration was observed in patients receiving a 100mg dose. Daclatasvir caused a decrease in serum HCV RNA levels by about two orders of magnitude within 6 hr of administration. Daclatasvir at daily dose of 60mg has a plasma half-life of 12.8hr, Cmax 1726 ng/ml, Cmin 255 ng/ml, AUC 15121 ng.hr/ml, Protein Binding rate more than 99%. It is transported by P-glycoproteins and metabolizing by CYP3A4 liver enzyme [157,158].

3. LEDIPASVIR (GS-5885)

CHEMICAL STRUCTURE & DESCRIPTION

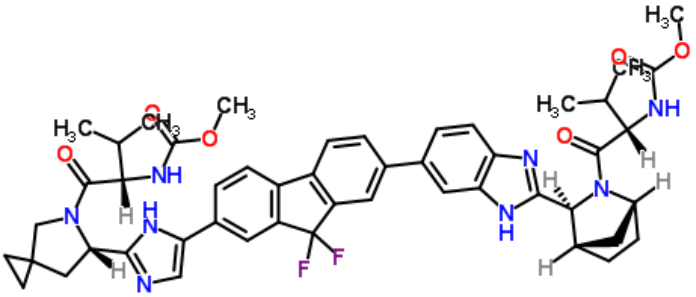


Figure (31): structure of ledipasvir

Ledipasvir inhibits an important viral NS5A phosphoprotein, which is involved in viral replication, assembly, and secretion. Ledipasvir is used for the treatment of chronic HCV infection [159]. After completing Phase III clinical trials, on October 10, 2014 the FDA approved the combination product ledipasvir 90 mg/sofosbuvir 400 mg (***Harvoni***) for genotype 1 hepatitis C [160].

INDICATION: Harvoni is a therapeutic regimen which interferes with HCV replication and can be used without PEG-IFN-α or ribavirin to treat genotype 1a or 1b infected patients. A triple regimen of *Ledipasvir, Sofosbuvir and Ribavirin* produced a 12-week post-treatment sustained virological response (SVR12) rate of 100% in both treatment-naive patients and prior non-responders with HCV genotype 1. This data presented at the 20th Conference on Retroviruses and Opportunistic Infections in March 2013[161,162].

PHARMACOKINETICS: Ledipasvir has oral bioavailability of 76%, half-life of 47hr, and plasma protein binding of about 99%. It is not metabolized by cytochrome.

ADVERSE EFFECTS: Ledipasvir plus sofosbuvir is a therapeutic regeimen which has been very well tolerated. According to clinical trials, the most common side effects of this regimen, fatigue and headache reported [163].

DRUG INTERACTION: Harvoni has the most drug instruction with St.John's Wort or rifampicin and other P-glycoprotein-inducers. The coadministration will cause decrease the blood concentration and thus therapeutic effects of Ledipasvir/sofosbuvir [163].

4. ELBASVIR (MK-8742)

CHEMICAL STRUCTURE & DESCRIPTION

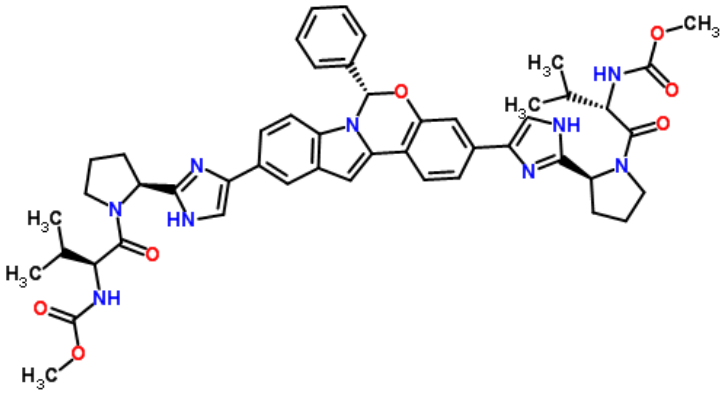


Figure (32): structure of elbasvir

Elbasvir interferes with the HCV protein NS5A. In laboratory experiments with cells and HCV, elbasvir is active against most strains of HCV, including genotypes 1a, 1b, 2a, 3a and 4a. Side effects are similar to those reported for grazoprevir [120].

INDICATION: Elbasvir is experimental drug that combind with grazoprevir for the treatment of CHC infections. It is currently in Phase III trials of Merck developing anti-HCV drugs. It is used in combination with the NS3/4a protease inhibitor grazoprevir, either with or without ribavirin [121].

5. SAMATASVIR (IDX719)

CHEMICAL STRUCTURE & DESCRIPTION

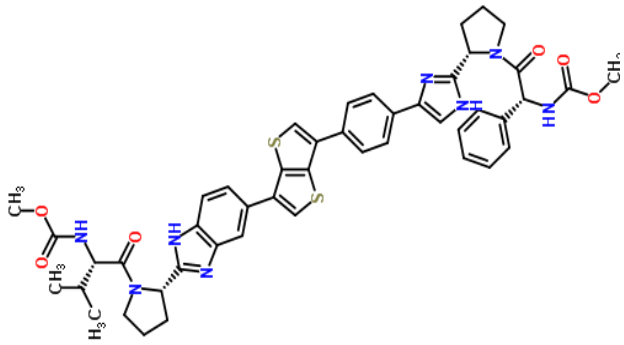


Figure (33): structure of samatasvir

Samatasvir is a NS5A inhibitor which selectively inhibits the HCV replication with high activity across genotypes, potentially affording a once-daily single-pill dosing regimen for all genotypes. Samatasvir inhibited genotype 1a and 1b HCV replicons at picomolar concentrations, with a mean CC_{50} value of >100 µM in the Zluc genotype 1b replicon cell line. Study has schown that the activity of samatasvir is decreased at the presence of human serum albomin and alpha-1 acid glycoprotein (AAG). Because, samatasvir like other antiviral drugs binds to the serum proteins, and this binding has been associated with reduced drug efficacy [164].

COMBINABILITY: Samatasvir has a mild synergistic effect in the interferon combination therapy but a moderate antagonistic effect with boceprevir or telaprevir, and a mild antagonistic effect with ribavirin combination therapy regimens has been repoted [164].

PHARMACOKINETICS: Following oral administration in HCV-infected subjects at daily doses of 25, 50, and 100 mg, samatasvir exhibited dose-related plasma exposures. Peak exposures were reached with a median time of 3 to 4 hours postdose. With a half-life of approximately 20hr, plasma samatasvir increased over time with a mean accumulation ratio of approximately 50% based on trough exposures for QD dosing. For the same total daily dose, samatasvir 50 mg twice daily achieved higher trough exposures than did the 100 mg QD dose although no marked

differences in antiviral activity were noted between the two regimens. Both 100 mg QD and 50 mg BID reached trough concentrations that were at least 7 fold above the protein-binding adjusted 90% effective concentration (EC_{90}) of samatasvir against the least susceptible HCV genotype (genotype 2a, EC_{90} = 2.3 ng/ml), while plasma concentrations of samatasvir remained above the EC_{90} over the entire dosing interval after multiple dosing for all doses/regimens [165].

ADVERSE EFFECTS: the most frequent AEs reported were constipation, headache, and nausea, catheter site pruritus, dyspepsia, decreased appetite, insomnia. All AEs were mild or moderate in intensity and did not appear to be dose related [165].

HOST FACTORS AS TARGETS FOR TREATMENT of HCV INFECTIONS

HCV depends on various host factors throughout its life cycle. One of the most important host factors is cyclophilins. The cyclophilins are a group of proteins with peptidyl-prolyl isomerase enzymatic activity, localised in different cellular compartments and involved in a variety of functions related to cell metabolism and energy homeostasis, having enhanced expression in inflammation or malignancy. Cyclophilin A (CypA), the most abundantly expressed cyclophilin, is present mainly in the cytoplasm and is a host factor involved in the life cycle of multiple viruses. The extracellular fractions of CypA and CypB are potent pro-inflammatory mediators. Cyclosporines are the prototype cyclophilin inhibitors. Cyclic peptides, which bind and inhibit cyclophilins without having immunosuppressive properties, have been generated by chemical modifications of cyclosporin A. Many tissues of the human body expressing the Cyp B. This molecul has a cis-trans isomerase activity that supports the folding and function of many proteins. Cyp B enhances HCV replication through the modulation of NS5B activity, but the complete mechanisms of action of Cyp is currently not derstood [3,166].

NON-IMMUNOSUPPRESSIVE CYCLOPHILIN INHIBITORS

Cyclophilin inhibitors with non-immunosuppressive properties have been generated by chemical modifications of the Cyp A molecule to produce alisporivir (DEB-025), NIM-811 and SCY-635. Alisporivir differs from the parent molecule Cyp A by purposely substituting two amino acids - sarcosine with D-alanine at position 3, and N-methyl-leucine with N-ethyl-valine at position 4 [166].

ALISPORIVIR (DEBIO-025)

CHEMICAL STRUCTURE & DESCRIPTION

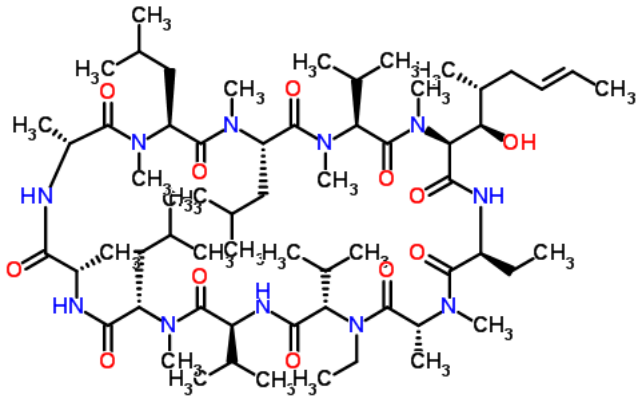


Figure (34): structure of Alisporivir

Alisporivir is Cyp B inhibitor exerting an antiviral impact on both HCV and also HIV replication. Alisporivir with 1200 mg twice daily dose for two weeks, in clinical trials in HCV/ HIV-coinfected patients, led to a mean maximal log10 reduction of HCV RNA and HIV DNA [3] and [167].

INDICATION: Alisporivir at therapeutic doses of 200 mg, 600 mg and 1000 mg in combination with PEG-IFN α-2a for 4 weeks in treatment naïve HCV genotypes 1, 2, 3 or 4 infected patients evaluated. The result showd a mean log10 reductions in HCV RNA level at 1000 mg dose than 600 mg and 200 mg doses in the PEG-IFN α-2a combination therapy and also than the PEG-IFN α-2a alone and 1000 mg alisporivir monotherapy groups. A triple combination therapy regimen of Alipasvir with PEG-IFN α-2a/RBV in a Phase II clinical trial in treatment-naïve HCV genotype 1 for 24-48 weeks resulted high sustained virologic response rates (69-76% compared to 55% in the control group). Also an interferon-free regimen of alipasvir in combination with ribavirin has been conducted. According to the promising obtained data, the development of alisporivir was put on hold due to rare cases of severe pancreatitis in combination therapy with PEG-IFN α-2a. Recently further development of alisporivir is re-initiated [3,48,168].

MIRAVIRSEN (SPC3649)

DRUG DESCRIPTION: Miravirsen is a locked nucleic acid-based antisense oligonucleotide or ribonucleotides interspaced throughout a DNA phosphorothioate sequence. It is selectively delivered to the liver and inhibites the HCV replication through effectively inhibition of liver miR-122.

MECHANISM OF ACTION: MicroRNAs (miRNAs) are short noncoding RNAs, which bind to messenger RNAs (mRNAs) and regulate protein expression. The miR-122 is a liver-specific miRNA which play a significant role in the replication of HCV. Miravirsen is an anti-miRAN which target MiR-122 following intravenous injection. Miravirsen may work mainly by hybridizing to mature miR-122 and blocking its interaction with HCV RNA. As well as the biosynthesis of miRNAs includes two precursors, a primery miRNA (pri-miRNS) transcript, and a shorter pre-miRNA, thus Miravirsen also target sequence in pri- and pre-miR-122. It is currently in clinical trails for treatment of chronic hepatitis C Virus Genotype 1 infection [167].

THIRD CHAPTER

COMBINATION THERAPIES

I. INTERFERON FREE COMBINATION THERAPIES AND TRIALS

Currently many direct acting antivirl agents as monotherapy or mostly in combined therapy with each other or in combination with standerd therapy members namely interferon and ribavirin, for the treatment of chronic hepatitis C infections, are either worldwide have already aproved or they are trail stages. At present studies for the successful treatment of HCV infections are focused on two main areas. (1) Viral factors, mainly the non-stractural proteins of HCV. (2) Host or cellular factors. Many classes of DAA drugs are effectively targeting the non-stractural HCV proteins which are responsible for HCV proteins cleavage and thus replication. For instance, the NS3/4a protease inhibitors that are combining with an agent targeting the HCV polymerase complex, eg, NS5B nucleoside or non-nucleoside polymerase inhibitors, or NS5A inhibitors Nis. Have a look on pages 11 & 12 for more information.

A. DUAL *COMBINATIONS of DAAs ± RBV*

Sofosbuvir (*Sovaldi*)* + *Ledipasvir (Harvoni): Harvoni is the first interferon-free DAA regimen to be issued a notice of compliance (NOC) in Canada. This regimen is indicated for the treatment of genotype 1 CHC. Sofosbuvir 400mg is combined with Ledipasvir 90mg daily in a fixed-dose combination (FDC) pill for HCV genotype-1 treatment-naïve patients. This therapeutic regimen in a single oral daily fixed dose resulted an SVR of more than 93% after 8 weeks and more than 97% after 12 weeks in HCV-1 treatment-naïve patients. It also resulted in SVR rates >93% after 12 weeks in previous non-responders with or without RBV. Evidence

suggests that Harvoni is well tolerated, with reduced treatment duration and high SVR rates [170,171,172].

Sofosbuvir + Simeprevir (*Olysio*): Sofosbuvir at a dose of 400mg daily in combination with 150mg daily dose of Simeprevir which is a protease inhibitor, was evaluated ± RBV for 12 or 24 weeks. Result showed the 12 weeks of sofosbuvir and simeprevir without RBV appears to be a highly effective regimen with a good safety profile in HCV-1 patients. The most common advers effects of this regimen were headache, fatigue, nausea and increased blood amylase concentration [170,184].

Sofosbuvir + Daclatasvir (Daklinza): Sofosbuvir 400mg daily in combination with daclatasvir 60mg daily ± RBV in treatment-naive HCV genotype-1 patients without cirrhosis. The successful result of treatment (SVR rate: 100%) obtained from sofosbuvir plus daclatasvir for 12 weeks without RBV. Adding RBV or increasing the duration of treatment did not seem to increase the SVR rates [170,171].

***Sofosbuvir* + GS-5816:** Administration of Sovosbuvir 400mg plus 25mg or 100mg of GS-5816 which is NS5A inhibitor, with and without ribavirin, for 12 weeks in a large number of patients (154 patients), including 13/14 HCV-4 resulted in an overall SVR rate more than 95%. Rates of SVR-12 ranged from 88% to 100% among those receiving SOF plus GS-5816 100mg for 12 weeks [171,173].

Grazoprevir + Elbasvir: The therapeutic regimen of *grazoprevir* 100mg daily plus *elbasvir* 25mg and/or 50mg daily ± ribavirin have been shown high rates of SVR rates. The efficacy of this regimen was not enhanced by addition of RBV or increasing the treatment duration to 18 weeks. The safety profile was good. Grazoprevir/Elbasvir regimen is under evaluation in large ongoing phase III trials [120,121,122,170].

Danoprevir + Mericitabine: The experimental hepatitis C drugs Danoprevir plus Mericitabine for 12 weeks in combination with PEG-IFN/RBV in CHC infected patients, resulted a profound and greater antiviral suppression than the additive effects of either monotherapy

In a triple therapy, Danoprevir with 100mg twice-daily plus Mericitabine 1000mg twice-daily ± ribavirin 1000-1200mg daily, showed good safety and efficacy in previously treated HCV genotypes 1a or 1b patients. Muscle and joint aches, fatigue, headache, and as well as gastrointestinal symptoms are reported as most common advers effects of this regimen [177,185,186].

Faldaprevir + Deleobuvir: Recently, the antiviral effect and safety of Faldaprevir at a dose of 120 mg once daily plus Deleobuvir at doses of 400 or 600 mg three times daily with RBV 1000–1200mg daily for 4 weeks were evaluated. A higher rate of response was observed in patients with genotype 1b than in genotype 1a infections. The most frequent adverse events were mild gastrointestinal disorders, rash and photosensitivity [177, 178].

Tegobuvir + GS-9256: Gilead developed an oral combination therapeutic regimen of Tegobuvir 40 mg twice daily plus 75 mg twice daily GS-9256, a NS3 serine protease inhibitor, and/or plus RBV 1000–1200 mg daily or tegobuvir and GS-9256 plus PEG-IFNalfa-2a/RBV [177].

Daclatasvir + Asunaprevir (Sunvepra): Japan approves first all-oral, interferon- and ribavirin-free hepatitis C treatment dual regimen [174].

B. *TRIPLE and QUADRUPLE COMBINATIONS of DAAs ± RBV*

Daclatasvir + Asunaprevir + Beclabuvir: Daclatasvir 60mg once daily, Asunaprevir 100mg twice daily and Beclabuvir 75mg or 150mg twice daily, and/or this combinations with different fixed-doses (daclatasvir 30mg plus asunaprevir 200mg plus beclabuvir 75mg) for the treatment of non-cirrhotic and cirrhotic HCV-1 infectid patients, are an ongoing clinical trial study investigating 12-week regimens. [175,176].

Paritaprevir + Ombitasvir + Dasabuvir: It is a multitargeted, all-oral, IFN-free regimen for 12-weeks treatment. Paritaprevir coformulated with the ombitasvir and the dasabuvir twice daily, with or without RBV with high efficacy. This is in phase III trials with excellent results [170].

Sofosbuvir + Grazoprevir + Elbasvir: The triple regimen of Sofosbuvir 400mg once daily in combination with Grazoprevir 100mg plus Elbasvir 50mg is being evaluated in a phase II study for 4, 6 and 8 weeks. The result showed that this regimen was generally well-tolerated.

Headache, fatigue and nausea are the most common reported advers effects of HCV the therapy regimen [120,121,122,170].

Ombitasvir + Paritaprevir + Dasabuvir with Ritonavir (Holkira): This combination is used for treatment of Chronic Hepatitis C. Health Canada has recently approved the Holkira Pak (ombitasvir/paritaprevir /Ritonavir + Dasabuvir) for the treatment of CHC genotype 1 infected pateints. This therapy regimen is specifically approved without ribavirin in non-cirrhotic patients with genotype 1b infection, and in combination with ribavirin in non-cirrhotic patients and those with compensated cirrhosis with genotype 1a infection. In Holkira Pak the three DAAs with differing mechanisms of action (12.4mg ombitasvir, 75mg paritaprevir, and 50mg ritonavir) are prepared in one combination tablet and 250mg dasabuvir is compressed in a separet tablet. These tablets are co-packaged in weekly cartons of each daily dose for convenience [172].

Paritaprevir/Ritonavir + Ombitasvir + Dasabuvir: look viekira Pak.

Table (3): Combinations of one DAA with PEG-IFN/RBV [179]

Combination	Patients	SVR	Reference
Vaniprevir+PR vs PR	Treatment-naïve G1 treatment exp G1 treatment exp & cirrhotics	53-77% in different study groups vs. 14% in PR group	Manns *et al* [61] Lawitz *et al* [62] Rodriguez-Torres *et al* [63]
Daclatasvir+PR vs PR	Naïve G1-4 Naïve G2 & G3 Naïve/Non-Resp G1 Naïve/Non-Resp G1	G1b: 87%, G4: 100% G2 : 83% vs. 63% G3: 67-69% vs. 59% 89-100%/50-78% vs. 75% 68-90%/22-33% vs. 62%	COMMAND-*1* [64] COMMAND: Dore *et al* [65] Izumi *et al* [66] Suzuki *et al* [67]
Asunaprevir+PR	Naïve G1 and G4	G1: 63,5% vs. 45% G4: 89% vs. 43%	Bronowicki *et al* [68]
Danoprevir/r+PR	Naïve G1,G4 Null responders Naïve G1	66-89% vs. 38.5% (PR) 67% 68-85% vs. 42% (PR)	DAUPHINE: Everson *et al* [56] Gane *et al* [69] Marcellin *et al* [70]
MK-5172+PR vs PR	Naïve G1 non cirrhotics	86-91% vs. 61%	Manns *et al* [71]
Mericitabine+PR vs PR	Naïve G1/G4 Naïve G1/G4	57% vs. 36% 51% vs. 32%	Pockros *et al* [72] Weldemeyer *et al* [73]

G, genotype

Table (4): Combinations of two DAAs with PEG-IFN/RBV [179]

Combination	Patients	SVR	Reference
VX-222+telaprevir+PR	Naïve G1	82-93%	Nelson *et al* [74]
Daclatasvir+asunaprevir±PR	Null responders G1a and 1b	78-95%	Lok *et al* [57]
MATTERHORN: Mericitabine+PR (triple) or+danoprevir+PR (quadruple) or+danoprevir+RIBA (IFN-free)	Treatment-experienced G1	95% triple or 100% quadruple or 44-72% IFN-free	Feld *et al* [75]
Ledipasvir+GS-9451(PI)+PR vs. PR	Naive G1 *IL28B* CC Treatment-experienced G 1	79% 6 wks vs. 98% 12 wks vs 73% (PR) Relapser 80% (1a)-94% (1b) Partial respondres 52%(1a)-100% (1b) Null 47%(1a)-100% (1b)	Thompson *et al* [76] Everson *et al* [77]

G, genotype

Table (5): DAAs families with their specific target sites on HCV [179]

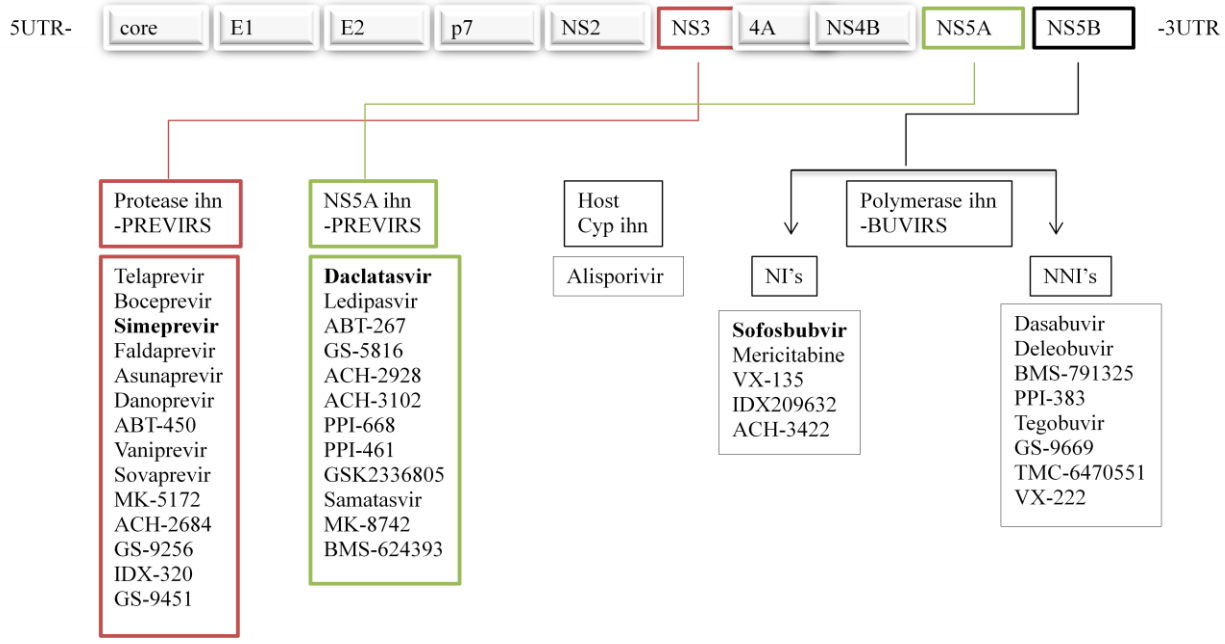


Table (6): Selected DAAs and host-targeting agents in the pipeline [179,180,181,182]

No	Drug name	Company	Trarget/active site	Phase or statuse
	NS5A inhibitors			
1	Daclatasvir (BMS-790052)	Bristol Mayers Squibb	NS5A domain 1 inhibitor	Approved [154]
2	Ledipasvir (GS-5885)	Gilead	NS5A protein	Approved [154]
3	Samatasvir (IDX719)	Idenix	NS5A protein	II [162]
4	Ombitasvir (ABT-267)	Abbott	NS5A protein	Applied [154]
5	Elbasvir (MK-8742)a	Merck	NS5A protein	III [154]
6	BMS-824393	Bristol Mayers Squibb	NS5A protein	I
7	PPI-461	Presidio Pharmaceuticals	NS5A protein	I
8	PPI-668	Presidio Pharmaceuticals	NS5A protein	III
9	GS-5816	Gilead	NS5A protein	II
10	ACH-2928	Achillion	NS5A protein	
11	ACH-3102	Achillion	NS5A protein	
12	GSK2336805	Gilead/ Merck	NS5A protein	
13	BMS-624393	Bristol Mayers Squibb	NS5A protein	
14	ABT-530	Abbott	NS5A protein	II
No	**Drugs tarageting host factors**			
1	Alisporivir (Debio-025)	Novartis	Cyclophilin inhibitor	Halted
2	NIM811	Novartis	Cyclophilin inhibitor	Halted
3	SCY-635	Scynexis	Cyclophilin inhibitor	II
4	Miravirsen	Santaris	miRNA122 antisense RNA	II

Grazoprevir (MK-5172) and Elbasvir (MK-8742) grouped together as MK-5172A.

Table (7): Selected DAAs and host-targeting agents in the pipeline [179,180,181,182,183].

No	Drug name & Target	Company	Trarget/active site	Phase or status
	NS3/4A protease inhibitors			
1	Ciluprevir (BILN2061)	Boehringer Ingelheim	Active site/macrocyclic	Stopped
2	Telaprevir (VX-950)	Vertex	Active site/linear	Approved
3	Boceprevir (SCH503034)	Schering-Plough	Active site/linear	Approved
4	Simeprevir (TMC435)	Janssen/Medivr	Active site/macrocyclic	Approved
5	Danoprevir (R7227)	Roche/InterMune	Active site/macrocyclic	III
6	Vaniprevir (MK-7009)	Merck	Active site/macrocyclic	Halted/II
7	Narlaprevir (SCH900518)	Schering-Plough	Active site/linear	Halted/ III
8	Asunaprevir (BMS-650032)	Bristol Mayers Squibb	Active site	Approved
9	Faldaprevir (BI 201335)	Boehringer Ingelheim	Active site/linear	Stopped
10	Sovaprevir (ACH-1625)	Achillion	Active site/macrocyclic?	Applied
11	Grazoprevir (MK-5172)	Merck	Active site/macrocyclic	III
12	Paritaprevir (ABT-450)	Abbott	Active site	Approved
13	PHX1766	Pheromix	Active site	Halted
14	GS-9256	Gilead	Active site	II
15	GS-9451	Gilead	Active site	Ib
16	IDX-320	Idenix	Active site	II
17	ACH-2684	Achillion	Active site	
No	**Nucleoside analog NS5B polymerase inhibitors (NI's)**			
1	Valopicitabin (NM283)	Idenix/Novartis	Active site	Stopped
2	Mericitabine (R7128)	Roche/Pharmasset	Active site	III [154]
3	Sofosbubvir (GS7977 former PSI7977)	Pharmasset	Active site	Approved [154]
4	R1626	Roche	Active site	Stopped
5	PSI-938	Pharmasset	Active site	Stopped
6	IDX-184	Idenix	Active site	halted
7	ALS-220	Alios/Vertex	Active site	I
8	VX-135	Vertex	Active site	
9	IDX209632	Idenix	Active site	
10	ACH-3422	Achillion	Active site	
No	**Non-nucleoside analog NS5B polymerase inhibitors (NNI's)**			
1	Sterobuvir (ANA-598)	Anadys	NNI site 3/palm 1	II
2	ABT-072	Abbott	NNI site 3/palm 1	Halted
3	Dasabuvir (ABT-333)	Abbott	NNI site 3/palm 1	III [159]
4	Deleobuvir (BI-207127)	Boehringer Ingelheim	NNI site I/thumb 1	Stopped [155]
5	BLIB-1941	Boehringer Ingelheim	NNI site I/thumb 1	Stopped
6	MK-3281	Merck	NNI site I/thumb 1	Stopped
7	TMC-6470555	Janssen	NNI site I/thumb 1	I
8	Filibuvir (PF-00868554)	Pfizer	NNI site 2/thumb 2	II
9	VX-759	Vertex	NNI site 2/thumb 2	Halted
10	VX-916	Vertex	NNI site 2/thumb 2	Halted
11	VX-222	Vertex	NNI site 2/thumb 2	II
12	Tegobuvir (GS9190)	Gilead	NNI site 4/palm 2	II
13	HCV-796	ViroPharma/Wyeth	NNI site 4/palm 2	Stopped
14	IDX-375	Idenix	NNI site 4/palm 2	II
15	PPI-383	Presidio Pharmaceuticals		
16	TMC-6470551	Janssen		
17	GS-9669	Gilead		II
18	Beclabuvir (BMS-791325)	Bristol Mayers Squibb		III

Table (8): Approved or in late stages of development DAAs for HCV treatment [183]			
Multi-class combination drugs			
Brand Name	**Generic Name**	**Status**	**Pharmaceutical Company**
Harvoni	Sofosbuvir + ledipasvir (GS-7977 + GS-5885)	Approved	Gilead Sciences
Viekira Pak	(ombitasvir + paritaprevir + ritonavir) + (dasabuvir)	Approved	Abbvie
Viekirax	Ombitasvir (ABT-267) + paritaprevir (ABT-450) + ritonavir	Phase III	Abbvie
n/a	Asunaprevir + daclatasvir + *Beclabuvir* (BMS-791325)	Phase III	Bristol-Myers Squibb
n/a	Grazoprevir + elbasvir (MK-8742 + MK-5172)	Phase III	Merck
Pegylated interferon alpha			
Pegintron	Peginterferon alfa-2b	Approved	Merck
Pegasys	Peginterferon alfa-2a	Approved	Genentech
Nucleoside analogs			
Copegus, Moderiba, Rebetol and Ribasphere	Ribavirin	Approved	Genentech, abbvie, Merck and Kadmon
NS3/4A protease inhibitors			
Olysio (Galexos/Sovriad)	Simeprevir (TMC435)	Approved	Janssen and Medivir AB
Victrelis	Boceprevir	To be discontinued 12/2015	Merck
Sunvepra	Asunaprevir (BMS-650032)	Phase III	Bristol-Myers Squibb
n/a	Vaniprevir (MK-7009)	Phase III	Merck
n/a	Paritaprevir (ABT-450)	Phase III	Abbvie
n/a	Grazoprevir (MK-5172)	Phase III	Merck
Incivek	Telaprevir	Discontinued as of 16/10/2014	Vertex
Nucleoside and non-nucleoside NS5B polymerase inhibitors			
Sovaldi	Sofosbuvir (GS-7977)	Approved	Gilead Sciences
Non-nucleoside NS5B polymerase inhibitors			
Exviera	Dasabuvir (ABT-333)	Phase III	Abbvie
n/a	Beclabuvir (BMS-791325)	Phase III	Bristol-Myers Squibb
n/a	ABT-072	Phase II	Abbvie
NS5A inhibitors			
Daklinza	Daclatasvir (BMS-790052)	Phase III	Bristol-Myers Squibb
n/a	Ledipasvir (GS-5885)	Phase III	Gilead Sciences
n/a	Ombitasvir (ABT-267)	Phase III	Abbvie
n/a	GS-5816	Phase III	Gilead
n/a	Elbasvir (MK-8742)	Phase III	Merck

Conclusion

Viral hepatitis is worldwide main problem. Site 1960s and particularly 1970s when hepatitis types A, B and C (a mostly progressive disease that often advanced silently to cirrhosis and even cancer) respectively were recognized. Attention was focused to seeking drug treatments that might impede this inexorable advanced disease. Acyclovir, one of the first antiviral agents to be evaluated, failed to show a positive effect. Numerous drugs were evaluated, but many had little positive effect or actually caused harm. Among them, interferon (IFN) appeared to be the most effective.

Aim of HCV treatment is to cure the virus infected pateins. To achieve this goal, one of the most effectiv therapy methods is combination of anti viral drugs with different mechanism of action. Administration of specific drugs and the duration of treatment depend on many factors, including HCV genotypes, past treatment experience, degree of liver damage and ability to tolerate the prescribed treatment. For instance, ribavirin is an effective antivial against HCV infection. This medication prevents the HCV RNA synthesis and viral mRNA capping. RBVshould not be used as monotherapy because of its limited effect on HCV viral load and relapse elevation in transaminase levels. Therefore, it has always combined with peg-IFN-α or recombinant interferons.

Recombinant IFN-α was used to treat HCV. IFN-α alone had a SVR rate of approximately 6% to 16%. Meanwhile, ribavirin as monotherapy lowers the alanine aminotransferase levels but not HCV RNA levels. However, when it was added to IFNα, RBV raised SVR rates to approximately 34% to 42% after 24 and 48 weeks of treatment. The next step was enhancing the half-life of IFN via pegylation, which improved the virological response rates and reduced the injection frequency. PEG- IFN alone for 48 weeks induced a positive SVR rate of approximately 39%, and this rate increased to approximately 54% to 56% when PEG was coupled with RBV. Although the interferon based combinations have many limitations, like tolerability, contraindications (*e.g.* patients with ascites) and severe impairment of other organs (*e.g.* severe heart and autoimmune diseases, atherosclerosis and psychiatric disorders), but since are standard regimens for HCV treatment.

Now there are a number of approved therapies to treat HCV, such as Harvoni, Viekira Pak, Viekirax and many DAAs classes and combination regimens, which may be prescribed with or without ribavirin and in some cases peginterferon as well. The availability of short duration, safe and highly effective regimens has created new challenges in the treatment of patients with HCV infection, and especially in groups in which the SVR was low with prior therapies, or in which IFN-based strategies were contraindicated. Despite the optimism for the near future, certain areas require further evaluation to make IFN-free regimens effective in all patients. Finally, efforts should be made to make IFN-free cost-effective in all clinical scenarios and accessible to all patients.

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Key skills

Linguistic proficiency

Dari:	Mother Language
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Pashto:	good spoken and written

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