Recent Advances in Antiviral Agents: Established and Innovative Therapies for Viral Hepatitis

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Abstract: The management of HBV or HCV has improved dramatically over the last decade with the development of new drugs. This paper provides a review of new available and developing treatment options for HBV and HCV associated liver diseases. In the closer future the most realistic therapeutical option for most of the patients with HBV and HCV infection will be combination and/or long-term usage of the new, stronger antiviral drugs, if they maintain good safety profiles, achieve low resistance rates and will be available at lower prices.

Key Words: HBV, HCV, IFN, lamivudine, adefovir, ribavirin, STAT-C, resistance.

INTRODUCTION

Worldwide, 350-400 million people are estimated to be infected with hepatitis B virus (HBV) [1] and 170 million people with hepatitis C virus (HCV) [2, 3]. One of the most important clinical problem associated with HBV/HCV chronic infections is the fact that a considerable number of patients develop end-stage liver disease and hepatocellular carcinoma (HCC). Finding ways to prevent HCC occurrence is therefore one of the most important clinical question linked to these two infections. Regarding the HBV infection, in spite of the availability of safe and effective vaccines for more than two decades, the infection is still a global health problem. On the contrary, a vaccine protecting against HCV infection is not available yet. Actually, the approach for the treatment for chronic HBV and HCV infections has dramatically changed over the past decade and the current availability of a number antiviral drugs adds to the complexity of these viral chronic diseases management. The goal of the treatments for HBV, HCV infections and their associated liver diseases is to achieve a clinical cure in a period as short as possible without producing side effects and resistance mutation of the virus.

Pathogenetic Notes of the HBV and HCV Viral Infections

HBV and HCV infections mainly occur *via* the intravenous route, such as blood transfusions, tattooing and piercing, or drug abuse [4].

The immune response to HBV plays a crucial role in the control of viral infection. A vigorous, polyclonal and multispecific peripheral blood T-cell response can be observed in patients with acute self-limiting HBV infection [5, 6]. Activated HBV-specific helper and cytotoxic T-cells are still present for several years after recovery from acute hepatitis B and seem to be maintained by continuous stimulation by low amounts of persisting virus. Therefore, resolution of disease does not imply complete eradication of infection but merely reflects the capacity of HBV-specific-T-cells to persistently control HBV infection [7, 8]. Viral persistence is believed to be associated with helper T-lymphocytes and cytotoxic T-lymphocytes functional tolerance to HBV [9]. In chronic HBV-infected patients, levels of HBV-specific helper T-cells and cytotoxic T-lymphocytes are generally very low or undetectables [10].

On the contrary, the most relevant feature of HCV infection is that the majority of patients become persistent carriers. In acute HCV hepatitis, complete virus clearance occurs in 20-30% of infected patients as a result of vigorous host immune response to HCV. However, 70-80% of infected patients become persistent carriers. Most of these HCV infected patients do not show any episodes of acute hepatitis and the infection , runs in an unapparent way in the majority of the cases. Once chronic hepatitis is established, most patients gradually progress to liver cirrhosis and finally to HCC (annual occurrence: 3.2% in Europe) [11]. The data reported until now, suggest the following pathogenic mechanism adopted by HCV to contribute to HCC development. During HCV viral infection many mutations accumulate in hepatocytes providing the substrate for hepatocarcinogenesis. Furthermore, during HCV infection some of the viral proteins lead to bypass some stages, thereby accelerating the development of HCC in HCV infection. The overall effect achieved by the expression of the viral proteins would be the induction of HCC, even in absence of a complete set of genetic aberrations required for carcinogenesis [12]. Thus, antiviral therapy has a key role in preventing progression of chronic liver disease to HCC development.

This article reviews both the established treatments and the innovative available and effective antiviral agents to treat both the HBV and HCV infections, focusing on those that might become available in the next several years.

A. ESTABLISHED TREATMENTS

Interferons

Interferons are naturally occurring cytokines with immunomodulatory, antiproliferative and antiviral properties [13].

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IFNs are largely used to treat both HBV and HCV-associated liver diseases. Clinically available IFNs at present are IFN alpha, IFN beta, consensus IFN and Pegylated IFN. The functional mechanisms of IFN involve suppression of the viral replication through phosphorylation of the synthesis initiation factor Elf2 protein after its activation *via* serine/threonine and tyrosine phosphorylation of the most important IFN-dependent signal transduction molecules such as STAT1 and STAT2. The inhibition of protein synthesis by Elf 2 activation impairs the initiation of viral protein translation. In addition, IFNs enhance immune activities, which promote the clearance of the infecting virus.

To stimulate the immune activity is extremely important to cure HBV infection. HBeAg-positive patients IFN-alpha results in loss of HBeAg in 25 to 40% of patients. A part of the patients has a durable response while reactivation occurs in the 10 to 20% [13-17]. Standard IFN-alpha induced responses are less durable in HBeAg-negative chronic HBV, with sustained response in 10 to 47% (average 24%) at 12 months after cessation of therapy [18, 19]. Long-term follow-up studies show better overall survival and lower incidence of hepatic failure and HCC in responders to IFN-alpha therapy [19-21].

The addition of a polyethylene glycol (PEG) molecule to IFN significantly prolongs half-life and results in more sustained IFN activity. Two pegylated IFNs have been studied for HCV treatment for first and then for HBV infection; a large branched 40kDa PEG linked to IFN alpha-2a (PEG IFN alpha-2a) and a small linear 12kDa PEG linked to IFN alpha-2b (PEG IFN alpha-2b) [22]. Both these interferons have shown similar tolerability and higher rates of sustained viral response compared with conventional IFN [23, 24]. Actually, both PEG-IFN alpha-2a and PEG-IFN alpha-2b are registered for the treatment of chronic HCV and HBV in Europe and in the United States and should be given by subcutaneous injection once weekly for 24-48 weeks in a dosage of 180 microgram or 1.5 mg/kg respectively in both HCV and HBV patients.

HBV genotype appears to predict response to PEG-IFNs, with a higher probability of HBeAg loss and HBsAg loss for patients with genotype A and B compared with genotype C and D [23, 25, 26]. In HBeAg-positive patients, treatment with PEG-IFN was found superior to conventional IFNs, with loss of HBeAg in 35% and seroconversion to anti-HBe in 29 to 32% of patients [23, 24]. In HBeAg-negative HBV only one large randomized controlled trial using PEG-IFN has been conducted so far: at the end of the follow-up, a combined response, with serum HBV DNA suppression and ALTs normalisation , was observed in 36% of patients [26]. HBsAg seroconversion occurs in 3 to 7% of PEG-IFN treated patients, which represents 10 to 20% of virological responders [23, 25, 26, 27].

The HCV therapy has significantly changed in the last few years: the monotherapy with IFN-alpha, defined in 1996 by the AISF (Italian Association for the Study of the Liver) consensus guide lines, has been completely replaced by a more effective combination therapy with IFN or PEG-IFN and Ribavirin, which is the actual "gold standard" and has been well outlined in 1999 by the Consensus Conference in Paris [28] organized by EASL (European Association for the Study of Liver).

Several international randomized trials reported that, either the PEG IFN alpha-2b (0.5-1.0 to 1.5 microg/kg) or the PEG IFN alpha-2a (180 microg), administered once weekly, are significantly more effective than standard IFNs administered three times weekly [29, 30], in patients with chronic hepatitis C or cirrhosis (Child-Pough grade A).

Adverse events observed during treatment with PEG-IFNs are similar to those observed with conventional IFNs. The interferon treatment side effects associated mainly reported in literature are: influenza like symptoms, fatigue, headache, myalgia, gastrointestinal symptoms (nausea, anorexia, weight loss), alopecia and local reaction at the injection site. These side effects are frequently observed, but rarely lead to discontinuation of treatment.

More serious adverse events such as myelosuppression, neuropsychiatric symptoms (irritability, depression and insomnia), neurological symptoms and thyroid dysfunction may require dose reduction or treatment discontinuation [31].

The proportion of patients requiring treatment discontinuation for safety reasons is comparable for PEG-IFN and standard IFN.

Nucleoside Analogs for HBV and HCV

HBV

HBV is a small, partially double-stranded DNA virus and a prototype member of the hepadnavirus family. Its 3.2 kb genome possesses four overlapping open reading frames encoding the envelope (pre-S/S), core (precore/core), polymerase and X proteins. Among these hepadnavirus proteins, a single protein contains the enzyme catalysing RNA- and DNA-dependent DNA polymerase RNAse H and protein priming activities. Hepadnavirus polymerase plays a critical role in the hepadnavirus genome replication. Analogously to HIV, the viral reverse transcriptase is a good target for inhibiting hepadnavirus replication. In fact, nucleoside reverse transcriptase inhibitors (NRTIs) have an anti-HBV activity.

Nucleoside analogues are chemically synthesized drugs able to mimic natural nucleosides. They inhibit viral replication because of their incorporation into newly synthesized HBV-DNA that causes chain termination. Another important feature is their ability to block the viral polymerase, if phosphorylated within cells to their triphosphate counterparts, competitively inhibiting the DNA dependent and reverse transcriptase activity. Nucleoside analogues can be produced in their natural R- or unnatural S-configuration, and these are often considered as enantiomers. Template-dependent DNA polymerases add both S- and R-enantiomers of dNTP analogues to DNA with equal efficiency [32, 33]. Interestingly enough, the HBV polymerase has a preference for the S- over the R- enantiomers configuration [33]. S- enantiomers configuration appears to have equal antiviral activity than R-counterparts, but they are less toxic and have greater metabolic stability.

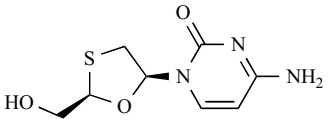
Three chemical groups within the class of nucleos(t)ide analogue can be recognised: a) L-Nucleoside Group (lami-

vudine, emtricitabine, telbivudine, clevudine); b) Acyclic Phosphonate Group (adefovir, tenofovir); c) Cyclopentene/ Cyclopentane Group (entecavir/abacavir) (Fig. (**1**)).

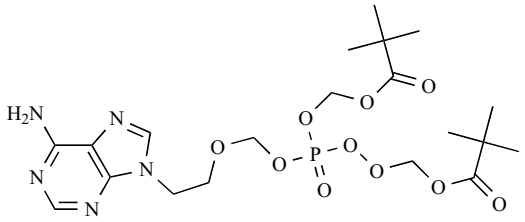
Nucleoside analogues may interfere with the following steps in the HBV life cycle: synthesis of the (-)-DNA strand by reverse transcription, synthesis of the (+)-DNA strand and cccDNA formation in newly infected cells. There are some controversial opinions on the ability of nucleoside analogs to interfere with the presence of HBV cccDNA. In fact, an appreciable number of data report that nucleoside analogue treatment does not have an appreciable effect on the cccDNA pool in hepatocytes [34]. The acetylation status of HBV cccDNA influences the replication rate of HBV, thus in the near future it will be interesting to develop an antiviral therapy using acetylation inhibitors [35]. Interestingly enough, it seems that cccDNA declines, in DHBV congenitally infected ducks treated with lamivudine and a dideoxyguanosine prodrug (entecavir), preferentially in liver biopsies [36].

Lamivudine

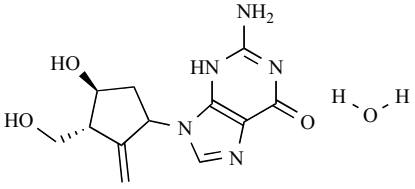
Lamivudine, also known as 3TC (Epivir) is the deoxycytidine analogue S-enantiomer 2′,3′-dideoxy-3′-thiacytidine (Fig. (**1**)). This agent is the first specific antiviral therapy to become available for the treatment of chronic hepatitis B. Lamivudine is absorbed rapidly after oral administration and it is phosphorylated acquiring potent antiviral effects [37];



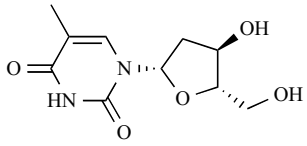
Lamivudine
IUPAC: 4-amino-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2-one



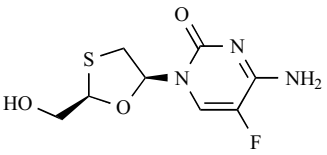
Adefovir dipivoxil
IUPAC: [2-(6-aminopurin-9-yl)ethoxymethyl-(2,2-dimethylpropanoyloxymethoxy)phosphoryl] oxymethyl 2,2-dimethylpropanoate



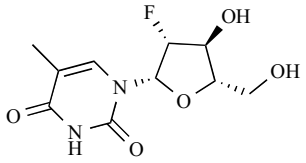
Entecavir
IUPAC: 2-amino-9-[(3R,4S)-4-hydroxy-3-(hydroxymethyl)-2-methylidenecyclopentyl]-3H-purin-6-one hydrate



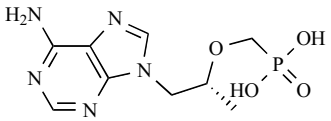
Telvibudine
IUPAC: 1-[(2S,4R,5S)-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl]-5-methylpyrimidine-2,4-dione



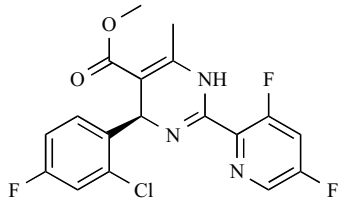
Emtricitabine
IUPAC: 4-amino-5-fluoro-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]pyrimidin-2-one



Clevudine
IUPAC: 1-[(2S,3R,4S,5S)-3-fluoro-4-hydroxy-5-(hydroxymethyl)oxolan-2-yl]-5-methylpyrimidine-2,4-dione



Tenofovir
IUPAC: [(2R)-1-(6-aminopurin-9-yl) propan-2-yl]oxymethylphosphonic acid



BAY41-4109
IUPAC: methyl (4R)-4-(2-chloro-4-fluorophenyl)-2-(3,5-difluoropyridin-2-yl)-6-methyl-1,4-dihydropyrimidine-5-carboxylate

Fig. (1). Old and new nucleoside analogs for HBV.

the recommended dose for adults is 100 mg per day, whereas that for children is 1 mg/kg per day rising to 100 mg/day.

Lamivudine is metabolized in the hepatocytes to the active triphosphate, by stepwise addition of phosphate groups (Fig. (**1**)). The drug contains a sulphur atom instead of carbon at the 3′ position of the sugar ring, which does not allow chain elongation by phosphodiester bond formation, in absence of the normal 3′ hydroxyl group. Since lamivudine acts by terminating viral DNA synthesis and competitively inhibiting the viral polymerase/reverse trascriptase [38], it is equally effective in patients of any race, but also against both the wild-type virus and precore/core promoter variants [39, 40]. In addition, there is evidence that lamivudine treatment may overcome cytotoxic T cell hyporesponsiveness seen in chronically infected patients [41].

Lamivudine has few side effects even after long time administration, however, HBV polymerase coded gene mutation (YMDD) occurs frequently when lamivudine is administered for more than one year [42]. The YMDD mutation consists in the substitution of isoleucine or valine for methionine within the tyrosine-methionine-aspartate-aspartate (YMDD) motif and is associated with virologic and clinical resistance to lamivudine therapy. The side groups of isoleucine and valine of the YMDD mutants sterically prevent lamivudine from appropriately configuring into the nucleotide binding site of the reverse transcriptase. Aminotransferase flare is associated with lamivudine therapy and may signify clinical resistance with emergence of YMDD mutants. Anyway, it is important to remember that aminotransferase flare may also herald the recovery phase with seroconversion and viral eradication [43]. Finally, post-treatment flare often occurs when the drug is discontinued, so patients who begin therapy have to continue avoiding discontinuation.

Adefovir Dipivoxil (ADV)

Adefovir or bis-pivaloyloxymethyl-9-(2-phosphonylmethoxyethyl) adenine (PMEA) is a phosphonate of an acyclic nucleotide analogue (Fig. (**1**)). It is NRTI with a smaller sugar ring that seems acting better for the inhibition of YMDD mutants [44]. The drug, unlike other nucleoside analogues, contains a phosphate group already and requires an additional phosphorylation step (diphosphate), before it becomes active. This is preceded by the removal of the bis-pivaloyloxymethyl moiety. It is rapidly converted to adefovir by an esterase in the intestine or serum, and is then transported to the cells and phosphorylated to the active diphosphate form, which acts as an alternative substrate inhibiting HBV polymerase. Incorporation of this agent into the viral DNA results in chain termination. Thus, adefovir is a potent inhibitor of HBV replication and is also thought to stimulate natural killer cell activity and to induce endogenous interferon production [45]. Adefovir, at a daily dose of 10 mg, is well tolerated and the efficacy is similar to that of lamivudine, however, this drug is cleared primarily by excretion of unchanged drug into the urine, and special care is required in the case of renal dysfunction. Actually, both lamivudine and adefovir have become standard antiviral agents for HBV-associated liver diseases as specific inhibitors of HBV DNA polymerase [23, 44, 46].

Recent clinical trials conducted in HBeAg-negative patients treated for 144 and 240 weeks, report that benefits obtained with adefovir treatment (improvement in hepatic fibrosis, durable HBV replication suppression , liver enzymes normalization and delayed development of resistance) were maintained, with infrequent emergence of viral resistance. Resistance mutations rtN236T and rtA181V were identified in 5.9 and 29 percent of patients after 144 and 240 weeks, respectively [46, 47].

Entecavir

Entecavir is a nucleoside analogue of deoxyguanosine and is a very potent inhibitor of HBV DNA polymerase (Fig. (**1**)). The antiviral efficacy of this drug has been reported to be superior respect to lamivudine [Colonno RJ *et al.* AASLD October 27-31, 2006. Abstract 110]. The reduction in HBV DNA and serum ALT at a dose of 0.5 mg daily is significantly better than with lamivudine. Only a few resistant mutations have been reported in naïve patients [49], whereas, in lamivudine resistant patients has been observed an increasing resistance over time: year 1: 1%; year 2: 9%; year 3: 15% [Colonno RJ *et al.* AASLD October 27-31, 2006. Abstract 110]. This agent has been recently licensed by FDA, in United States.

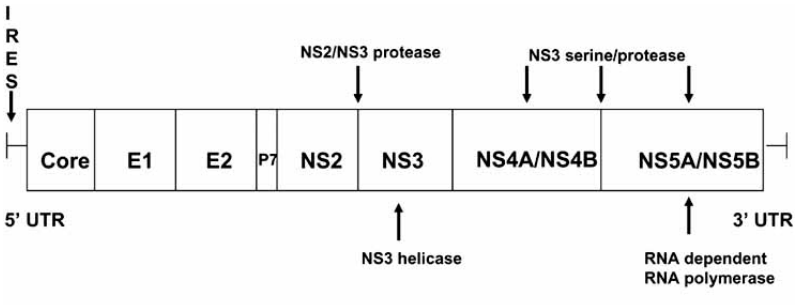
HCV

Combination therapy with PEG IFN-alpha and Ribavirin (RBV) is currently the standard of care for treating patients with chronic hepatitis C [48]. However, in despite of substantial advances in the use of IFN-alpha based therapy over the past decade, HCV is still associated with a failure to eradicate the infection in 20% patients infected by HCV genotype 2 and 3 and 40-60% patients infected by HCV genotype 1. The reason for this failure rate include: dose reduction or withdrawals due to side effects, non-adherence to treatment schedules, stage of liver disease, patients and virus characteristics.

The HCV is an enveloped, single-stranded RNA-virus. Once the viral RNA gets into the cell, it is translated into a polyprotein, which is cleaved at multiple sites by cellular and viral proteases to produce 3 structural proteins and 6 non-structural (NS) proteins [49]. The structural proteins include core, E1 and E2 envelope proteins, which are glycoproteins. The non-structural proteins include NS2, NS3, NS4A, NS4B, NS5A and NS5B. Between the HCV structural and non-structural proteins is located p7, a small hydrophobic polyprotein that may have a role as an ion channel. A membrane-associated replication complex is formed, which includes viral proteins, replicating RNA, and altered cellular membranes [50, 51].

Actually, HCV gene sequences or HCV proteins are considered ideal drug targets (Fig. (**2**)). Drug development has been delayed because of lack of effective cell culture systems and small animal models for HCV. However, a HCV subgenomic replicon system using hepatoma cells [51] that allows to screen candidated drugs has been now developed.

As mentioned before, currently, the initial treatment of chronic HCV infection is PEG IFN alpha 2a or PEG IFN alpha 2b in combination with oral Ribavirin. In the following



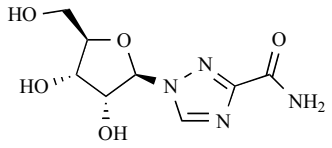
IRES inhibitors	*NS3/4 inhibitors*	*NS5 inhibitors*
ISIS-14083	BILN 2061	NM-283 (Valopicitabine)
Heptazyme	VX 950	R1626
	SCH 503034	HCV 796
	ITMN 191	JTK 003

Fig. (2). Targets sites and STAT-C antiviral agents described in the text.

part of this article we will describe this latter drug for treatment of HCV infection. PEG IFN has been described previously.

Ribavirin

Ribavirin (RBV), developed in 1972, is a nucleoside analog of guanosine (Fig. (**3**)) with full antiviral activities. Ribavirin is not effective against HCV when administered as a single agent [52]. The antiviral mechanisms of this drug remain not completely understood. Several potential mechanisms of action has been proposed: inhibition of viral capping; inhibition of RNA polymerase by ribavirin triphosphate (RTP); modulation of immune response, increasing Th1 cellular immune response; inhibition of host inosine monophosphate dehydrogenase (IMPDH) by ribavirin monophosphate (RMP), allowing depletion of cellular guanosine; lethal mutagenesis due to incorporation of RTP into the viral polymerase [52, 53].



Ribavirin
IUPAC: 1-[(2R,3R,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-1,2,4-triazole-3-carboxamide

Fig. (3). Ribavirin.

The most serious problem in the use of ribavirin is hemolytic anemia, which occurs in almost all cases when administered. About 90% of orally administered ribavirin enters the bloodstream *via* the intestine and then spreads throughout the body. The drug is rapidly absorbed and is phosphorylated inside the cells; then it is rapidly dephosphorylated by nucleated cells. Ribavirin incorporated into erythrocytes accumulates in these cells and is not excreted outside because of the absence of dephosphorylating enzymes in the erythrocyte. Agents without such side effects are needed as replacements for ribavirin. Several promising new therapeutic approaches for the treatment of chronic hepatitis C using novel agents are currently in the early stages of clinical development and will be described below.

B. INNOVATIVE TREATMENTS

New Interferons

New recombinant forms of IFN alpha molecules are currently being developed. The new drugs might have better immunomodulatory and antiviral effects and a better tolerability. They also have better pharmacokinetic and pharmacodynamic features. These new molecules will be available for both of the HBV and HCV infections in the next several years.

IFN R7025/RO5014583

Roche's enhanced interferon R7025/RO5014583 maintains antiviral activity over the entire weekly dosing interval. Preclinical data show that it has several better antiviral features than PEG-IFNs such as: *in vitro* IFN-stimulated gene expression, antiviral activity, immunomodulatory activity (Th1 cytokine induction, dendritic cell maturation), antiproliferative activity, antiproliferative activity, potential immunogenicity [AASLD, October 27-31, 2006].

Infergen (or Interferon Alfacon-1 or Consensus Interferon)

Infergen (Table **1**) is a recombinant non naturally occurring type-I interferon. The 166-amino acid was derived by scanning the sequences of several natural IFNs alpha subtypes. DNA sequence was constructed using chemical synthesis methodology. It is produced in *Escherichia coli (E. coli)* that have been genetically altered by insertion of a synthetic interferon alfacon 1 sequence [54]. Infergen purity is obtained by sequential passage over a series of chromatogra-

Table 1. The Most Promising Molecules, in Phase I, II, III or IV Clinical Trials, Actually Under Investigation for HCV Infection. Here, we Report Only Few of them Extensively Described in the Text

Phase I	Phase II	Phase III	Phase IV
R7025/R05014583 (Maxygen/Roche)	**Oral IFN-alpha** (Amarillo Biosciences)	**Albuferon** (Novartis)	**Infergen/Consensus/Alfacon 1** (Valeant Pharmaceutical Int.)
Heptazyme (RPI, Boulder)	**ISIS 14803** (Isis Pharmaceutical)	**Levovirin**	
R1626 (Roche)	**NM-283 (Valopicitabine)** (Idenix Pharmaceutical)	**Viramidine (taribavirin)** (Valeant Pharm. Int.)	
HCV 796 (Viro-Pharma Inc.)	**JTK-003** (Akros Pharma Inc.)		
Isatoribine (ANA975, TLR 7) (ANADYS)	**SCH 503034** (Schering Plough)		
GI 5005 Vaccine (GlobeImmune)	**VX 950 (telaprevir)** (Vertex Pharmaceutical)		
XTL-6865 (XTL)	**Actilon (CpG 101101, TLR9)** (Coley Pharm. Group Inc.)		
	E-1 Vaccine (Innogenetics)		

phy columns. Infergen exhibited at least 5 times higher specific activity *in vitro* than IFN alpha 2a and IFN alpha 2b. Recently, a clinical trial confirmed the higher activity of Infergen compared with IFN alpha 2a or IFN alpha 2b. The recommended dose of Infergen for treatment of HCV infection is 15 mcg/twi plus ribavirin 1g/day for 24-48 weeks (according to the genotype) [55].

Albuferon

Albuferon (Table **1**) is a novel 85.7 kilodalton recombinant protein consisting of interferon alpha genetically fused to human serum albumin. Recombinant human albumin is a carrier protein with no intrinsic activity, but with a long circulating half life; the human serum albumin is expected to extend systemic circulation of recombinant interferon alpha 2b and to improve its therapeutic activity. The long half life of the drug (~150 hours or 6 days) supports dosing every 2 or 4 weeks [56].

New Nucleoside Analogs for HBV

Telbivudine (L-deoxythymidine, LdT)

Telbivudine (Fig. (**1**)) has a potent antiviral activity against HBV, but is cross-resistant with lamivudine. Unfortunately, it selects for the methionine to valine (M2041) mutation in the YMDD motif as reported in a phase II study including 104 HBeAg-positive patients treated with 400 or 600 mg daily of telbivudine alone, or lamivudine 100mg daily or in combination. M2041 mutation occurred in 4.5%, 9.8% and 15.8% patients, respectively [57]. Telbivudine, throughout a two years study, seems to be superior to lamivudine in suppressing HBV-DNA in HBeAg-positive and HBeAg-negative patients, but the number of patients with normal ALT and HBeAg loss were similar [Lai CL *et al.* AASLD October 27-31, 2006, Abstract 9]. Preliminary results of a phase II clinical trial (135 HBeAg-positive patients treated with ADV 10 mg/die or telbivudine 600 mg/die) demonstrate that telbivudine is more potent than adefovir in head-to-head comparison. An increased resistance, at the second year, in patients receiving telbivudine monotherapy has been reported [Bzowej N., *et al.* AASLD October 27-31, 2006, Abstract 1005]. Thus, actually the role of telbivudine monotherapy in hepatitis B treatment is limited, but have to be considered for the future combination treatment with IFNs.

Emtricitabine

Emtricitabine is a 5-fluorinated derivative of lamivudine, [(–)-ß-2',3'-dideoxy-5-fluoro-3'-thiacytidine] (Fig. (**1**)), which is converted to triphosphate by cellular enzymes and competes with dCTP as a substrate for HBV polymerase [58]. Emtricitabine was found to be a potent inhibitor of HBV replication in human hepatoblastoma cell line 2.2.1.5, in primary human hepatocytes and in nude mice [58, 59, 60]. This agent is now in clinical phase II or III trials. A clinical trial of emtricitabine 25-200mg for 48 weeks showed antiviral effects. However, as for the lamivudine, resistant mutations were observed either in the 100mg or in 200mg groups [61].

Clevudine (L-FMAU)

Clevudine, 2'-fluoro-5-methyl-β-L-arabinofuranosyluracil (L-FMAU), is a pyrimidine nucleoside analogue (Fig. (**1**)) that inhibits HBV replication. Actually, this drug is in clinical phase II or III trial. Clevudine (30mg/daily for up to 24 weeks) induced, after 4 weeks, a 2.5-3.0 x $\log^{10}$ copies/ml decrease of viral load in a clinical trial. A feature of clevudine is the durability of virus suppression persisting for 24 weeks after withdrawal of treatment in some patients [62].

Tenofovir

Tenofovir disoproxil fumarate exhibits a more potent viral inhibitory effect than the currently approved drugs (IFNs, lamivudine and adefovir dipivoxil). Tenofovir disoproxil fumarate is an acyclic nucleoside analogue closely related to adefovir, which is directly incorporated into DNA (Fig. (**1**)). Current clinical trials reveal its effective safety and the frequencies of viral resistance apparition. Tenofovir has recently been shown to cause significant (4 x $\log^{10}$ copies/ml) reduction in serum HBV DNA and seroconversion to anti-HBe in 25% of patients treated for one year [63]. Moreover, it is active against lamivudine-resistant strains [64].

Non-Nucleoside Analogues for HBV

Bay 41-4109

Bay 41-4109, which belongs to the heteroaryl dihydropyrimidine (HAP) family, has shown potent antiviral activity to HBV in an *in vitro* study. This agent exerts its antiviral effects by inhibiting nucleocapsid maturation through the binding to core protein in the HBV replication process. The HBV capsid is an icoesahedral complex of 120 capsid protein dimers. Recent data suggest the existence of two functionally distinguishable classes of drug-binding sites on HBV capsids. One site stabilizes capsid whereas the second site induces structural changes that cannot be tolerated during viral maturation. Bay 41-4109 inhibits virus replication by inducing assembly inappropriately and by interfering with viral assembly decreasing the stability of normal capsid [65].

Alternative "Ribavirin-Like" Drugs for HCV

As reported above, some problems cause a low rate of response in HCV infected patients (40-50%); thus, new therapeutic strategies are needed to treat chronic HCV infection. Several HCV therapies are currently in development (Table **1**).

Levovirin

These agents are ribavirin derivatives and have no side effects such as hemolytic anemia [66]. Levovirin is an L-enantiomer of ribavirin (Fig. (**4**)) and is excreted in non-phosphorylated form with urine and therefore it does not accumulate in erythrocytes. This agent has not antiviral activities and has not effect on viral polymerase while it is phosphorylated. However, like ribavirin, it induces Th1/Th2 modulation, which stimulates HCV-specific T cell proliferation.

Viramidine (Taribavirin)

Viramidine has a carboxamidin structure and differs from ribavirin (Fig. (**4**)), which is a carboxamide, as regards intra cellular transport . However, this agent is converted to ribavirin rapidly and thus it has antiviral and immunomodulatory effects similar to ribavirin. A question of interest is whether combination therapies of IFN with these ribavirin derivatives have antiviral effects similar to the IFN-ribavirin combination therapy ones,, but with fewer side effects. Two recent trials report that dosing viramidine at levels higher than 18 mg/kg yielded overall sustained virologic response (SVR) rate similar to that of the current standard ribavirin treatment (52%) with a lower rate of anemia. In fact, anemia rates in patients with HCV were lower (5% *vs* 24%) in viramidine combined with PEG IFN alfa 2b treatment compared with weight-based ribavirin plus PEG IFN alfa 2b [Jacobson I *et al.*, AASLD, October 27-31, 2006, Abstract 1133. Benhamou Y. *et al*., EASL, April 26-30, 2006, Abstract 751].

Viral Enzyme Inhibitors or STAT-C (Specifically Targeted Antiviral Therapy for HCV)

The HCV replicon system and the elucidation of the crystalline structure of HCV proteins, such as serine protease (NS3 protease) and RNA-dependent RNA polymerase (NS5B) have allowed the new anti HCV drugs development (Fig. (**3**), Table **1**). The specific inhibitor of HCV targeted: IRES sequence, NS3 protease, NS3 helicase, NS3 bifunctional protease/helicase, NS5B RNA-dependent RNA polymerase. The future role of these agents may be to meet currently unmet clinical needs and improve overall standard cares.

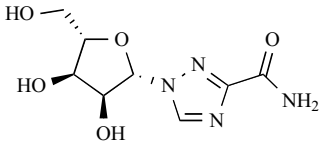
Here we will only describe the most promising molecules under investigation, but many others are in preclinical development.

Anti HCV Drugs Which Target the 5' Untranslated Region

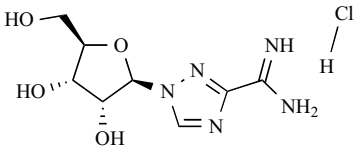
IRES is a functional stem-loop RNA structure located in 5' non-coding region of the HCV genome that also spans the first core-coding nucleotides and drives HCV polyprotein translation. The 5' untranslated region has been a good target for new drugs development because of the important roles it plays in HCV protein translation or HCV replication. Actually three main classes of IRES inhibitors exist: antisense oligonucleotides, ribozymes and small-interfering RNAs (siRNAs).

ISIS-14803 and Heptazyme

ISIS-14803 is an antisense DNA that consists of a 20-base phosphorothioate oligonucleotide. It inhibits HCV rep-



Levovirin
IUPAC: 1-[(2S,3S,4R,5S)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-1,2,4-triazole-3-carboxamide



Viramidine
IUPAC: 1-[(2R,3R,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl) oxolan-2-yl]-1,2,4-triazole-3-carboximidamide hydrochloride

Fig. (4). Alternative "ribavirin-like" drugs for HCV.

lication and protein expression in cell cultures and mouse models. However, in a small number of patients it exerts only a moderate (?) reduction of HCV viral load [67]. Actually, investigators are intrigued by a possible immunomodulatory mechanism of action *via* its GC motif.

Heptazyme is a HCV specific ribozyme, designed to cleave the HCV-IRES. Ribozymes are catalytic RNA molecules that bind to and cleave specific RNA sequences. Both are now being evaluated in Phase I or II clinical trials. However, development of Hepatazyme slowed down because of its toxicity in primates [68]. siRNAs lead to the selective destruction of HCV IRES sequence, suppressing HCV replication *in vitro*, and suggesting their potential use in future HCV therapy [69].

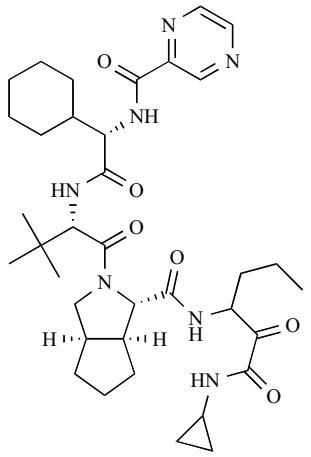
NS3A/4 Protease Inhibitors

The NS3 protease structure confirmed early homology modeling efforts and provided the necessary detailed insight to permit rational inhibitory design. The NS3 protease is a typical serine protease barrel, containing the canonic asp-his-ser catalytic triad signature. A peculiarity of NS3 protease is an extended polydentate substrate binding cleft, which ensures substrate specificity. The major protease-substrate interactions, which span the protease domain, include hydrogen bonds with the substrate backbone and complementary electrostatic or hydrophobic contacts in the binding site. Extensive studies to develop new anti HCV drugs targeting NS3 are being carried out to date. New agents, which target the NS3 helicase are also being studied [70].

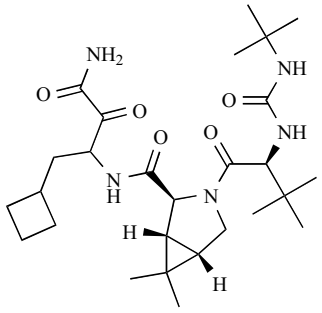
BILN 2061, VX 950, SCH 503034, ITMN 191

BILN 2061 is a very specific serine protease inhibitor, which showed a > 3 x $\log^{10}$ reduction in viral load after only 2 days of the highest doses in patients infected with HCV genotype 1 [Roberts S, *et al*. 41st EASL. April 26–30, 2006. Abstract 731]. The antiviral effects are less pronounced *in vitro* and *in vivo* in patients infected with HCV genotype 2 and 3. Its target is the catalytic site of NS3 enzyme, where it competes with its natural substrates. This drug is to going to be developed because of its myocardial toxicity in animal model [71].

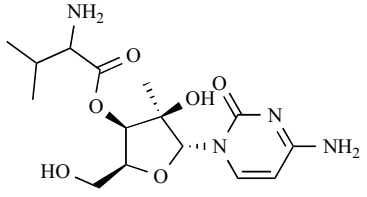
VX 950 (telaprevir) is a small protease inhibitor molecule of a new class of antiviral drugs that inhibits HCV (Fig. (**5**)). VX 950 has shown potent antiviral activity and promising pharmacological properties in preclinical testing that began in early 2002. VX 950 is an orally-bioavailable inhibitor of



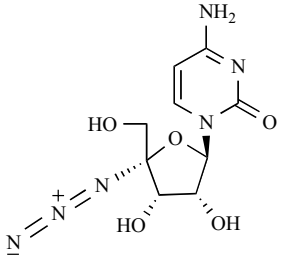
VX-950
IUPAC: (1S,3aR,6aS)-2-[(2S)-2-[[(2S)-2-cyclohexyl-2-(pyrazine-2-carbonylamino)acetyl]amino]-3,3-dimethylbutanoyl]-N-[1-(cyclopropylamino)-1,2-dioxohexan-3-yl]-3,3a,4,5,6,6a-hexahydro-1H-cyclopenta[c]pyrrole-1-carboxamide



SCH503034
IUPAC: (1R,2S,5S)-N-(4-amino-1-cyclobutyl-3,4-dioxobutan-2-yl)-3-[(2S)-2-(tert-butylcarbamoylamino)-3,3-dimethylbutanoyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2-carboxamid



Valopicitabine
IUPAC: [(2R,3R,4R,5R)-5-(4-amino-2-oxopyrimidin-1-yl)-4-hydroxy-2-(hydroxymethyl)-4-methyloxolan-3-yl] (2S)-2-amino-3-methylbutanoate



R-1626
IUPAC: 4-amino-1-((2*R*,3*R*,4*S*,5*R*)-5-azido-3,4-dihydroxy-5-(hydroxymethyl)-tetrahydrofuran-2-yl)pyrimidin-2(1*H*)-one

Fig. (5). Chemical structure of the most promising STAT- C agents for HCV.

the HCV NS3A/4 protease. In *in vitro* studies it has been demonstrated reversible and tight binding to the HCV protease active site, forming a stable enzyme-inhibitor complex. VX 950 is active in replicon assays, replication assays, and in animal models, both as a single agent and in several different combinations. VX 950 is now being evaluated in clinical trials. In a 14-day study in chronic hepatitis C patients, VX 950 was well-tolerated and showed rapid and dramatic antiviral activity [Resink H.W. *et al*., DDW 2005, 14-19 May, Abstract 7]. VX 950 currently appears to be one of the most promising of specific HCV inhibitors drugs in clinical development.

Drug design efforts targeting the active site resulted in the discovery of SCH 503034 (Fig. (**5**)), a structurally novel ketoamide HCV NS3 protease inhibitor [72]. SCH 503034 demonstrated efficacy in the HCV replicon system with virtually no cellular toxicity and has consequently been advanced for clinical evaluation. As reported for other NS3 inhibitors, SCH 503034 acts rapidly to suppress proteolytic activity and prevent formation of new replisomes. Moreover, this drug has been demonstrated to be able to interfere with innate immunity. The kinetics of NS3 protease inhibition observed with SCH 503034 in the replicon system is consistent with an initial rapid decline in HCV RNA observed in the clinic with VX 950.

Finally, ITMN 191, used in a pre-clinical studies, has shown an activity against NS3/4 mutants resistants to BILN 2091 and VX 950 [73].

NS5B RNA-dependent RNA polymerase inhibitors

The NS5B region of the HCV genome encodes an RNA dependent RNA polymerase and has been crystallographically analyzed. Actually, several molecules are studied in Phase I and II trials to evaluate the efficacy and the safety of the following RNA-dependent RNA polymerase inhibitors (Fig. (**2**)) and Table **1**.

Valopicitabine (NM-283)

Valopicitabine is a nucleoside analog that targets NS5B polymerase active site (Fig. (**5**)). Valopicitabine is administered once a day orally and blocks HCV replication by specifically inhibiting the HCV RNA-dependent RNA polymerase. Valopicitabine appears to act in two ways: it inhibits the viral polymerase directly and it is incorporated into growing strands of viral RNA, which terminates RNA chain extension. Initial Phase I clinical trial showed that valopicitabine is active in patients infected with the genotype 1 strain of HCV [Godofsky E et al., AASLD, April 2004, Berlin, Germany, Abstract 96]. The ongoing clinical trials are designed to evaluate the combination of valopicitabine and pegylated interferon in hepatitis C, genotype 1 patients who previously failed to respond to antiviral treatment, as well as in genotype 1 patients who have not been treated previously [Afdbal N. *et al.*, AASLD, April 2005, Paris, France, Abstract].

R1626

R1626 is a novel oral nucleoside analogue and is an HCV polymerase inhibitor (Fig. (**5**)). It was developed to significantly increase bioavailability and HCV inhibition. The R1479 prodrug has demonstrated potent anti-HCV activity in prior studies and no significant negative safety issues. A recent Phase I clinical trial reports some data obtained in chronic genotype 1 HCV patients. The study shows that R1626 is efficiently converted to R1479 following oral administration and R1626 monotherapy suppressed HCV RNA at all tested doses (500, 1500, 3000 mg) at day 15 [Roberts S. *et al.*, AASLD October 27-31, 2006. Abstract LB2].

HCV 796 and JTK-003

HCV 796 is an investigational non-nucleoside RNA polymerase inhibitor compound for the treatment of hepatitis C.

A Phase 1b trial, evaluated the efficacy of an orally dosed HCV 796 non nucleoside viral polymerase inhibitor with the potential to interfere with the replication of hepatitis C virus [Godofsky E. *et al.*, DDW 2004. May 15–20, 2004. Abstract 407]. Monotherapy with HCV 796 is well tolerated with no dose limiting toxicities and displays clear antiviral activity across multiple HCV genotypes. Peak antiviral response was achieved with doses of 500 mg and higher twice daily. The safety profile of this study indicates that oral doses of HCV 796 are generally well tolerated with no serious treatment-emergent adverse affects when given for 14 days. Mild to moderate headache was the most frequently reported adverse event.

JTK-003 is also an inhibitor of RNA-dependent RNA polymerase and has been evaluated in Phase II trials with promising results [Chandra P. *et al.*, DDW 2006. May 20–25, 2006. Abstract 1].

Host Immunomodulators

A crucial question that future clinical studies need to address is whether combination therapy with solely HCV targeted drugs will be sufficient to cure patients or whether the stimulation of the host immune system by immunomodulators will be necessary to obtain a complete eradication of the virus.

HCV infection could be eradicated by agents that stimulate the host innate and adaptive immunity. With this purpose synthetic agonists of Toll-like receptors (TLRs) 7 and 9 have recently demonstrated their potential in controlling HCV infection. TLRs are expressed by immune cells, which include macrophages, monocytes, dendritic and B cells. They recognize the presence of exogenous microorganisms through the recognition of molecular patterns characteristic of pathogens such as bacteria, viruses and parasites [74, 75]. The TLRs stimulation initiates acute inflammatory responses by induction of antimicrobial genes and pro-inflammatory cytokines and chemokines. Preliminary data showed a statistically significant reduction in viral load, stimulating either TRL 9 or 7, during HCV infection, leaving a hope for future combined therapies.

Isatoribine (ANA975, TLR 7 Agonist) and Actilon (CPG 1010101, TLR 9 Agonist)

A novel immunomodulator, isatoribine, is currently undergoing Phase I b development for HCV. TLR 7 agonist has demonstrated potent immunogenicity *in vitro* and *in vivo*, providing significant protection against a broad spectrum of viral challenges. Interim clinical data in patients with chronic

HCV have indicated that daily i.v. doses of 800 mg significantly reduce plasma HCV RNA levels, modulate interferon-responsive genes and are effective against genotypes 1 and 3 [75].

CPG 10101 (Actilon) is a TLR 9 agonist for treatment of HCV infection. It is able to stimulate immune system, inducing secretion of cytokines and chemokines (IFN-alpha, IFN gamma etc.) with known antiviral properties, and activating NK, T, pDC, NKT and B cells. Recently, it has been reported that combination therapy with PEG-IFN + RBV and CPG 10101 (0,20 mg/kg SC, weekly) improves early antiviral activity in non responders, thus, CPG 10101 appears acting synergistically with PEG-IFN + RBV and to be generally well tolerated [McHutchison J.G. *et al.*, EASL 41st Meeting, April 26-30, 2006].

The pleiotropic effects of IFN-alpha on both infected hepatocytes and immune system cells, suggest that combination therapy with protease inhibitors will likely remain preferable to simple monotherapy to effect a rapid sustained viral response and minimize the potential emergence of protease resistance.

HCV Vaccines

GI 5005 and E-1 Vaccines

GI 5005 is a form of therapeutic vaccine that is believed to stimulate the immune system to help fighting a variety of diseases. An on-going Phase 1b double-blinded, placebo controlled, dose-escalating, multi-center trial evaluating the safety, immunogenicity, and efficacy of GI-5005 is underway. GGI-5005 induces viral load reductions approaching 1 x $\log^{10}$, achieves a strong cellular immune response and, finally, is well-tolerated with no serious safety events, dose reductions, and no discontinuations due to adverse events [AASLD October 27-31, 2006]. Currently E1 vaccine is examinated in trials to evaluate its effectiveness in slowing down fibrosis progression [Horsmans Y. *et al.*, AASLD November 21-23, 2005].

XTL-6865

In a study presented at AASLD 2005, patients were given XTL-6865 during and after liver transplantation, which resulted in a HCV RNA reduction. A single antibody version of this product was tested in a pilot clinical program that included both Phase I and Phase II clinical trials.

C. COMBINATION THERAPY FOR HBV AND HCV

The hypothetical advantages of combination therapies are the synergistic antiviral effects and viral resistance decreasing or delay.

To date, in HBV infected patients, no combination therapy has been demonstrated to be really superior to monotherapy. Addition of lamivudine to PEG-IFN therapy does not increase response rates in either HBeAg-positive or HBeAg-negative patients, as it has earlier been shown in conventional IFN therapy [23, 24]. Nevertheless, long-term follow-up of patients treated with PEG-IFNs and lamivudine combination therapy showed durable response at 72 weeks post-treatment in over 80% of responders as well as the association between PEG-IFNs and adefovir for 48 weeks led to a marked decrease in serum HBV-DNA and intrahepatic cccDNA [30].

Adefovir plus lamivudine was superior to adefovir alone in preventing adefovir resistance, suggesting that the benefit of combination therapy may be observed with continued treatment [44]. Finally, it has been reported a superior virologic outcome and decrease of adefovir resistance obtained treating HBeAg negative patients with high baseline HBV-DNA with adefovir plus lamivudine *vs* adefovir alone [44]. All these data seem to support the idea that adding adefovir is better than switching to adefovir monotherapy for patients who develop resistance to lamivudine treatment.

As mentioned earlier, once HCV infection occurs, natural clearance of the viruses is rare, and once chronic hepatitis is established few patients recover naturally. In fact, most of patients progress to liver cirrhosis and finally to HCC. Thus, combination therapies of IFNs or PEG IFNs with alternative "ribavirin like" drugs will have better antiviral, antiproliferative and immunomodulatory effects than those of standard anti-HCV therapy, but with fewer side effects. Moreover, new anti HCV drugs which target specific segments of the HCV genome or STAT-C drugs are also expected to be clinically useful in combination with IFN. In fact, preliminary results from Phase II clinical trials to date have demonstrated that the antiviral effect of valopicitabine is enhanced when this agent is used in combination with PEG IFNs [Afdbal N. *et al.*, AASLD, April 2005, Paris, France, Abstract]. HCV-796 is currently in Phase 1b testing in combination with PEG- interferon [Roberts S, *et al.* 41st EASL. April 26–30, 2006. Abstract 731] (Table **1**).

CONCLUSION

Studies that elucidate the mechanisms of action of novel anti-HBV/HCV compounds, together with the rapid progress of hepadnavirus virology, will provide the basis for the design of more effective antiviral therapies, which are required to treat the enormous number of patients with chronic HBV and HCV infections. It is expected that the complete eradication of both HBV/HCV viruses will be possible in most cases in the close future.

Regarding the hepatitis B virus, the anti HBV drugs that are clinically available at present, besides IFN, are lamivudine and adefovir, which can be now administered without serious side effects. However, patients treated with lamivudine for more than one year often develop a YMDD mutation, which reduces the effectiveness of the drug. Presently, adefovir is given to those patients with or without lamivudine with good results. In the future entecavir, for which clinical trials have now been completed, is expected to be administered widely because of its high antiviral effects and because it produces few resistant mutations. Treatments for HBV-associated liver diseases aim to induce a state of clinical cure by long-term administration of antiviral drugs decreasing viral load on one hand, and to enhance immunological response by using IFN or HBV vaccine on the other hand.

In the case of HCV infection, antiviral treatments have been carried out only for HCV-associated chronic liver diseases. However, these treatments are now being considered

also for non-symptomatic HCV carriers with normal serum ALT because these carriers often develop liver injury in future. Actually, although the urgent need is to eradicate HCV infection only a partially effective combination therapy including ribavirin and PEG IFN alpha is available for C hepatitis. Given the prevalence of C hepatitis, together with the fact that almost half the chronically infected HCV patients are refractory to current therapy, it is important to identify an efficacious immunoprophylactic drug that protects individuals from HCV infection, as well as drugs that hamper the viral life cycle effectively eradicating the infection. The development of specifically anti-HCV targeted drugs (STAT-C) represents the beginning of a new era in anti-HCV therapy. Direct antivirals may enhance the action of IFN, thus reducing the duration of PEG IFN-based therapy. In addition, these targeted agents offer a potential in personalizing therapy according to patient-specific factors. HCV-infected patients, including those who are difficult to treat such as patients infected with genotype 1, patients at risk of anemia, prior non responders, and patients coinfected with HIV, will be able to find new options for enhanced response to treatment.

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