HEPATITIS C

November 2008

Review of Current Treatments and Market Opportunities

The market for hepatitis C treatment is considered in the context of disease prevalence and projected commercial values. Current treatments are reviewed, together with drugs in clinical trials and investigational drugs.

Hepatitis C

REVIEW OF CURRENT TREATMENTS AND MARKET OPPORTUNITIES

EXECUTIVE SUMMARY

Hepatitis C virus (HCV) infection is widespread, grossly under-diagnosed, and drastically under-treated. Globally, infection with HCV is a major cause of acute hepatitis and chronic liver disease. Although infection rates in major markets have slowed, there is significant number of people who were infected before 1990 who are now at risk of cirrhosis and liver cancer. Existing therapies have serious limitations and new therapies are urgently needed.

VIRUS

Structure

Hepatitis C virus (HCV) was first identified in 1989 (Choo, Kuo, Weiner, Overby, Bradley, & Houghton, 1989) and, since then, has been classified as a member of the *Hepacivirus* genus within the family *Flaviviridae*. This family also includes the flaviviruses, such as yellow fever virus, dengue virus, and west Nile virus. HCV is a small (40 to 60 nM diameter), enveloped, single-stranded, positive-sense RNA virus. The virus consists of a core of genetic material (RNA), surrounded by an icosahedral protective shell of protein which is further encased in a lipid envelope of cellular origin (Figure 1). Two viral envelope glycoproteins, E1 and E2, are embedded in the lipid envelope. The genome consists of a single open reading frame of some 9600 nucleoside bases. At the 5' and 3' ends of the RNA are the untranslated regions (UTRs) that are not translated into proteins but which are important for translation and replication of the viral RNA. The 5' UTR has a ribosome binding site (IRES - Internal Ribosomal Entry Site) that starts the translation of the viral polyprotein.

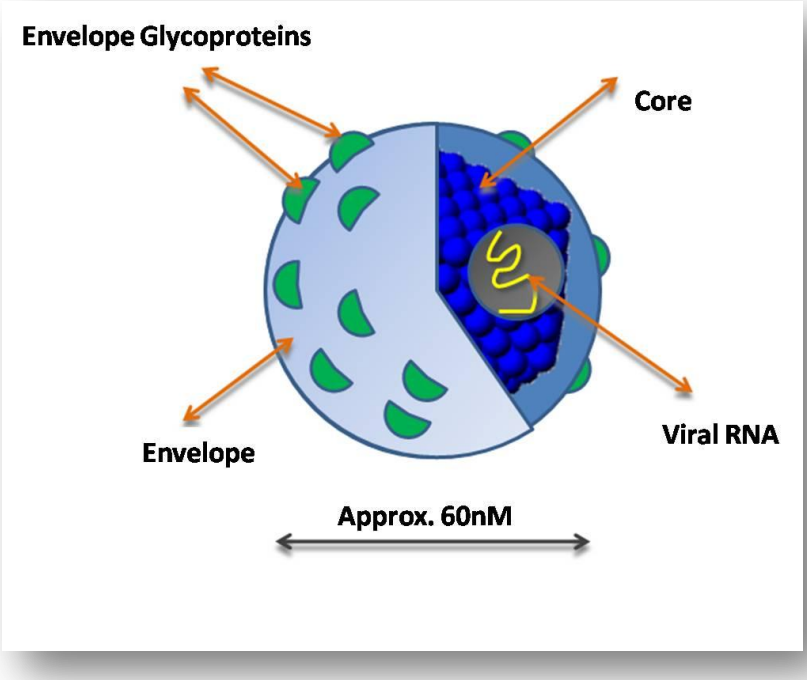

FIGURE 1: STRUCTURE OF HCV

Replication

HCV replicates mainly within hepatocytes in the liver and has a high rate of replication, with approximately one trillion viral particles produced each day in an infected individual. Three HCV cell entry receptors, CD81 (Pileri, et al., 1998), (Flint, et al., 1999), (Cormier, Tsamis, Kajumo, Durso, Gardner, & Tatjana, 2004), human scavenger receptor class B1 (SR-BI) (Maillard, Huby, Andréo, Moreau, Chapman, & Budkowska, 2006), (Scarselli, et al., 2002), and claudin-1 (Evans, et al., 2007) have been identified. Since these receptors are not exclusively expressed in the liver, their involvement in cell entry does not explain the observed HCV liver tropism, and efforts are ongoing to identify hepatocyte-specific cofactors.

Following endocytosis, the virus is uncoated and releases the single-strand 9.6 kilobase genome. The single open reading frame is translated into a polyprotein (approximately 3000 amino acids, depending on the HCV genotype). This polyprotein is then proteolytically processed by viral and cellular proteases to produce three structural and seven non-structural proteins. HCV encodes two proteases; the NS2-3 protease mediates a single cleavage at the NS2/NS3 junction, whereas the NS3-4A serine protease cleaves at four downstream sites in the polyprotein (Figure 2). The non-structural proteins then recruit the viral genome into an RNA replication complex which is associated with rearranged cytoplasmic membranes. RNA replication takes place *via* the viral RNA-dependent RNA polymerase of NS5B, which produces a negative-strand RNA intermediate. The negative strand RNA then serves as a template for the production of new positive-strand viral genomes. Nascent genomes can then be translated, further replicated, or packaged within new virus particles. New virus particles are thought to bud into the secretory pathway and are released at the cell surface.

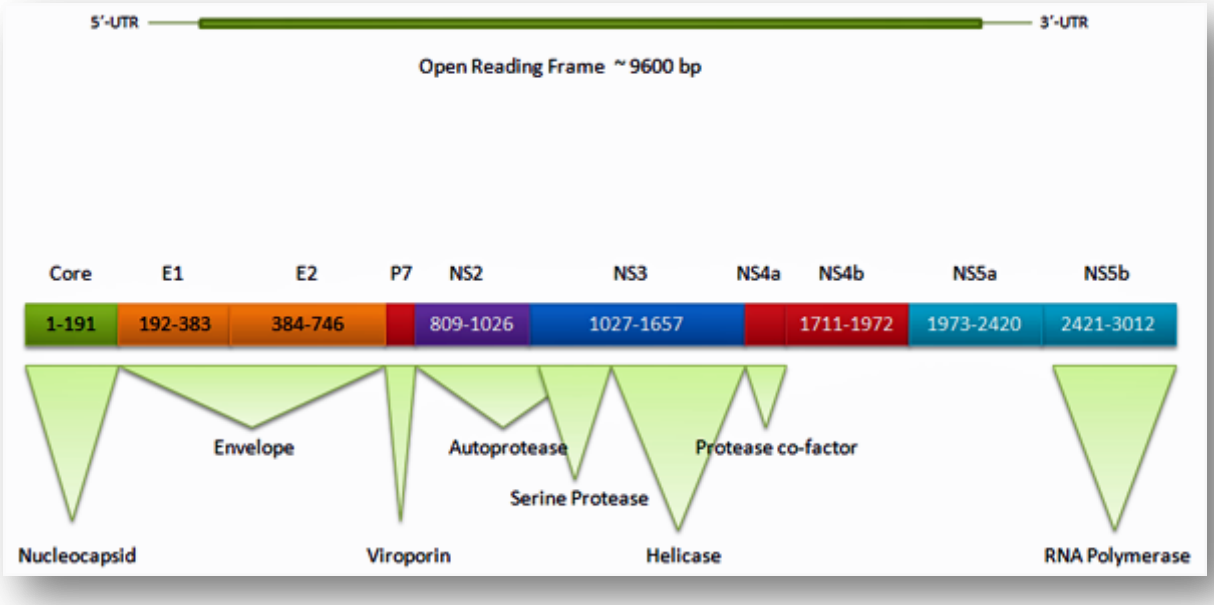

FIGURE 2: HCV GENOME ORGANISATION

Genotypes

There are at least 6 known HCV genotypes, with several subtypes and quasispecies within each genotype. The different genotypes have different geographical distributions; genotype 1 is the most prevalent in the USA followed by genotypes 2 and 3. Table 1 shows the global prevalence of different HCV genotypes. HCV genotype information is particularly important because different genotypes respond differently to the current standard of care treatment and may respond differently to new treatments.

TABLE 1: PREDOMINANT HEPATITIS C GENOTYPE BY REGION (I-BASE, 2008)

Region	Predominant HCV genotype
Europe, North America, Japan	1a or 1b (genotypes 2 and 3 are less common)
Southeast Asia	3
Egypt, the Middle East, Central Africa	4
South Africa	5
Asia	6

DISEASE

Transmission

HCV is spread primarily by unscreened blood transfusions and use of contaminated needles and syringes; the highest risk groups are intravenous drug users and people who received blood transfusions before 1990 when screening for HCV was introduced. Factors that have been reported to influence the rate of HCV disease progression include age (increasing age is associated with more rapid progression), gender (males have more rapid disease progression than females), alcohol consumption (associated with an increased rate of disease progression), HIV co-infection (associated with a markedly increased rate of disease progression), and fatty liver (the presence of fat in liver cells has been associated with an increased rate of disease progression).

Acute Hepatitis C Infection

In the acute phase of infection, which lasts around six months, 60-70% of infected individuals will not develop any symptoms; in the minority of people who do experience acute phase symptoms, these are generally mild and non-specific and rarely lead to a diagnosis of HCV infection. A proportion (15-25%) of individuals infected with HCV clear the virus from their bodies during the acute phase whilst the remaining 75-85% will go on to develop a chronic infection.

Chronic Hepatitis C Infection

Clinically, chronic infection is often asymptomatic until extensive liver damage has occurred. After a latent period lasting for decades, chronic infection leads to cirrhosis, hepatocellular carcinoma, and hepatic failure. 10-20% of infected individuals develop cirrhosis and 1-5% develop liver cancer within 20-30 years (Centers for Disease Control and Prevention, 1998). HCV-associated end-stage liver disease is the most frequent indication for liver transplantation among adults.

Host Response

Although infection with HCV triggers both B-cell and T-cell immune responses, in most cases these are insufficient to clear the virus. There is a growing consensus that acute control of HCV infection is associated with vigorous and multiepitope-specific antiviral CD_4^+ and CD_8^+ T-cell responses which seem to be essential for spontaneous HCV clearance. The experience with interferon therapy has also demonstrated that chronic HCV infection can be controlled and HCV can possibly be eradicated by stimulating the host immune response through adoptive immunotherapy. The exact mechanisms by which HCV evades the immune system are still being investigated, but may include inadequate or un-sustained immune response, rapid viral mutation during the acute phase of infection, and viral interference with the immune response.

Prevalence

HCV is a global disease and, in combination with HBV, now accounts for 75% of all cases of liver disease around the world. Although not all nations have adequate means to carry out thorough surveys, epidemiological studies in different regions of the world suggest wide variance in HCV prevalence patterns, with higher incidences of HCV among less developed nations. The highest incidence regions are China, South America and parts of Africa (Figure 3). In total, over 200 million people around the world are thought to be infected with hepatitis C - an overall incidence of around 3.3% of the world's population. Statistically, as many people are infected with HCV as are with HIV. Co-infection with HIV is common and rates of HCV infection among HIV positive populations are higher.

According to the Center for Disease Control (CDC), there are approximately 3.2 million people in the US with chronic HCV infection, the highest prevalence being amongst individuals born between 1945 and 1965. Although only 802 cases of acute infection were confirmed in 2006, the CDC estimates that approximately 19,000 new HCV infections occurred that year, after adjusting for asymptomatic infection and under-reporting. HCV is the leading cause of liver transplantation in the United States and 8,000-10,000 deaths a year are estimated to be caused by the disease. Although the rate of new infections is declining, a relatively large number of people have now been infected for 20 years or more, and the number of patients seeking treatment is expected to peak within the next 10-20 years.

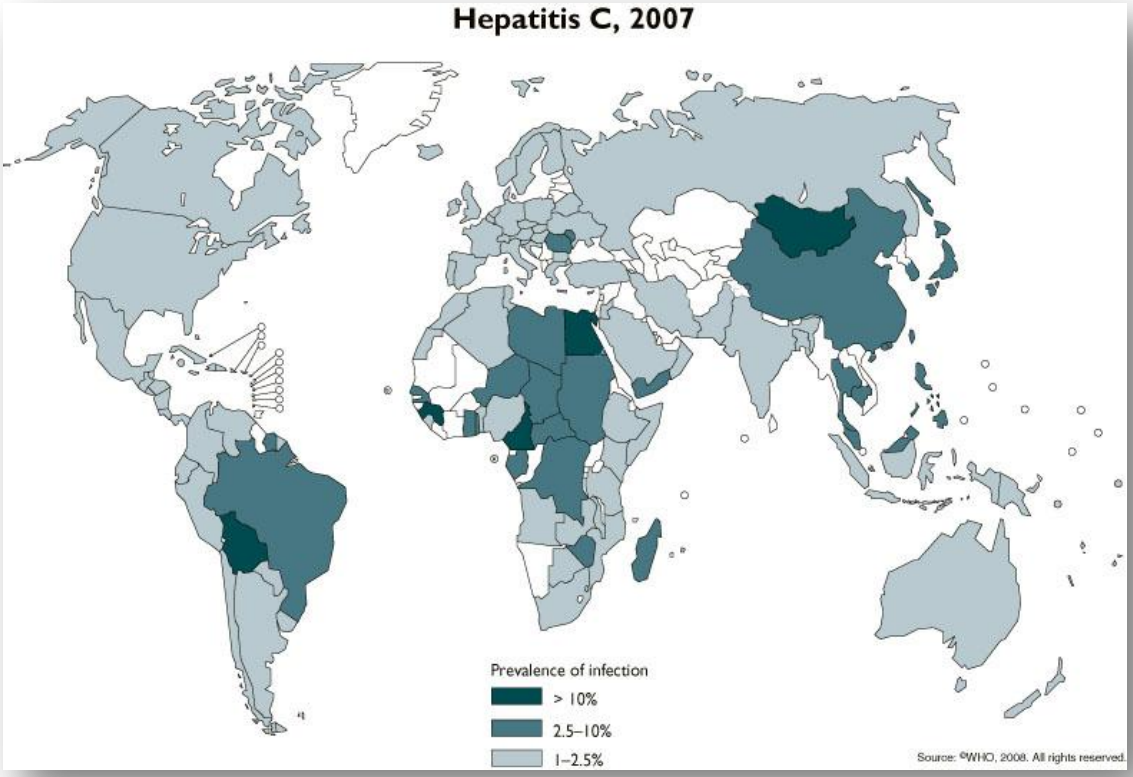

FIGURE 3: GLOBAL INCIDENCE OF HCV (WHO DATA)

DIAGNOSIS

Diagnostic tests for HCV are used to prevent infection by screening donor blood and plasma, to establish clinical diagnoses, and to make better decisions regarding medical management of patients. The diagnosis is rarely made during the acute phase of the disease because the majority of people infected experience no symptoms. Those who do experience acute phase symptoms are rarely ill enough to seek medical attention. The diagnosis of chronic phase HCV infection is also challenging due to the absence or lack of specificity of symptoms until advanced liver disease develops, which may not occur until decades after initial infection.

Chronic hepatitis C may be suspected on the basis of medical history (particularly if there is a history of intravenous drug abuse), a history of piercings or tattoos, or abnormal liver enzymes or liver function tests found during routine blood testing. Occasionally, hepatitis C is diagnosed as a result of targeted screening such as blood donation (blood donors are screened for numerous blood-borne diseases including hepatitis C) or contact tracing.

Diagnostic tests commercially available today are based on enzyme immunoassays (EIA) or enhanced chemiluminescence immunoassays (CIA) for the detection of HCV specific antibodies. Anti-HCV antibodies can be detected 15 weeks after exposure in 80% of patients, 5 months after exposure in 90% of patients and 6 months after exposure in more than 97% of patients. Anti-HCV antibodies indicate exposure to the virus, but cannot determine if ongoing infection is present.

A recombinant immunoblot assay (RIBA) that identifies antibodies which react with individual HCV antigens is often used as a supplementary test to confirm a positive EIA or CIA result.

All persons with positive anti-HCV antibody tests undergo additional testing to determine whether current infection is present. The presence of the virus is detected using polymerase chain reaction (PCR), transcription mediated amplification (TMA), or branched DNA (b-DNA) assays. These tests can also measure the amount of virus present in the blood (the HCV viral load). The HCV viral load is an important factor in determining the probability of response to therapy, but does not indicate disease severity or the likelihood of disease progression. The FDA has recently approved a new, more sensitive HCV test. The COBAS® AmpliPrep/COBAS® TaqMan® HCV Test system, made by Roche Molecular Diagnostics, uses real-time PCR technology to quantify the amount of HCV RNA in a patient's blood. The new test offers a broad dynamic range from high levels of virus (25,000,000 IU/mL or higher) down to the "undetectable" levels of 18 IU/mL.

In people with confirmed HCV infection, genotype testing is generally recommended. HCV genotype testing is used to determine the required length and potential response to interferon-based therapy.

TREATMENT

There is no prophylactic or therapeutic vaccine to prevent or treat HCV infection and current treatments are very expensive, of limited efficacy and have serious side effects. The current cost of 48 weeks of standard of care treatment is approximately $35,000 (Melnikova, 2008). There is thus a significant existing unmet medical need, and the emergence of novel therapies may increase treatment uptake since available options make treatment in the asymptomatic phase unattractive for many patients and physicians.

The current standard of care treatment is a combination of pegylated interferon-α and the antiviral drug, ribavirin. The main treatment goal is to achieve a sustained virological response (SVR), defined as undetectable HCV RNA in peripheral blood 24 weeks after the end of treatment. In practical terms, this is effectively equivalent to eradication of HCV infection and removes the risk of further liver damage. SVR rates for pegylated interferon plus ribavirin depend on HCV genotype. SVR rates for genotypes 2 and 3 are higher (75-90%) compared with genotype 1 (ca 50%), which is particularly difficult to treat. The recommended dose of ribavirin and duration of treatment also depend on the genotype. For patients with genotypes 2 and 3, a 24-week course of combination treatment using pegylated interferon and 800 mg of ribavirin daily is currently recommended, whereas for patients with genotype 1, a 48-week course and higher dose of ribavirin (1,000 or 1,200 mg daily based on body weight) are recommended. Further clinical studies to optimise treatment regimens are being undertaken.

An association has been demonstrated between SVR and the rapidity of viral clearance (Ferenci, et al., 2005)(Fried, Hadziyannis, Shiffman, Messinger, & Zeuzem, 2008). Patients who achieve a rapid virological response, defined as undetectable HCV RNA levels *via* polymerase chain reaction by week 4, have an excellent chance of attaining SVR if therapy is completed (Ferenci, et al., 2005). In addition, patients who become HCV RNA-negative by week 12 have a 60-72% chance of achieving SVR if therapy is completed, whilst patients who become HCV RNA-negative only by week 24 have a less than 50% chance of achieving SVR (Ferenci, et al., 2005).

Viral levels as measured by HCV RNA do not correlate with the severity of the hepatitis or with a poor prognosis (as in HIV infection) but viral load does correlate with the likelihood of a response to antiviral therapy. Response rates are higher in patients with HCV RNA levels in serum of less than 800,000 IU/ml (generally considered to be low). Response rates are higher in younger patients than older people and higher in women than in men. Response rates are also higher among Caucasian and Asian Americans than among African American patients. Average overall response rates in individuals with HCV genotype 1 infection are 50-60% among Caucasian

Americans, but only 25-30% among African American patients. The reasons for these ethnic differences are not understood (NDDIC, 2006).

Interferon

Interferons are cytokines produced by a wide variety of cells in response to the presence of double-stranded RNA, a key indicator of viral infection. Interferons aid the immune response by inhibiting viral replication within host cells, activating natural killer cells and macrophages, increasing antigen presentation to lymphocytes, and inducing the resistance of host cells to viral infection. Several recombinant forms of interferon-α (α-2a, α-2b, consensus interferon) have been used as therapy for hepatitis C, but these have been largely replaced by pegylated interferons (peginterferons). Peginterferons are interferon-α molecules that have been chemically modified by the addition of chains of polyethylene glycol (PEG). Pegylation changes the uptake, distribution, and excretion of interferon, and prolong half-life. Peginterferon can be given once weekly and provides a constant level of interferon in the blood, whereas standard interferon must be given several times weekly and provides intermittent and fluctuating levels. In addition, peginterferon is more active than standard interferon in inhibiting HCV, and gives higher sustained response rates with similar side effects.

Two forms of peginterferon, with roughly equivalent efficacy and safety, have been developed and approved for use: peginterferon α-2a (Pegasys: Roche) and peginterferon α-2b (Pegintron: Schering-Plough).

Interferon Side Effects

Peginterferons have multiple neuropsychiatric effects. Prolonged therapy can cause marked irritability, anxiety, personality changes, depression, and even suicide or acute psychosis. Patients particularly susceptible to these side effects are those with pre-existing serious psychiatric conditions and patients with neurological disease. Patients with depression may benefit from antidepressant therapy using selective serotonin reuptake inhibitors. Generally, the psychiatric side effects resolve within 2 to 4 weeks of stopping therapy.

Peginterferon therapy can also induce autoantibodies, and a 24- to 48-week course triggers an autoimmune condition in about 2% of patients, particularly if they have an underlying susceptibility to autoimmunity. Exacerbation of a known autoimmune disease (such as rheumatoid arthritis or psoriasis) occurs commonly during peginterferon therapy. Peginterferon has also been shown to have bone marrow suppressive effects.

Ribavirin

Ribavirin is an orally active anti-viral drug that is active against a number of DNA and RNA viruses, although its exact mechanism of action in HCV therapy remains unclear. Ribavirin is converted in the body into the 5' triphosphate which is the active species. By itself, ribavirin has little effect on HCV, but when added to interferon therapy it increases the SVR by 2-3 fold.

Ribavirin Side Effects

Ribavirin causes red cell haemolysis to varying degrees in almost all patients. Some patients develop symptoms of anaemia, including fatigue, shortness of breath, palpitations, and headache. Ribavirin has also been found to cause itching and nasal stuffiness. These are histamine-like side effects which occur in 10-20% of patients and are usually mild to moderate in severity.

Given the limited efficacy and adverse events associated with the combination of interferon and ribavirin, it is clear that other treatments for HCV infection are urgently needed. There are also very limited options available for patients who either do not respond to current therapy or who respond and later relapse.

EMERGING THERAPIES

Experts agree that, in the short term at least, it is likely that new treatments will be added on to interferon/ribavirin treatment rather than replacing it.

Therapies that are being developed to treat HCV infection can be subdivided into those that act on a viral protein and those that act by modifying the host response.

Viral Inhibitors in Clinical Development

Ribavirin Analogues

Tarabavirin (previously Viramidine, Valeant Pharmaceuticals) is a prodrug of ribavirin that is preferentially absorbed by the liver and converted into ribavirin by adenosine deaminase. This results in lower accumulation of ribavirin-5'-triphosphate in red blood cells and consequently less haemolytic anaemia. Although phase III clinical trials failed to show equivalent efficacy to Ribavirin (Benhamou, et al., 2006), a subgroup analysis of one of these trials indicated that higher SVR rates were seen in patients receiving more than 15 mg/kg of taribavirin and that the rate of anaemia in this group was approximately half of the rate of patients treated with ribavirin (Pockros, 2008).

Other viral targets for which inhibitors have progressed to clinical studies are described below.

NS3 Protease

The NS3 protease is a serine protease with a shallow hydrophobic substrate binding region which thus represents a significant challenge for rational drug design. Nevertheless, inhibitors of NS3 protease have entered clinical development.

Ciluprevir (BILN 2061, Boehringer-Ingleheim) was the first NS3 inhibitor reported to show efficacy in human studies (Lamarre, et al., 2003), but its development was discontinued when cardiac toxicity was observed in laboratory animals. Two other NS3 inhibitors, telaprevir (VX-950, Vertex) and boceprevir (SCH503034, Schering-Plough) are currently undergoing phase III clinical trials. There are also three other NS3 inhibitors, ITMN-191 (R7227, Intermune/Roche) TMC435350 (Medivir/Tibotec) and MK-7009 (Merck) in earlier stages of clinical studies as well as a number of compounds in preclinical development. NS3 protease inhibitors reported to have entered clinical development are shown in Table 2.

TABLE 2: NS3 PROTEASE INHIBITORS THAT HAVE ENTERED CLINICAL DEVELOPMENT

Compound	Company	Phase
VX-950 (Telaprevir)	Vertex	3
BI 201335	Boehringer Ingelheim	2
TMC435350	Medivir/Tibotec	2
SCH503034 (Boceprevir)	Schering-Plough	2
ITMN-191, R7227	Intermune/Roche	1
MK-7009	Merck	1
VX-500	Vertex	1
VX-813	Vertex	1

In 2007, the World Community Grid (UTBM, 2007) launched a programme to screen *in silico* a combined drug and lead-like library containing 2.2 million compounds against HCV NS3 protease and proteases of other flaviviruses.

RNA Polymerase

Polymerase inhibitors form the largest group of antiviral drugs, with proven efficacy in treating hepatitis B virus, herpes simplex virus, and HIV infection. The HCV RNA-dependent RNA polymerase provides another very attractive drug discovery target, although it is also proving very challenging. Polymerase inhibitors can be divided into nucleoside analogues and non-nucleoside analogues. The nucleoside analogue inhibitors are converted by cellular enzymes into the nucleoside triphosphates which inhibit viral nucleic acid synthesis. The non-nucleoside polymerase inhibitors do not need to be modified by phosphorylation to inhibit the viral polymerase. The non-nucleoside inhibitors bind allosterically on the enzyme surface near to its active site and so disturb its structure and function.

The first-in-class nucleoside analogue polymerase inhibitor, R1626 (Roche), showed an impressive end-of-treatment response rate when given in combination with peginterferon α-2a and ribavirin (Nelson, et al., 2008), but development was terminated in the third quarter of 2008 due to new and unexpected safety findings from a phase IIb study (natap, 2008).

Other nucleoside analogue polymerase inhibitors that have entered clinical development include IDX184 (Idenix Pharmaceuticals), MK-0608 (Isis Pharmaceuticals/Merck) and R7128 (prodrug of PSI-6130, Roche/Pharmasset).

Non-nucleoside polymerase inhibitors that have entered the clinic include HCV-796 (Wyeth/ViroPharma), GS 9190 (Gilead Sciences), GSK625433 (GlaxoSmithKline), PF-00868554 (Pfizer), VCH-759 and VCH-916 (ViroChem Pharma), ANA598 (Anadys Pharmaceuticals) and ABT333 (Abbott).

TABLE 3: POLYMERASE INHIBITORS THAT HAVE ENTERED CLINICAL DEVELOPMENT

Compound	Mechanism	Company	Phase
IDX184	Nucleoside	Idenix Pharmaceuticals	1
MK-0608	Nucleoside	Isis/Merck	1
R7128 (prodrug of PSI-6130)	Nucleoside	Pharmasset/Roche	1
HCV-796	Non-nucleoside	Wyeth/ViroPharma	2
GS9190	Non-nucleoside	Gilead Sciences	1
GSK625433	Non-nucleoside	GlaxoSmithKline	1
PF-00868554	Non-nucleoside	Pfizer	1
VCH-759	Non-nucleoside	ViroChem Pharma	1
VCH-916	Non-nucleoside	ViroChem Pharma	1
ANA598	Non-nucleoside	Anadys Pharmaceuticals	1
ABT-333	Non-nucleoside	Abbott	1

NS5A

NS5A is an HCV non-structural protein that possesses no enzymatic activity and reportedly regulates viral replication and host cell interactions. NS5A inhibitors that have entered the clinic include BMS-790052 (Bristol-Myers Squibb), A-689 (AZD7295) and A-831 (AZD2836) (both Arrow Therapeutics).

Internal Ribosome Entry Site (IRES)

The IRES is a functional stem-loop RNA structure located in the 5' non-coding region of the HCV genome that also spans the first core-coding nucleotides and drives HCV polyprotein translation. Inhibiting IRES function should therefore block the formation of the translational complex involving the ribosomal subunits and cellular proteins and inhibit translation of the HCV polyprotein. To date, only nucleic-acid based strategies have been used, with essentially three classes of IRES inhibitors: antisense oligodeoxynucleotides, ribozymes, and small-interfering RNAs (siRNAs).

Antisense oligonucleotides are unmodified or chemically modified single-stranded DNA or RNA molecules designed to prevent the translation of viral RNA. Chemical modification of oligonucleotides is intended to optimise stability and specificity. Specific binding between an antisense oligonucleotide and its target RNA results in a hybrid RNA molecule that is subsequently degraded by the cellular enzyme, RNase H.

IRES inhibitors that have entered the clinic include ISIS 14803, a 20-unit antisense phosphorothioate oligodeoxynucleotide (Isis Pharmaceuticals) and Heptazyme, an IRES-specific ribozyme (Ribozyme). Both agents were subsequently suspended because of adverse events and limited efficacy.

Cyclosporin Analogues

Cyclosporin-A is an immunosuppressive agent widely used in the management of liver transplant recipients. Evidence has emerged that cyclosporin-A also exerts an inhibitory effect on HCV replication (Firpi, et al., 2006). Cyclophilin has been demonstrated to be an important host factor that supports HCV replication and the anti-HCV activity of cyclosporin-A is mediated through specific blockade of cyclophilin. Novel cyclophilin blockers have the potential to treat HCV

infection and Debiopharm have recently reported positive efficacy results in a phase IIa study for the selective cyclophilin inhibitor, DEBIO-025 (Debiopharm, 2008).

Another cyclophilin blocker that has entered early clinical development is SCY-635 (Scynexis).

Immunomodulators in Clinical Development

Interferons

A number of companies are developing modified interferons or novel interferon delivery systems with the hope of achieving enhanced pharmacokinetic and pharmacodynamic properties, more potent immunomodulatory effects, and better tolerability. Intarcia Therapeutics is developing a sustained release (implanted osmotic mini-pump) delivery system for omega interferon (Omega DUROS®) and Biolex/Octopus are developing Locteron®, a controlled release formulation of interferon. Albuferon® (Human Genome Sciences/Novartis) is an albumin-interferon α-2b fusion protein with a long duration of action. Amarillo Biosciences/Cytopharm are developing a low dose oral interferon.

Nonspecific Immunomodulatory Agents

The immunomodulator, thymalfasin (Zadaxin, SciClone) is a synthetic version of thymosin α-1. Thymalfasin stimulates the immune system by affecting T cells and NK cells, which are the body's most potent defence against infectious diseases. A phase III clinical trial was started to explore the effect of adding thymalfasin to pegylated interferon α-2a and ribavirin for the treatment of hepatitis C. Unfortunately, it has been recently reported that the thymalfasin treatment group did not achieve statistical significance for the primary endpoint of sustained virological response (SVR) as assessed in the primary analysis population (Datamonitor, 2008).

Caspase/Pancaspase Inhibitors

Increased rates of hepatocyte apoptosis and activated caspases have been observed in HCV infection. IDN-6556 (PF-03491390, Pfizer) is a pan caspase inhibitor that entered clinical trials for the treatment of hepatitis C induced liver disease and liver transplantation. In a phase II clinical study, the drug was well-tolerated and there were improved markers of liver function in the patients who were treated. Another caspase inhibitor that has entered clinical trials is GS9450 (LB84451) (Gilead Sciences).

Toll-Like Receptor Agonists

Toll-like receptors (TLRs) are a class of receptors expressed on immune cells that recognize structurally conserved molecules derived from microbes. They are believed to play a key role in the innate immune system, stimulating production of pro-inflammatory cytokines and chemokines. CPG10101 (TLR9 agonist, Actilon™, Pfizer), ANA773 (TLR7 agonist prodrug, Anadys Pharmaceuticals), and IMO-2125 (TLR9 agonist, Idera Pharmaceuticals) have all entered clinical development. Dynavax also have a TLR9 agonist undergoing clinical evaluation.

Other Agents Undergoing Clinical Trials

A number of other drugs are being studied in HCV-infected patients to treat fibrosis, liver cancer, and hepatitis C-associated thrombocytopenia. Compounds with the potential to act directly against the virus or improve the host immune response are also being evaluated. A list of these is provided in Appendix 1.

Viral Inhibitors in Pre-Clinical Development

There are a number of attractive targets that are being evaluated for the treatment of HCV, but for which no clinical candidates have yet been identified.

NS3 Helicase

Although the NS3 helicase is a potentially attractive target for anti-HCV drugs, no helicase inhibitors have yet entered clinical trials. A number of groups, including workers at the Universities of Cardiff (Brancale, Vlachakis, Kandil, Biondaro, Berry, & Neyts, 2008) have research aimed at identifying inhibitors of the NS3 helicase.

HCV p7

The HCV p7 protein is a small hydrophobic protein of 63 amino acids comprising two trans-membrane α-helices separated by a short positively charged cytoplasmic loop which functions as an ion channel. It has been shown to be essential for HCV infectivity and blockers of p7 have been described (Pavlovic, et al., 2005). The Australian company Biotron also has a programme targeting HCV p7 (Biotron, 2008).

Inhibitors of IRES

Several approaches have been undertaken in the attempt to inhibit HCV translation. Antisense oligonucleotides have proven to be invaluable in the characterization of the HCV internal ribosome entry site (IRES). Chemical modification of oligonucleotides has resulted in optimized stability and specificity. Artificial ribozymes have also been developed to target the HCV IRES. Both techniques have demonstrated efficacy *in vitro* and *in vivo*. Various studies have identified cellular cofactor proteins that are required for IRES function, which may present themselves as intervention targets. Recent experiments have revealed that the HCV IRES uses a novel mechanism of recruiting translational components. These new advances in understanding the mechanism of HCV translation could lead to the development of novel IRES inhibitor strategies. PTC/Schering have an early stage programme targeting IRES inhibitors.

Entry Inhibitors

Another potentially promising approach to treating HCV infections would be the inhibition of viral entry into the cell. Replicor, Progenics Pharmaceuticals, Samaritan Pharmaceuticals and iTherX (Immusol)/Novartis have early stage HCV fusion/entry inhibitor programmes.

As well as interest in new targets, there is also considerable effort being devoted to developing novel NS3 protease inhibitors and polymerase inhibitors. Some preclinical activities are summarised in Appendix 2.

Immunomodulators in Pre-Clinical Development

Cytokines

Actokine Therapeutics is developing cytokines, including ActoKine-2, which may have potential for the treatment of HCV infection.

VACCINES

There is currently no approved vaccine against HCV. Recombinant vaccine candidates have proved largely unsuccessful in eliciting a protective response in chimpanzees. Much research is in progress but the high mutability of the HCV genome complicates vaccine development. There are

currently three vaccines undergoing clinical evaluation and a number of others in preclinical development.

GI-5005 is a targeted molecular immunogen (Tarmogen®) from GlobeImmune designed to elicit an HCV-specific T-cell response. Tarmogens are whole, heat-killed recombinant *S. cerevisiae* yeast that express antigens from one or more disease-related proteins. Four-week phase 2 clinical trial data show that GI-5005 doubled viral clearance overall and doubled the rapid virological response (RVR) rate in naïve patients with high viral load (Globeimmune, 2008).

A phase II Study to evaluate Civacir® (human hepatitis C immune globulin) from Biotest Pharmaceuticals in liver transplant recipients was initiated in January 2007.

In February 2006, Intercell initiated a phase II trial with IC41in HCV-infected patients, and has entered into a collaboration with Novartis to develop other HCV vaccines.

Other vaccines in clinical and preclinical development are listed in Table 4.

TABLE 4: VACCINES IN DEVELOPMENT

Compound	Mechanism	Company	Phase
ChronVac-C	DNA-based Therapeutic Vaccine	Inovio/Tripep	1/2a
PeviPROTM	Therapeutic Vaccine	Pevion Biotect	1
TG4040	Therapeutic Vaccine	Transgene	1
HuMax-HepC™	HepC antibody	Genmab	Preclinical
	Therapeutic vaccine	Argos Therapeutics	Preclinical
	Vaccine	GenPhar	Preclinical

MARKET

An estimated 10 million people in the seven major markets[1] have chronic HCV infection. The market to treat hepatitis C virus will grow by nearly five-fold during the next decade, increasing from approximately $2 billion in 2007 to $10-15 billion in 2017 (Melnikova, 2008).

Geoffrey Porges at Sanford Bernstein likewise forecasts that the HCV market will grow, from about $2.5 billion in 2007 to $11 billion in 2012 (CNBC, 2007).

The increase in value will be driven in part by new drugs being added to existing standard of care treatments, and in part by greater uptake of therapy.

Decision Resources forecast that the Chinese market will more than double from $64 million in 2007 to $150 million in 2012 (Redorbit, 2008). Likewise, the Indian market is expected to grow from $21.6 million in 2007 to more than $171 million by 2012, although the population will remain a largely untapped opportunity for growth (Pharmalicensing, 2008).

[1] United States, France, Germany, Italy, Spain, United Kingdom and Japan.

CHALLENGES IN DEVELOPING TREATMENTS FOR HEPATITIS C

Cellular Assays and Animal Models

Barriers to the study of HCV include its very narrow host range. The natural host is human and the only well-established non-human host is chimpanzee, which poses ethical problems for drug testing. There are also a number of mouse models that can be used. These include the SCID-Alb-uPA chimera mouse model (Mercer, et al., 2001), the HCV-Trimera mouse (Ilan, et al., 2002) and a model that utilizes a mouse-adapted replicon-containing Huh-7 human hepatoma cell line expressing a luciferase reporter linked to the HCV subgenome (Zhu, et al., 2006).

Cell culture systems have also been very difficult to establish for HCV. The most commonly used *in vitro* assay system is based on subgenomic replicons that persistently replicate in hepatoma cell lines (Krieger, Lohman, & Bartenschlager, 2001). Recently, advances have been made in establishing systems to study the entire HCV lifecycle (Silberstein & Taylor, 2008). The fact that HCV, as with almost all RNA viruses, exists as viral quasispecies also makes it very difficult to isolate a single strain or receptor type for study.

Resistance

Because HCV mutates rapidly, changes in the envelope proteins may help the virus to evade the immune system. The high mutation rates and number of quasispecies have also made vaccine development very difficult.

A ribavirin-resistant NS5B mutation of hepatitis C virus has been observed during ribavirin monotherapy (Young, et al., 2003). Resistance to both NS3 protease inhibitors (Dahl, Sandstrom, Akerblom, & Danielson, 2007), (De Clercq, 2007), (Cubero, et al., 2008) and NS5B polymerase inhibitors (Le Pogam, et al., 2008), (De Clercq, 2007) has also been observed. One way to attempt to prevent the emergence of drug-resistant strains is to use a combination of drugs targeting different mechanisms. Another approach may be to design drugs aimed at highly conserved proteins where mutations lead to severely compromised replication capacity. Drugs that interact with host targets are also less likely to select for resistant variants.

Patient Populations

HCV patients who have not responded to treatment with peginterferon/ribavirin (non-responders) or who have had a rebound in viral load after treatment (relapsers) represent a significant market opportunity. As with HIV treatment, the future of HCV therapy is likely to involve combination therapy with novel drugs that have different modes of action. In the short-term, such drugs would likely be added to the current interferon-based regimens. With time, potent antiviral combinations could displace interferons altogether. A first step towards interferon-free treatment has been made with the recent announcement that Roche/ InterMune and Pharmasset are planning a study to explore the safety and efficacy of the polymerase inhibitor, R7128 and protease inhibitor, R7227 (ITMN-191) in treatment-naive patients infected with HCV Type 1(Medical News Today, 2008). However, lessons learned from the HIV epidemic suggest that, owing to the high heterogeneity and high mutation rate of HCV, drug resistance is likely to emerge during treatment with specific inhibitors of the viral protease and polymerase even in a combination setting. Therefore, exploring additional targets that are vital for various stages of the viral life cycle remains important.

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APPENDIX 1: ONGOING CLINICAL TRIALS IN HCV-INFECTED PATIENTS

Compound	Generic Name	Mechanism	Company	Phase
	Colchicine	Inhibitor of microtubule polymerization (fibrosis)	Schering-Plough	4
BAY43-9006	sorafenib	Multikinase inhibitor	Onyx Pharmaceuticals/Bayer	4
Procrit		Epoetin-Alfa	Ortho Biotech	3
	Eltrombopag	Thrombopoeitin Receptor Agonist	GSK	3
	Infliximab	TNFa blocker	Schering	3
IPH 1101		Agonist of Tγ9δ2 lymphocytes	Innate Pharma	2
MX3235	Celgosivir	Alpha-glucosidase I inhibitor	Migenix	2
GI262570		Anti-fibrotic	GSK	2
JKB-122		Anti-inflammatory	Jenkin Biosciences	2
SCV-07	gamma-D-glutamyl-L-tryptophan	Broad spectrum immune stimulator	SciClone	2
	Silymarin	Extract of milk thistle		2
	Silibinin	Extract of milk thistle		2
	Prazosin	Fibrosis		2
	Oglufanide disodium	Immunomodulator	Implicit Bioscience	2
MitoQ	mitoquinone	Inflammation/Fibrosis Inhibitor	Antipodean Pharmaceuticals	2
	IET	Interferon Enhancing Therapy	Transition Therapeutics	2
GV1001	Heptovax	Liver cancer	Pharmexa	2
CTS-1027		MMP inhibitor	Conatus	2
TCM-700C		NK cell stimulation	TCM Biotech International	2
MBI-3253	Celgosivir	oral prodrug of castanospermine	Migenix	2
	Methylene blue	Prevents replication of nucleic acids	Bioenvision	2
	Lenocta	Protein tyrosine phosphatase inhibitor	VioQuest Pharmaceuticals	2
	Fluvastatin	Statin	Novartis	2
UT-231B	Miglustat	Therapeutic iminosugars	United Therapeutics/Unither Pharmaceuticals	2
	nitazoxanide	Thiazolides	Romark Laboratories	2

Compound	Generic Name	Mechanism	Company	Phase
KPE02003002			Kemin Pharma	2
CF102		A3AR AGONISTS	CAN-FITE	1
MDX-1106 (ONO-4538)		Anti-PD1	Medarex/Ono	1
	Bavituximab	Anti-Phospholipid antibody	Peregrine Pharmaceuticals	1
CYT107		glyco-r-hIL-7	Cytheris	1
ECH18		Immune Regulator	Enzo Biochem	1
NOV-205		Immunomodulator	Novelos Therapeutics	1
SPC3649	LNAantimiRTM-122	MicroRNA	Santaris Pharma	1
PEG-rIL-29		PEGInterferon lambda	ZymoGenetics	1
LGD-4665		Thrombopoeitin Receptor Agonist	Ligand Pharmaceuticals	1

APPENDIX 2: COMPANIES WITH PRECLINICAL ACTIVITIES IN HCV RESEARCH

Compound	Mechanism	Company
PYN18	Antiviral plant extract	Phynova
PRO 206	Entry inhibitors	Progenics
SP-30	Entry inhibitors	Samaritan Pharmaceuticals
	Entry inhibitors	Itherx (Immusol)/Novartis
	HCV-p7	Biotron
	Helicase Inhibitor	Vertex
HuMax-HepC™	HepC antibody	Genmab
	IRES inhibitor	PTC/Schering
	micro-RNA antagonism of miR-122 host gene	Regulus Therapeutics (Alnylam/Isis)
HepaCide-I™	Nanoviricide	NanoViricides
GL60667	Non-nucleoside polymerase inhibitor	Genelabs (acquired by GSK)
	Non-nucleoside polymerase inhibitor	Migenix
ACH-1095 (GS-9525)	NS4A antagonist	Gilead/Achillion
	NS5A inhibitor	Presidio
	Nucleoside analogue polymerase inhibitor	Boehringer Ingelheim/Biota
	Nucleoside analogue polymerase inhibitor	Genelabs (acquired by GSK)
PSI-7851	Nucleoside analogue polymerase inhibitor	Pharmasset
REP 9C	Phosphorothioate oligonucleotides	REPLICor
	Polymerase Inhibitor	Arrow Therapeutics (AZ)
	Polymerase inhibitor	Biocryst
	Polymerase inhibitor	Enanta
	Polymerase inhibitor	Inhibitex
	Polymerase inhibitor	Medivir/Roche
	Polymerase inhibitor	Medivir/Tibotec
	Polymerase inhibitor	Merck/Metabasis
	Polymerase inhibitor	Phenomix
	Polymerase inhibitor	Roche/Medivir
	Polymerase inhibitor	Tibotec
	Protease Inhibitor	Enanta/Abbott
	Protease Inhibitor	Idenix
PHX1766	Protease Inhibitor	Phenomix
SCH446211	Protease inhibitor	Schering-Plough
	Replication inhibitor	Avexa/TargetDrug
	RNA interference TT033	Benitec/Tacere Bio/Pfizer
SIRNA-034	RNAi	Sirna Therapeutics (acquired by Merck)
	siRNA	Tekmira
	Small molecule antivirals	Alios BioPharma
KPE00001133		Kemin Pharma