X. The Future of HCV Therapy: Viral Targets and Drug Development

Introduction

The standard treatment for hepatitis C virus (HCV), combination therapy with pegylated interferon alfa and ribavirin, has limited efficacy, poor tolerability, and significant expense. New treatment options that are more potent and less toxic are desperately needed. Anecdotal reports suggest that many people with HCV are choosing—often based on the advice of their doctors—to defer treatment due to the limitations of interferon and ribavirin, in the hopes that better options will become available in the next few years. The complicated calculus of when and whether to initiate HCV treatment begins with an assessment of one's current disease state and risk of disease progression. However, for many, these considerations are superseded by the difficulties of managing treatment, including factors such as the duration of treatment, the risk of depression, the potential quality of life impairment, and the necessity of injecting interferon. Candidates for treatment who have HCV genotype 1, HCV viral loads exceeding two million, and/or coinfection with HIV must also confront the lower likelihood of achieving a sustained virological response (SVR). Most people in the United States with chronic HCV infection have one or more of these poor prognostic factors. New and better treatments could mitigate at least some of these concerns and make therapy for HCV more acceptable and, ultimately, more successful. Moreover, more effective treatments are an urgent priority for those who relapse or do not respond to current regimens.

The next advances in HCV treatment will improve on success rates but are unlikely to supplant pegylated interferon alfa and ribavirin as the backbone of therapy. Many of the new agents proposed for HCV treatment are being developed for use in combination with these existing drugs, though some compounds are under investigation as substitutes for or improvements over current formulations of interferon alfa and ribavirin. Neither interferon alfa nor ribavirin were developed specifically to treat HCV infection; both had been developed and approved for other indications well before the actual discovery of the hepatitis C virus. The mechanisms of action of interferon and ribavirin as treatment for HCV are not fully understood, and most likely involve multiple immunomodulatory and antiviral effects (J. Y. Lau 2002; Tanabe 2004; Taylor 2001b). Ongoing research aimed at clarifying these mechanisms may shed light on the nature of viral resistance and the causes of treatment failure. Such studies could provide new insights into strategies to improve treatment response and successfully retreat non-responders and relapers. Studies of viral kinetics and changes in gene expression in individuals on treatment have begun to produce some answers, and have fueled hypotheses about the optimal timing, duration, and dosing of current therapy.

Most current drug development programs are oriented towards antiviral compounds that specifically inhibit HCV by targeting aspects of its replication cycle. Informed by a substantial body of basic research into the molecular virology of HCV, researchers and drug companies are exploring several viral targets and drug candidates. Progress has been delayed by the lack of cell culture and small animal models, standard tools to screen potential drugs. Recent advances in HCV replicon systems have finally enabled in vitro testing of a candidate compound’s ability to inhibit viral replication, while chimpanzees have been used to study pathogenesis and immune response, yielding data that may contribute to efforts at finding a vaccine against HCV.
Several drugs currently in development target aspects of the HCV replication cycle, including translation initiation (antisense oligonucleotides and synthetic ribozymes), cleavage and processing (serine protease inhibitors), and RNA synthesis (RdRp inhibitors, helicase inhibitors, and nucleoside analogues). Other drugs with broad antiviral and immunomodulatory properties are also under investigation (Pawlotsky 2004b; Tan 2002). Most of these drugs are in very early stages of preclinical or clinical development; many of the most promising candidates, including NS3 serine protease inhibitors, will not be available outside of clinical trials until 2008 at the earliest (McHutchison 2002). A few therapeutic vaccine candidates, aimed at improving immune responses to HCV among people with chronic infection, have entered small clinical trials. Yet for the most part, the development of a vaccine to prevent HCV infection has not advanced beyond a few animal studies, and vaccine research faces significant hurdles due to HCV’s genetic diversity (Himoudi 2002; Pancholi 2003).

Government and academic researchers, large pharmaceutical companies, and small biotechnology firms have undertaken dozens of HCV drug and vaccine development programs representing a diverse range of targets and strategies. Beyond the established medical need and public health importance of these efforts, a significant financial incentive underlies the substantial investment by industry in this field. Industry figures estimate the current size of the worldwide hepatitis C treatment market at $2.5 billion. The U.S. HCV market alone is currently calculated at $1.4 billion, representing 100,000 people treated each year. Commonly cited projections of the potential annual market for HCV therapeutics by 2010 typically range from $3 billion to $7 billion or higher. Based on these figures, a new HCV treatment would likely meet the pharmaceutical industry’s criteria of a blockbuster drug: anticipated annual sales of $1 billion.

While commercial motives have generally benefited HCV research, they have also at times hindered progress due to the nature of the patent system. During the 1990s, some drug and vaccine studies were delayed by lawsuits initiated by Chiron against other companies, claiming that their drug development programs infringed Chiron’s patents related to HCV (Cohen 1999). Similarly, access to ribavirin—a compound originally discovered in 1970—had been limited by exclusive licensing arrangements between ribavirin’s developer, ICN Pharmaceuticals (through its former subsidiary, Ribapharm) and Schering-Plough, the manufacturer of standard and pegylated forms of interferon alfa-2b. This limited the ability to study and market potentially superior forms of interferon alfa in combination with ribavirin, while other actions kept the price of HCV therapy artificially high by delaying the introduction of generic, and hence cheaper, forms of ribavirin, which finally reached the market in April 2004. While these issues appear to be largely resolved for now, the potential inhibitory effect of intellectual property disputes on the development of new drugs, vaccines and diagnostics calls for scrutiny and vigilance, especially in emerging therapeutic areas such as RNA interference.

**Targeting the HCV Replication Cycle**

With better knowledge of the molecular virology of HCV, research has increasingly focused on viral targets specific to HCV, rather than general antiviral and immunomodulatory agents. Compounds identified through this work may ultimately increase sustained virological response rates. Except where noted, these drug candidates will be tested and used, at least initially, in combination with interferon alfa and, in most cases, ribavirin. In part, this relates to the perceived need for
combination approaches to therapy in order to offset the potential for developing drug resistance.

The high mutation rate of HCV poses a particular challenge to therapeutic strategies that directly target sections of the virus. For example, mutations in the NS3 serine protease could potentially abolish the antiviral efficacy of a protease inhibitor. As with immune escape, the selective pressure exerted by a protease inhibitor would favor the survival and replication of HCV virions bearing those mutations. Therefore multiple simultaneous strategies for inhibiting HCV replication—the use of interferon alfa and ribavirin alongside the serine protease inhibitor—would minimize the potential for the emergence of viral resistance. This approach to HCV therapy mirrors the standard treatment for HIV, which combines three antiretroviral drugs. In anticipation of future issues with HCV inhibitor resistance, the NIH's National Institute of Allergy and Infectious Diseases (NIAID) awarded a contract for the development of a resistance test for HCV protease and RNA-dependent RNA polymerase inhibitors to ViroLogic, a company that markets assays for HIV drug resistance.

Ideally, several new agents will become available over the next several years, increasing therapeutic options. New drugs could ultimately facilitate a shift away from interferon alfa-based treatment regimens, and hopefully allow patients and doctors to construct combinations of anti-HCV agents tailored to individual circumstances. Such a scenario will not conceivably emerge for several years. Even the agents described below that have already entered clinical trials will most likely not be approved by the FDA until the second half of this decade. Yet the broad range of approaches to drug development is encouraging, increasing the odds of finding successful agents. Despite some gaps in research, largely reflecting lingering questions from molecular virology, HCV drug development programs are targeting virtually every stage of the viral replication cycle.

New approaches to HCV drug development can be divided into three general categories: agents that target viral enzymes (protease, RNA-dependent RNA polymerase, and helicase), agents that target viral envelope proteins (E1 and E2), and agents that target viral RNA. Some research suggests that cellular proteins involved in HCV replication may also be appropriate targets for drug development, though little work has been done in this area. In all cases, the goal of therapy is the disruption of the viral replication cycle.

**Target: viral enzymes**

**NS3 serine protease inhibitors**

The success of protease inhibitors in HIV treatment has inspired numerous drug development programs aimed at producing a protease inhibitor effective against HCV. Virtually all of these efforts have targeted the NS3 serine protease. Drugs that inhibit the NS3 serine protease would block cleavage, making viral replication impossible. NS3 serine protease inhibitors may further prevent the inhibition of interferon responses by HCV NS3 (Ferenci 2004; Foy 2003). Strategies to inhibit NS2-NS3 protease activity have received less attention, though a collaboration between Merck and Aurora Biosciences had made progress in identifying potential inhibitors prior to Aurora's acquisition in 2001 by Vertex (Waxman 2001; Whitney 2002). The candidate furthest along in clinical development, Boehringer Ingelheim's BILN 2061, has shown potent inhibitory effects both *in vivo* and *in vitro*, generating substantial enthusiasm for the prospects of this class of drugs.
Boehringer Ingelheim’s BILN 2061

BILN 2061 is an oral NS3 serine protease inhibitor. Three phase I studies of people infected with HCV genotype 1 have looked at safety and the effect on viral load of a two-day treatment period. Treated subjects in each group received twice-daily 200 mg doses of BILN 2061, administered in a solution of polyethylene glycol and ethanol. In a pilot study, eight subjects with significant fibrosis receiving BILN 2061 all experienced at least a 2-log drop (a 100-fold decrease) in HCV viral load while on treatment; two subjects had a greater than 3-log, or 1000-fold, drop in viral load (Benhamou 2002; Lamarre 2003). A study of cirrhotic patients also showed greater than 2-log drops in viral load in all eight treated subjects (Wedemeyer 2003).

Similar, if less dramatic, results were seen in a third study of individuals with milder liver damage treated at different doses; 7 of 9 who received the 25 mg twice-a-day dose achieved a temporary viral load reduction of at least 1 log (10-fold), while all 8 subjects receiving either 200 mg or 500 mg twice-daily experienced viral load decrease greater than one log (Hinrichsen 2002). A study using similar design but looking at individuals with genotypes 2 and 3 found that BILN 2061 was less effective in non-1 genotypes. Four of the 8 treated subjects experienced viral load declines of greater than 1 log; another subject experienced a smaller drop in viral load, while the remaining three saw no change (Reiser 2003). In all studies, viral loads returned to baseline levels within a week after the two-day treatment period. No significant adverse events were reported in these trials or in dose-escalation studies of healthy volunteers. Based on pharmacokinetic data, BILN 2061 could be dosed twice daily (Lamarre 2003).

Based on these encouraging results, phase II studies of BILN 2061 were scheduled to begin in 2003 in Europe and the United States. These trials have been placed on hold while Boehringer researchers attempt to resolve toxicities observed in monkeys taking high doses of BILN 2061, reportedly related to cardiac abnormalities. As of May 2004, Boehringer has not made any further announcements on the status of their HCV protease inhibitor program.

Other compounds in or nearing clinical trials

Vertex has announced that it plans to bring VX-950, an NS3 serine protease inhibitor originally developed in partnership with Eli Lilly, into clinical trials in the first half of 2004. Initial trials launched in June will examine safety and dosing in healthy volunteers, with plans for a pilot study in people with HCV expected later in 2004. Vertex researchers report that VX-950 appears to inhibit HCV replicons containing a mutation conferring resistance to BILN 2061; similarly, BILN 2061 remains active against VX-950-resistant strains (C. C. Lin 2004).

Schering-Plough also has an active protease inhibitor development program, and has published data on one compound, SCH6, that demonstrated potent in vitro inhibitory effects in HCV replicon systems (Foy 2003; J. J. Lu 2003). A related compound has entered phase I clinical trials in healthy volunteers.

Several other companies reportedly have HCV NS3 protease inhibitors in development, including Abbott, Agouron/Pfizer, Bristol-Myers Squibb, Chiron, Eli Lilly, Gilead, GlaxoSmithKline, InterMune (in partnership with Array Biopharma), and Merck. GlaxoSmithKline has evaluated an HCV NS3...
protease inhibitor in marmosets (a New World primate species related to tamarins) infected with GB virus B (GBV-B, a virus closely related to HCV—see Chapter VIII, The Molecular Virology of Hepatitis C). In this surrogate animal model for HCV infection, treatment with GW0014X, an HCV NS3 protease inhibitor, for four days (by subcutaneous injection twice daily) resulted in a transient 3 log (1,000-fold) reduction in GBV-B viral load (Bright 2004). A novel gene therapy approach exploits the protease activity of NS3 to induce apoptosis in HCV-infected cells. Researchers in Canada have developed a modified form of the BID (BH3 interacting domain death agonist) molecule, which causes cells to undergo apoptosis. The molecule has been modified to act as a substrate for the HCV NS3 protease; upon entering an HCV-infected cell, the modified BID is cleaved by the HCV serine protease and thus activated, triggering cell death. In theory, the cells containing HCV would die off, leaving only uninfected hepatocytes. So far, in vitro data and studies in chimeric mice bearing human hepatocytes support this hypothesis (Hsi 2003; E.C. Hsu 2003). Further studies are underway.

Drug design issues

In theory, the NS3 serine protease should offer several potential points of intervention for therapeutic development, such as blocking the active site which catalyzes cleavage, interfering with the cofactor activity of NS4A, and inhibiting a zinc-binding site that forms a crucial structural component of properly-folded NS3 (Bartenschlager 1999; De Francesco 1996). The latter two approaches have yielded few leads, though some preliminary work on zinc-dependent inhibition has been conducted by Axys (acquired by Discovery Partners), Celera, and Bristol-Myers Squibb (Sperandio 2002; Yeung 2001). The active or substrate-binding site of the NS3 serine protease poses its own challenges, since the cleft or pocket where the enzyme binds to its substrate (the region targeted for cleavage) is quite shallow, and thus a difficult target for drug design (Lindenbach 2003; McHutchison 2002; Penin 2004). However, a number of compounds with the potential to target NS3’s protease activity—at its active site or other susceptible regions—have been identified, including peptidic (peptide-based) inhibitors, non-peptidic inhibitors, and peptidomimetics (Bianchi 2002; Casbarra 2002; Fattori 2002; Ingallinella 2002; Narjes 2003).

These classes of compounds—peptidics, non-peptidics, and peptidomimetics—have different pharmacological properties, which can translate into differences in dosing and metabolism. Peptide-based compounds, which can mimic the NS3 protease’s substrate, range from dipeptides, which contain two amino acids, to hexapeptides, composed of a linear chain of six amino acids (Fischmann 2002; Llinàs-Brunet 2000 [Boehringer Ingelheim]; Tan 2002). Peptidic inhibitors face challenges in bioavailability, since they tend to be degraded rapidly in the body. Non-peptidic inhibitors typically have different methods of binding to NS3, and in general have improved bioavailability over peptidic compounds. Peptidomimetics are compounds developed through structure-based design that mimic or antagonize peptides, with non-peptide-like properties that in theory overcome some of the pharmacokinetics limitations of peptides (Poupart 2001 [Boehringer Ingelheim]; Priestley 2000 [DuPont]; X. Zhang 2003 [Bristol-Myers Squibb]). For HIV treatment, all currently approved HIV protease inhibitors are peptidomimetics, though the first non-peptidic HIV protease inhibitor, Boehringer Ingelheim’s tipranavir, is in late-stage clinical testing. BILN 2061 and VX-950 are peptidomimetic protease inhibitors, while SCH6 is a peptidic inhibitor.
The risk of viral resistance is likely to pose a major challenge to the development and clinical use of NS3 serine protease inhibitors, as it has with HIV protease inhibitors. A recent in vitro study using HCV replicons examined the potential for resistance to Compound 1, a Boehringer Ingelheim agent with protease inhibitor activity. HCV replicons were able to adapt in the presence of Compound 1 and develop resistance to its effects, with several mutations identified that conferred decreased susceptibility to inhibition (Trozzi 2003). Mutations conferring drug resistance have also been identified for BILN 2061 and VX-950 (C. Lin 2004; L. Lu 2004). These findings were not unexpected. Indeed, the potential for resistance is virtually guaranteed for compounds targeting the NS3 protease as well as other viral enzymes, given the quasispecies nature of HCV and its aptitude for evolution in response to selective pressure from the immune system. However, the ability to anticipate resistance mutations through such in vitro methods will enable the optimization of candidate agents and, in principle, the development of protease inhibitor combinations that offset the risks of resistance (Lindenbach 2003). Ideally, companies with compounds in or approaching early clinical development—such as Boehringer Ingelheim, Schering-Plough, and Vertex—will collaborate on researching combination approaches after initial safety and efficacy has been demonstrated.

Progress in developing NS3 serine protease inhibitors and other classes of drugs directly targeting HCV has been delayed considerably by actions taken by Chiron. In 1998, Chiron filed suit against Gilead Sciences and Agouron (and later Vertex and Eli Lilly) for infringing on Chiron’s HCV patents. Chiron filed a number of patents on the basis of its leading role in the discovery of HCV, including patents relating to the NS3 protease. The company maintained that research on protease inhibitors infringed on its patents, and demanded licensing fees from companies conducting research, and a guarantee of royalties from any products reaching the market. These claims had a chilling effect on the field, bringing several companies’ drug development programs to a halt pending resolution of legal issues (Cohen 1999). To date, most of the patent disputes have been resolved, with companies that conduct research on protease inhibitors paying licensing fees to Chiron.

### NS5B RNA-dependent RNA polymerase and NS3 helicase/NTPase

Both the NS5B RNA-dependent RNA polymerase (RdRp) and the NS3 helicase/NTPase constitute major targets for the development of antiviral therapies (Borowski 2002; McHutchison 2002; Tan 2002; Walker 2002; N. Yao 1998). Inhibition of either enzyme would disrupt HCV RNA strand synthesis, preventing the production of genomic HCV RNA for new virions. Part of ribavirin’s mechanism of action may operate at this stage of the viral replication cycle (see ‘Antiviral effects of ribavirin: from chain termination to lethal mutagenesis’ later in this chapter). Several groups are developing other nucleoside analogues for HCV treatment, with some compounds already in clinical trials. Alternately, the HCV NS5B polymerase enzyme could be targeted directly. The RNA binding cleft of RdRp offers potential for small molecule inhibitors, an approach that has been employed to develop compounds active against the RdRp of bovine viral diarrhea virus (BVDV), a pestivirus closely related to HCV (Baginski 2000; Sun 2003).

Other compounds, described by Shire BioChem Inc. and academic collaborators, are characterized as non-nucleoside inhibitors of HCV replication, binding at a distance from the NS5B RdRp active site and preventing protein conformations necessary for enzymatic activity (L. Chan 2003; Reddy
2003; M. Wang 2003). This strategy is roughly analogous to the presumed mechanism of action of the non-nucleoside reverse transcriptase inhibitor (NNRTI) class of antiretrovirals used in HIV treatment—which include efavirenz (Sustiva®) and nevirapine (Viramune™)—and reflects the functional similarities between HCV RdRp and the HIV reverse transcriptase enzyme, an RNA- and DNA-dependent polymerase (Esnouf 1995; Hsiou 1996; Temiz 2002).

Various targets and candidates for helicase/NTPase inhibition have also been proposed, but none are currently in human clinical trials and no major companies have announced drug discovery programs for these targets (Borowski 2000; Borowski 2001; Borowski 2002; Phoon 2001).

**RdRp inhibitors entering clinical trials**

Some HCV RdRp inhibitors have recently moved towards clinical trials.

Idenix Pharmaceuticals (formerly Novirio) has an oral nucleoside analogue, NM283, in clinical development. Novartis has an option to jointly develop NM283. NM283 has shown potent anti-HCV activity in a chimpanzee study reported at the 2003 HEP DART meeting. The first data studying NM283 in people with hepatitis C was presented at conferences in the spring of 2004. A phase I/II dose escalation study examined the safety, pharmacologic profile, and antiviral activity of NM283 given for 15 days to people with HCV genotype 1. At the highest dose (800 mg, once a day), study participants experienced on average a 1 log (10-fold) decline in HCV viral load; the most frequent side effects were nausea and vomiting, which generally faded after the first two days of treatment (Godofsky 2004). A follow-up study to be conducted in the summer of 2004 will evaluate combination treatment with pegylated interferon and NM283 for four weeks. A larger, long-term combination therapy trial will begin in the second half of 2004.

JTK-003, an HCV NS5B RdRp inhibitor developed by Japan Tobacco (JT), has entered into phase II trials in Japan. Phase I trials of JTK-003 have been initiated in the United States by JT’s U.S.-based subsidiary, AKROS Pharma. Japan Tobacco also has another RdRp inhibitor, JTK-109, in phase I studies.

Rigel Pharmaceuticals has begun clinical testing of its non-nucleoside RdRp inhibitor, R803. An initial phase I study in healthy volunteers was completed in January 2004. Rigel initiated a phase I/II trial of twice-daily R803 at different doses in people with HCV in May 2004, with results expected by the end of the year. Roche also has an HCV polymerase inhibitor, R1479, in phase I studies.

ViroPharma and Wyeth have collaborated in developing HCV RdRp inhibitors for several years. An early candidate called VP-50406, alternately described as a helicase and replication inhibitor, had advanced to phase II trials before ViroPharma and Wyeth discontinued further research on this agent due to poor in vivo antiviral activity (McHutchison 2002). ViroPharma used an HCV replicon system to screen for compounds with inhibitory activity against HCV RdRp. A compound dubbed HCV-371 entered phase I trials in January 2003, but ViroPharma terminated development of this compound due to study results showing no effect on HCV RNA in study participants, presumably due to poor pharmacokinetics.
ViroPharma initiated a phase I study in healthy volunteers of another candidate, HCV-086, in early 2004. Pending results from this initial trial, a phase Ib dose-ranging trial among people with HCV is planned for the second half of 2004, with phase II trials anticipated for 2005.

Compounds in preclinical development

Several nucleoside analogues are in development for HCV treatment. Merck, Pharmasset, and Ribapharm have all reported results from preclinical compound screening for anti-HCV nucleoside analogues (Carroll 2003; Murray 2003; Shim 2003; Stuyver 2003a). Roche has a nucleoside analogue, 1048297, in early clinical development. Metabasis Therapeutics received a grant from the National Institute of Allergies and Infectious Diseases to screen for nucleoside analogues active against HCV that specifically target the liver. Merck scientists have described a family of structurally related chain-terminating nucleoside analogues showing in vitro synergy with interferon alfa; a mutation conferring high-level resistance to the compounds has been identified (Migliaccio 2003).

A different version of nucleoside analogues, called nucleotide analogues, may also show promise in HCV therapy (Gallois-Montbrun 2003). Nucleosides must undergo phosphorylation (the addition of one or more phosphate groups) before achieving the form that enables them to intervene in strand synthesis. Nucleotides are nucleosides that have already been partly phosphorylated. In 2002, Biota and GlaxoSmithKline formed a collaboration to screen nucleotides for anti-HCV activity.

Merck scientists have identified two classes of nonnucleoside inhibitors of NS5B RdRp and used HCV replicon cultures to describe mutations that confer resistance to one class of these compounds while remaining sensitive to inhibition by other RdRp inhibitors; other types of NS5B polymerase inhibitors have also been described by this group (Summa 2004; Tomei 2003; Tomei 2004). Merck has formed an HCV drug development partnership with Metabasis. Metabasis will apply its proprietary liver-targeting prodrug technology to Merck compounds, which may reduce any systemic toxicities associated with these drugs and improve their efficacy. GlaxoSmithKline researchers have characterized an agent called Compound 4 that inhibits the initiation of RNA synthesis in vitro and is synergistic with interferon alpha; an NS5B mutation associated with resistance to Compound 4 has been identified (Gu 2003b; Johnston 2003; Nguyen 2003).

Several other companies have non-nucleoside HCV RdRp inhibitors in preclinical development. Scientists from Pfizer and Boehringer Ingelheim have each recently described non-nucleoside inhibitors of HCV RdRp (Beaulieu 2004; Love 2003; McKercher 2004). Israel’s XTL Biopharmaceuticals plans to develop non-nucleoside inhibitors identified and evaluated in preclinical testing by South Korea’s B&C Biopharm (Dagan 2003). XTL plans to file an application with FDA in the second half of 2004 to begin clinical trials of a polymerase inhibitor. Genelabs is screening nucleoside analogue and non-nucleoside RdRp inhibitors that can inhibit HCV replication in replicon models. BioCryst is also evaluating compounds identified by rational drug design methods for anti-RdRp activity. Roche recently entered a partnership with the Swedish company Medivir focused on discovery of HCV NS5B polymerase inhibitors. Abbott and Eli Lilly are also pursuing development of HCV polymerase inhibitors.


**Target: viral envelope proteins**

*Alpha-glucosidase inhibitors, entry inhibitors, monoclonal antibodies, and immunoglobulin*

The post-cleavage modifications of the HCV envelope proteins E1 and E2 may also present a target for drug development. E1 and E2 both undergo glycosylation (the addition of sugar molecules, or glycans) in the endoplasmic reticulum (ER). The glycosylation of E1 and E2 leaves glucose residues on the N-linked glycans that are trimmed off by cellular enzymes, the ER $\alpha$-glucosidases. The removal of these extra glucose residues is a prerequisite for proper folding, since it allows the glycoproteins to associate with the chaperone proteins calnexin and calreticulin (Helenius 2001). Therefore, inhibitors of ER $\alpha$-glucosidases should prevent proper folding and heterodimerization of HCV E1 and E2, ultimately blocking the assembly of infectious viral particles (Branza-Nichita 2001; Dwek 2002). Derivatives of imino sugars, compounds that mimic monosaccharides, have been evaluated *in vitro* as ER $\alpha$-glucosidase inhibitors, with potent antiviral activity that compares favorably to the effects of interferon alfa and ribavirin (Durantel 2004). Castanospermine, a glucose imino sugar, has potent inhibitory effects on glycosylation. Celgosivir (MBI-3253), a pro-drug of castanospermine, has been licensed by the Canadian company Micrologix from Virogen, a U.K. firm. Micrologix plans to initiate phase II trials of celgosivir as an HCV therapy in 2004.

The plethora of promising drugs in development to inhibit the entry of HIV into target cells, along with the FDA approval of the HIV entry inhibitor, enfuvirtide (Fuzeon™), offers encouragement for antiviral strategies targeting HCV entry. HCV entry inhibitors would likely attempt to prevent HCV from binding to its receptor(s), thus blocking the infection of new cells (Lahm 2002). However, the development of such inhibitors would require better knowledge of HCV receptor usage, binding sites on the E1 and E2 glycoproteins, and conformational changes—alterations in the three-dimensional structure of the proteins—induced by receptor binding (see Chapter VIII, The Molecular Virology of Hepatitis C).

Given the uncertainties about the mechanisms of HCV entry, little research has explored strategies for entry inhibition. A team at Kansas State University has synthesized compounds that block HCV envelope protein E2 from binding to CD81 *in vitro* by mimicking part of the CD81 receptor (VanCompernolle 2003). Compounds related to amantadine that may potentially block HCV binding to CD81 *in vitro* have also been reported by researchers at University of California-Irvine, but it remains unclear whether this mechanism will inhibit HCV entry and viral replication (Wagner 2003). Progenics has expressed an intention to develop therapies that block HCV from binding to L-SIGN (liver/lymph node-specific intercellular adhesion molecule-3-grabbing integrin), but the company does not have an active HCV drug development program, and L-SIGN has not been thoroughly validated as a target for HCV entry inhibition (see Chapter VIII, The Molecular Virology of Hepatitis C).

In lieu of targeting receptors used by HCV for cell entry, some researchers have attempted to target the envelope proteins themselves. Several groups have pursued the development of monoclonal antibodies (mAbs) that can neutralize HCV (Cerino 2001; C. Li 2001; Y. H. Zhou 2000). Monoclonal antibodies are derived from a B cell line, sometimes taken from individuals infected with HCV, that is engineered to produce identical antibodies, all targeting the same region of E1 or
E2. Monoclonal antibodies have also been used to identify sites of the properly folded E1-E2 heterodimer susceptible to targeting and neutralization (Burioni 1998; Clayton 2002; Cocquerel 2003a; Habersetzer 1998; Hadlock 2000).

XTL Pharmaceuticals, based in Israel, has begun clinical testing of a human monoclonal antibody (mAb) directed against E2. The mAb, designated HepeX™-C (formerly called XTL-002), is administered by infusion. HepeX-C demonstrated safety and efficacy in phase I trials, and has entered phase II studies to determine whether it can prevent recurrence of HCV viremia in chronically infected patients undergoing liver transplants. XTL has chosen to focus its drug development exclusively on liver transplant recipients, abandoning immediate plans to further research HepeX-C in the larger pool of individuals with chronic HCV infection due to “market conditions” (XTL 2002). In late 2003, XTL indicated that further clinical development will require additional financial support, and that the company will seek a development partner. XTL also halted one of the dosing arms in a HepeX-C study in May 2004, pending further investigation of the death of a trial participant (a liver transplant recipient). XTL has licensed other anti-HCV monoclonal antibodies, also directed against HCV E2, from Stanford University. XTL plans to initiate studies of HepeX-C in combination with one of the newly licensed mAbs.

The Danish company Genmab has an anti-HCV E2 monoclonal antibody, HuMax-HepC, in preclinical development for treating post-transplant HCV re-infection. HuMax-HepC is based on an antibody isolated from an individual with chronic HCV infection and was licensed from Connex GmbH and INSERM, the French National Institute for Health and Medical Research. Genmab is using a proprietary technology to produce fully human monoclonal antibodies from transgenic mice, rather than typical processes that generate monoclonal antibodies in mice which then need to be humanized to remove mouse proteins. Nabi Pharmaceuticals is studying its HCV immunoglobulin Civacir™, an infusion of antibodies to HCV collected from the blood of individuals who are HCV-antibody positive, for the prevention of HCV recurrence following liver transplantation. This approach has proved successful in preventing HBV recurrence; the initiation of a NIAID-sponsored randomized phase I/II trial was announced in December 2002. Nabi announced in February 2004 that preliminary results from this study demonstrated safety and a trend towards reduced ALT levels; further trials are under consideration. A similar effort by the Canadian company Cangene to prevent post-transplant recurrence through anti-HCV hyperimmune serum (blood containing highly reactive antibodies to hepatitis C) was terminated after failure in a phase II trial.

**Other viral protein targets: p7**

*Imino sugar derivatives*

The putative viroporin (pore-forming) activity of HCV protein p7, hypothesized to form ion channels on cell membranes, may provide another target for drug discovery. Some *in vitro* research shows that the antiviral drug amantadine can block p7’s ability to form ion channels, but amantadine does not appear effective in clinical trials as an HCV treatment (Griffin 2003; Griffin 2004; see ‘General Approaches to HCV treatment: Immune modulators and broad-spectrum antivirals’ later in this chapter). However, other compounds, including imino sugar derivatives, may effectively target p7. NN-DGJ is an imino sugar derivative that does not inhibit
α-glucosidase, but rather appears to interfere with viral replication by blocking the formation of ion channels by p7 (Pavlović 2003). Recent research suggests that NN-DGJ may also exert antiviral activity through other mechanisms, including induction of cellular defenses that included interferon-stimulated genes such as 2',5'-OAS (A. S. Mehta 2004; see Chapter IX, Immune Response, Persistence, and Pathogenesis).

While the antiviral potential of imino sugar derivatives has been recognized for several years, development of agents for HCV treatment has been slow, with a lead candidate only recently entering clinical trials. The convoluted path from laboratory to clinic for these compounds began with academic research conducted through collaborations between the Oxford University Glycobiology Institute and the Thomas Jefferson University Medical School in Philadelphia. In an effort to develop therapeutic uses for imino sugar derivatives, these scientists formed a research partnership in 1998—IgX Oxford Hepatitis—with a New Jersey-based biotech, the IgX Corporation, which subsequently changed its name to Synergy Pharmaceuticals. SP231B, a NN-DGJ compound resulting from this work, underwent further development by Synergy, which dubbed this class of imino sugar derivates “alkavirs” based on their alkyl side chains. Synergy, which was acquired by Callisto Pharmaceuticals (formerly Webtronics) in 2003, licensed SP231B to United Therapeutics for development as an HCV treatment. This compound—now called UT-231B—appears to function by blocking p7 ion channels rather than through α-glucosidase inhibition (Pavlović 2003). UT-231B completed phase I trials in healthy volunteers in 2003. A 12-week phase II dose-ranging “proof-of-concept” study in people with HCV who failed standard treatment is underway and expected to be completed by the end of 2004.

**Target: viral RNA**

Some drug discovery programs have focused on HCV RNA, rather than viral proteins. Conserved HCV RNA elements required for efficient viral replication make an attractive target for inhibition, and approaches that interfere with protein synthesis would disrupt an essential step in viral replication (Jubin 2001; Jubin 2003). This approach underlies Rigel Pharmaceuticals’ attempts to develop an inhibitor that targets the HCV internal ribosomal entry site (IRES), thus blocking translation initiation. PTC Therapeutics also has a drug development program aimed at disrupting HCV translation.

Most research to date has largely focused on a field called nucleic acid therapeutics, which studies the antiviral potential of RNA molecules that can bind to HCV RNA sequences. These RNA molecules include ribozymes and antisense oligonucleotides. Most recently, a new technique called RNA interference has generated new excitement for its therapeutic potential, though clinical applications remain years away. These strategies target HCV RNA after its release into the cytoplasm, and aim to block translation of viral proteins.

**Ribozymes**

Ribozymes are enzymes composed of RNA, unlike most enzymes, which are generally proteins. Ribozymes can bind to and catalyze the cleavage of specific mRNA sequences, thus blocking protein synthesis. Studies in the 1990s showed promising results in vitro for ribozymes constructed to target the HCV 5’ UTR and other regions involved in translation and replication (Ohkawa 1997;
More recently, sites accessible to ribozyme-mediated cleavage have been identified on the folded structure of the 5’ UTR, with the suggestion that multiple sites be targeted simultaneously (Nadal 2003). Alternately, trans-splicing ribozymes can introduce new genetic material into a targeted region of RNA (Long 2003). A South Korean group has used this technique to introduce a gene sequence encoding the diphtheria toxin A chain that undergoes HCV IRES-dependent translation, resulting in the death of HCV IRES-containing cells (Ryu 2003).

Heptazyme™, a synthetic ribozyme developed by Ribozyme Pharmaceuticals (RPI) in collaboration with Eli Lilly, targets the 5’ UTR and successfully inhibited replication in cell culture of a chimeric HCV-poliovirus that uses the HCV IRES to initiate translation (Macejak 2000; Macejak 2001a). Heptazyme could be administered by injection subcutaneously or intravenously; a phase I trial found that Heptazyme was relatively well tolerated (Macejak 2001b; Sandberg 2001). However after initiating a phase II trial (put on hold due to primate toxicology—loss of vision in one animal), RPI opted to discontinue development of this drug, presumably due to relatively weak preliminary data on clinical efficacy as well as toxicity issues.

**Antisense oligonucleotides**

Antisense oligonucleotides are short sequences of RNA or DNA, commonly 15 to 20 nucleotides in length, complementary to a sequence of viral RNA. The antisense oligonucleotide hybridizes with, or binds to, the complementary sequence, blocking translation initiation or targeting the RNA for enzymatic degradation by RNase H. In vitro studies from several research groups in the 1990s have demonstrated the potential of antisense oligonucleotides to identify targets on the 5’ UTR and inhibit HCV translation. Since then, continued evolution in technical knowledge has led to the construction of synthetic oligonucleotides which are themselves resistant to cellular degradation and show a high sequence specificity restricted to their target region (Braasch 2002; Faria 2001; Heintges 2001; McCaffrey 2003b; Tallet-Lopez 2003; Toulmé 2001).

One compound currently under investigation, ISIS 14803, has been developed by Isis Pharmaceuticals (originally in partnership with Elan Corporation) to hybridize with the HCV IRES, and showed safety and efficacy in initial phase II trials (Gordon 2002). In May 2003, Isis announced the initiation of phase II trials of ISIS 14803 (given by injection) in combination with pegylated interferon and ribavirin in a group of 30 patients, all with genotype 1 virus, who did not respond to prior pegylated interferon/ribavirin treatment. A phase I/II study is examining the prospect of intensifying standard pegylated interferon/ribavirin treatment by adding ISIS 14803. People who have not achieved a 2 log drop in HCV viral load by 12 weeks of standard combination therapy or an undetectable viral load by 24 weeks will receive 12 weeks of ISIS 14803 (given twice-weekly as a two hour intravenous infusion) while continuing with pegylated interferon and ribavirin. ISIS 14803 is also being studied as a single agent for HCV treatment. AVI BioPharma also has an antisense compound targeting HCV in preclinical development.

RNA aptamers, another type of oligonucleotide, may also inhibit HCV protein synthesis. RNA aptamers (also referred to as RNA ligands) are short RNA sequences that fold into a particular conformation. This folding allows aptamers to bind to the three-dimensional structures on viral mRNA, such as the stem and hairpin loops found in the HCV IRES. Thus, in contrast to the binding
RNA Interference

RNA interference (RNAi, also referred to as gene silencing) is an antiviral mechanism first discovered in plants. RNAi relies on small interfering RNA (siRNA—double-stranded RNA sequences barely over 20 nucleotides in length). These siRNA hybridize (bind) to a complementary sequence of mRNA, thereby targeting the mRNA for degradation by the cell. These siRNA can be designed to bind to specific mRNA sequences, and thus may have broad therapeutic potential.

Several groups have recently synthesized siRNAs that inhibit HCV protein synthesis and replication in cell cultures. Inhibition of a chimeric HCV NS5B/luciferase protein using RNAi has been demonstrated in mice (Kapadia 2003; McCaffrey 2002; Randall 2003; P. S. Ray 2004; Wilson 2003). Researchers from Chiron tested siRNAs targeting various regions of the HCV 5’ UTR in an Huh7 human hepatoma cell line, showing strong and specific inhibition of translation (Seo 2003). A recent study identified different degrees of susceptibility to siRNA inhibition across different regions of the HCV 5’ UTR (Krönke 2004). This study also used an engineered retrovirus vector to successfully deliver short hairpin RNA (shRNA, related to siRNA) effectively to cells, suppressing the replication of HCV replicons.

Mouse studies have shown the potential for siRNA as a therapy against liver disease. siRNA has been shown to protect mice from fulminant hepatitis, or acute hepatic failure, and fibrosis through blocking Fas-mediated cell death (E. Song 2003). A similar study protected mice from acute liver failure through siRNA targeting caspase 8, an enzyme involved in apoptosis (Zender 2003). Two reports have demonstrated inhibition of hepatitis B virus (HBV) through siRNA in a mouse model of HBV infection (Giladi 2003; McCaffrey 2003a).

As a new research tool and potential therapeutic target, the mechanics of RNAi are still being explored. Recent reports indicate that some siRNAs, despite their short length, can upregulate interferon-stimulated genes, at least in part via PKR, thus complicating analysis of their antiviral efficacy (Bridge 2003; Sledz 2003). Alterations in the techniques used to synthesize siRNA may reduce their potential for inducing interferon responses (D. H. Kim 2004). Other research has suggested that some siRNA can degrade target mRNA, albeit less effectively, even when slightly mismatched in complementary nucleotide sequences. A recent study also found that siRNA can affect expression levels of a non-targeted gene with a partial overlapping genetic sequence, raising concerns for RNAi therapeutics about selectivity and the risk of inadvertently targeting cellular genes (Jackson 2003; Pusch 2003; Saxena 2003; Scacheri 2004).

These questions have not dampened commercial interest in exploiting RNAi; companies reported to be developing RNAi therapies for hepatitis C include Acacia/CombiMatrix (collaborating with Spain’s Fundació irsiCaixa), Alnylam, Australia’s Benitec (which recently acquired the California-based Avocel), Nucleonics, and Sirna (Check 2003). Sirna expects to identify a lead candidate for entry into clinical testing in 2004; other companies have announced plans to file IND applications
with FDA as early as 2005.

Intellectual property battles threaten to overshadow the scientific challenges of siRNA therapeutics; a number of companies have filed potentially conflicting patent claims on RNAi technology. In theory, this could stall or jeopardize development efforts, as occurred with HCV drug development in general around Chiron’s HCV patents. Indeed, Benitec has already filed patent infringement suits against rival Nucleonics and two other companies, claiming violation of Benitec’s patented gene silencing technology. These issues should be resolved through a framework that grants broad and open access to technological innovations with reasonable but not burdensome provisions for licensing fees. If patent disputes restrict drug development efforts and suppress competition, people with hepatitis C will suffer.

Drug design issues

Little of the work on ribozymes and antisense oligonucleotides has translated into clinical research on HCV drug development. This reflects in part the need for more research to clarify the potential for safe and effective uses of these classes of therapeutic agents. For instance, ribozymes risk inadvertently cleaving cellular mRNA if they are not carefully designed to specifically and selectively target viral RNA. Therapeutic ribozymes must therefore be highly selective for viral RNA so as not to disrupt important cellular processes and risk toxicity and side effects. Drug delivery—the effective targeting of therapeutic agents to the appropriate cells and tissues—also poses a substantial challenge for clinical applications of ribozymes, since these molecules are easily degraded in the body before reaching the target cell. Drug delivery and tissue targeting remain important obstacles, though some progress has been made on these technical issues.

By the 1990s, both ribozymes and antisense oligonucleotides had attracted considerable interest and investment as potential therapeutics for a broad range of conditions. Ribozymes and antisense oligonucleotides still play important roles as research tools for molecular biology, but enthusiasm about their clinical value has diminished in many quarters. Some of this apparent retreat from the optimism surrounding RNA-based therapeutics reflects the vicissitudes of the biotech investment market, which punished companies for failing to live up to the excessive hype surrounding these technologies in the early-to-mid 1990s.

Skepticism has also mounted in the face of disappointing results from clinical trials—particularly the failure in a phase III trial of ISIS Pharmaceutical’s antisense compound to treat Crohn’s disease. The field has not fully overcome concerns about side effects and issues with drug delivery (Dove 2002; Opalinska 2002). To date, FDA has only approved one antisense oligonucleotide ( Isis’ Vitravene®, for the treatment of CMV retinitis, approved in 1998) for marketing, though several other compounds are in clinical trials. FDA has not approved any ribozyme-based therapies.

The hype that once surrounded ribozymes and antisense oligonucleotides now centers on RNA interference. In some quarters, excitement about RNA interference has all but eclipsed interest in ribozymes and antisense oligonucleotides as potential therapeutics for HCV. Indeed, Ribozyme Pharmaceuticals has shifted its focus entirely from developing ribozymes to RNA interference. The company has gone so far as to rename itself as Sirna Therapeutics, to reflect its new focus on small interfering RNA (siRNA).
Approaches using siRNA, while promising, also face challenges in drug delivery (getting the siRNA to the target cell) and the durability of their therapeutic effect, which tends to be transient using current methods (Kitabwalla 2002). Chemical modifications to siRNA may allow these molecules to resist degradation until they reach target cells (Chiu 2003; Czauderna 2003; Dorsett 2004; Layzer 2004; Muratovska 2004). Based on mouse studies, one group recommends exploring the use of siRNA in a solution of lipiodol (iodine in poppy-seed oil), injected directly into the portal vein of the liver (Zender 2003; Zender 2004). Another potential strategy would involve using viral vectors containing genetic sequences designed to express siRNA; the viral vectors would infect target cells and deliver the genetic sequences enabling intracellular production of siRNA, as in gene therapy (Check 2003; Devroe 2004; Dorsett 2004; Krönke 2004; Yokota 2003). Despite unresolved questions about translating siRNA approaches into therapeutic applications, RNA interference has attracted considerable interest, drawing many researchers into the nascent field, and research is advancing rapidly. Still, the therapeutic potential of siRNA will not be realized for several years, assuming that research overcomes obstacles similar to those facing groups developing antisense oligonucleotides as therapeutic agents (Dove 2002; Jubin 2003; Opalinska 2002; Robinson 2004).

Resistance may also pose a challenge for RNAi therapy. As with ribozymes and oligonucleotide analogues, siRNA molecules must be designed with high specificity for the targeted region of HCV RNA, yet that specificity also makes RNAi approaches vulnerable to the emergence of mutations that escape siRNA hybridization. Such resistance has already been seen in vitro during studies of siRNA targeting HIV and poliovirus (Boden 2003; A. T. Das 2004; Gitlin 2002). Resistance could in theory be prevented by using multiple siRNAs with different viral targets (Lieberman 2003; Saksela 2003). Recent in vitro research using HCV replicons has demonstrated the viability of this strategy, using multiple siRNAs targeting the HCV 5’ UTR and HCV coding sequences within the open reading frame to inhibit replication (Krönke 2004). It has not been determined whether HCV and other human viruses possess other defensive strategies to evade RNA interference, as has been described with plant viruses (Vargason 2003; K. Ye 2003).

**Target: cellular proteins**

HCV replication could potentially be blocked by interfering with cellular proteins required for efficient translation of viral proteins or strand synthesis, as an alternative to directly targeting the HCV viral enzymes and HCV RNA. In theory, agents that target cellular factors may reduce the risk of drug resistance from viral mutation, since the human genes encoding a targeted cellular protein would not mutate in response to therapy. However, recent evidence from poliovirus suggests that drug resistant mutations that obviate the antiviral effects of compounds targeting cellular proteins can occur in some situations (Crotty 2004).

A number of cellular proteins have been implicated in HCV replication and may provide effective targets for drug development. The recent recognition that inhibition of geranylgeranylation can disrupt HCV replication in vitro suggests that prenylation inhibitors, a broad class of drugs that includes statins, may have potential for HCV treatment (J. Ye 2003; see also Chapter VIII, The Molecular Virology of Hepatitis C). Prenylation inhibitors are currently under investigation for the treatment of the hepatitis delta virus and may have activity against a broad range of viruses (Einav 2003b).
Research targeting cellular factors has attracted little activity to date, in part because the specific cellular proteins involved in HCV replication have not been fully identified, and their roles are still being explored. In addition, strategies that target cellular proteins risk interfering with important cellular processes. Nevertheless, some researchers have pursued strategies aimed at cellular cofactors involved in HCV IRES-directed translation.

A 60-nucleotide RNA molecule isolated from yeast (Saccharomyces cerevisiae) can selectively inhibit the cap-independent translation initiation of various viral IRES, including HCV (S. Das 1998a; S. Das 1998b; Venkatesan 1999). The small RNA molecule, dubbed inhibitor RNA (IRNA), does not appear to disrupt inhibition by binding to viral RNA, as in the case of antisense oligonucleotides. The IRNA has been shown to bind to the La protein, apparently mimicking the region of the HCV IRES to which La binds (S. Das 1996; S. Das 1998b). The researchers hypothesize that HCV translation is inhibited through competition between the HCV 5’ UTR and the IRNA for the La protein, which may be required for efficient protein synthesis (S. Das 1998a).

While the yeast RNA itself may not be readily adopted for clinical use, interference with cellular factors involved in translation regulation may be a viable alternative approach to antiviral drug development. BioZak, Inc., a California-based company, hopes to develop or license BZK111, a peptide 18 amino acids in length derived from the IRNA research. BAK111 apparently inhibits viral protein synthesis through competitive binding to the HCV IRES, which blocks La protein and ribosome binding. Targeting cellular proteins such as La involved in HCV translation provides another strategy for inhibiting viral replication that remains largely unexplored, though Anadys Pharmaceuticals is collaborating with German researchers on identifying potential targets among cellular factors essential for HCV protein synthesis. The German company Axxima is also investigating target host cell proteins involved in regulating HCV replication, including gastrointestinal-glutathione peroxidase (GI-GPx). Hybrigenics, a French company, is mapping viral and cellular protein-protein interactions as part of its HCV drug target identification program.

Researchers associated with Immusol and the University of California San Diego School of Medicine have used ribozymes in research to identify a role for human 20S proteasome α-subunit PSMA7 in HCV translation. Their group found that a hairpin ribozyme designated Rz3’X, originally designed to target the HCV 3’ UTR, apparently exerts its effects on HCV IRES-directed translation by cleaving PSMA7 mRNA (Krüger 2001a; Krüger 2001b). The nature of PSMA7’s involvement in HCV translation remains unclear.
Mechanisms of Action for Current HCV Therapy: Interferon and Ribavirin

Introduction

The future of HCV therapy will build on the present—combination therapy with interferon alfa and ribavirin. In most cases, drugs in development for HCV treatment will be used, at least initially, with this combination, or attempt to improve on and substitute for current versions of interferon or ribavirin. Ironically, some of the antiviral properties and effects of the newer anti-HCV agents being developed through rational drug design approaches will, in many cases, be better defined than those of the drugs that have been used in HCV therapy for years. Interferon alfa (originally approved for HCV treatment in 1992) and ribavirin (approved in combination with interferon alfa in 1998) each have a range of antiviral and immunomodulatory effects, making it difficult to pinpoint specific mechanisms associated with response to HCV treatment. Patterns of virological response to combination therapy with interferon alfa and ribavirin also vary significantly according to viral and host factors, indicating that the effect of treatment with these drugs is not necessarily uniform. Nevertheless, a better understanding of the mechanisms through which interferon alfa and ribavirin exert their effects on HCV would aid in designing therapeutic strategies that can augment or refine current combination therapy (Gale 2003). Ultimately, research into the mechanisms of action of current combination therapy may create possibilities for adjunctive therapy that complements their effects, or for safer and more effective alternatives to these drugs.

Some probable mechanisms of action for interferon alfa and ribavirin can be hypothesized, despite much inconclusive or inconsistent research:

- Interferon alfa can trigger cellular defenses that block viral replication.
- Ribavirin may also disrupt or inhibit viral replication during the course of treatment.
- Interferon alfa and ribavirin may both modulate the immune response to HCV, restoring or enhancing an HCV-specific T_{H1}-type response and/or protecting against inflammation and fibrosis.

Interferon alfa and ribavirin appear to operate synergistically (Buckwold 2004; Tanabe 2004). When used alone, ribavirin does not reduce viral load, though it may reduce ALT levels and inflammation. Similarly, response rates to interferon monotherapy are substantially lower than to combination treatment.

Interferon alfa

Interferon alfa used in HCV treatment is a synthetic form of IFN-α secreted by cells in response to infection*. Endogenous IFN-α is a cytokine, a type of protein involved in immune responses, first discovered in 1957 and named to reflect its role in interfering with the viral replication cycle (see Chapter IX, Immune Response, Persistence, and Pathogenesis).

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* For clarity, ‘interferon alfa’ will be used to describe formulations used in treatment, and ‘IFN-α’ will refer to the endogenous substance produced by cells in response to infection.
IFN-α, along with IFN-β and the recently discovered IFN-ω, are classified as type I interferons, while IFN-γ is considered a type II interferon. IFN-α and other type I interferons bind to the IFN-α receptor (IFNAR, composed of two subunits, IFNAR1 and IFNAR2) expressed on cell surfaces. The IFN-α gene family consists of genes encoding over two dozen closely related but distinct IFN-α proteins, referred to as IFN-α subtypes and distinguished by number (IFN-α1, IFN-α2, etc.). For the most part, these subtypes are thought to act similarly, but differences in function and induction (by cell type and by viral stimulus, for example) have been noted (Castelruiz 1999; Foster 1998; Hilkens 2003; Larrea 2001). The liver may primarily express the IFN-α5 subtype, which has also been the predominant subtype found in the blood of individuals with HCV infection, though various other subtypes have also been detected alongside IFN-α5 (Castelruiz 1999; Larrea 2001). The clinical relevance of these observations is unclear; the two forms of alpha interferon most widely used in combination treatment for HCV infection both derive from IFN-α2.

The mechanisms of action of interferon alfa treatment are linked to the anti-HCV activity of endogenous IFN-α. Cells produce IFN-α very early in HCV infection, triggering cellular defenses and invoking immune responses. Endogenous IFN-α does not successfully control viral replication during acute HCV infection (Bigger 2001; Pavio 2003b). Exogenous interferon alfa—interferon alfa used as treatment—presumably operates through the same mechanisms as endogenous IFN-α. Treatment with interferon alfa would thus augment the antiviral and immunomodulatory effects of endogenous IFN-α to the level required for effective control of HCV, and overcome viral resistance to lower levels of IFN-α. The precise nature of the cellular defenses and immune responses induced by alpha interferon therapy remains unclear. Interferon alfa treatment also appears to reduce the risk of hepatocellular carcinoma, even in individuals who do not achieve a sustained virological response, through unknown mechanisms (Hino 2004).

**Antiviral effects of interferon: signaling pathways and interferon-stimulated genes**

Viral infection of a cell triggers a series of intracellular signaling events that stimulate the expression of genes and results in the secretion of endogenous IFN-α. The IFN-α secreted by the infected cell interacts with neighboring cells through interferon receptors, initiating another cascade of intracellular signals that direct the expression of antiviral genes. The induction of IFN-α expression is coordinated by interferon regulatory factors (IRFs), particularly IRF-3 and IRF-7; IRFs belong to a class of proteins called transcription factors that regulate gene expression. Multiple other signaling pathways also play a role in this process, including the NF-κB, JAK/STAT, and JNK pathways. The particular pathways through which interferon alfa therapy exerts its antiviral effects on HCV replication have not been well established. However, research in this area has already benefited from the increased use of microarray analysis—a powerful new tool for studying changes in gene expression—to examine the effects of interferon alfa in HCV replicon systems and in individuals undergoing HCV treatment.

IFN-α conducts its intracellular defense against HCV through the products of these interferon-stimulated genes (ISGs). The expression of ISGs induces an antiviral state in cells, by establishing conditions within the cell unfavorable for or actively hostile towards viral replication. Strategies used by ISGs that restrict viral replication range from the inhibition of cell growth, to suppression of protein synthesis, to apoptosis. Hundreds of ISGs have been identified, many of which may contribute to antiviral defenses; the full complement of ISGs may extend into the thousands (de
Veer 2001; Grandvaux 2002). Most research has focused on the three “classical” interferon-stimulated pathways: PKR, 2’,5’-OAS/RNase L, and Mx proteins (see Chapter IX, Immune Response, Persistence and Pathogenesis). These pathways, as well as ISG56 (also referred to as p56), have all been explored in relation to their contribution to the antiviral defense against HCV. Attempts to identify changes in ISG expression levels in chronic HCV infection and during interferon alfa therapy have produced mixed results that are difficult to correlate with treatment outcomes. Most likely the antiviral defense triggered by endogenous and exogenous forms of IFN-α involves multiple pathways and extends beyond the most frequently studied ISGs. Indeed, one group examined blood samples from seven individuals initiating HCV treatment and found changes in the expression of over one thousand genes within three hours after the dose of interferon alfa (Ji 2003). Any single ISG pathway may ultimately be less important than the interactions between the networks of genes regulated by IFN-α. Hopefully future studies will clarify the key ISGs involved in viral suppression and provide a foundation for optimizing interferon alfa-based therapy (Gale 2003). Some research already suggests that interferon alfa may facilitate viral clearance through routes that do not necessarily, or even primarily, lead to apoptosis (Guo 2003). In theory, the identification of key gene expression patterns associated with treatment success could indicate whether current treatments derived from the IFN-α2 subtype induce the most effective cellular defenses.

Antiviral effects of ribavirin: from chain termination to lethal mutagenesis

Ribavirin was first synthesized by researchers at ICN Pharmaceuticals (recently renamed Valeant) in 1970, and was approved for HCV treatment in combination with alpha interferon in 1998 (J. Y. Lau 2002). Ribavirin shows antiviral activity against a broad range of viruses, and is used as monotherapy to treat respiratory syncytial virus and Lassa fever; most recently it has been used with corticosteroids for the treatment of SARS (Koren 2003; Snell 2001). Ribavirin has limited effects on HCV viral load when administered alone, but appears to work synergistically with alpha interferon treatment (Bodenheimer 1997; Di Bisceglie 1995; Dusheiko 1996). However, ribavirin monotherapy may have value in the treatment of non-responders to combination therapy with interferon alfa. A placebo-controlled study of 48 weeks of ribavirin maintenance therapy in 34 individuals who did not respond to combination therapy found reductions in hepatic inflammation in 8 subjects receiving ribavirin (47%) and no members of the control group (Hoofnagle 2003).

Ribavirin belongs to a class of drugs called nucleoside analogues, which block viral replication during strand synthesis. Nucleoside analogues are compounds that mimic nucleotides and therefore can be incorporated by polymerase enzymes during chain elongation. Incorporation of a nucleoside analogue disrupts strand synthesis by terminating the growing chain of nucleotides. This is the mechanism of action of anti-HIV medications such as AZT (zidovudine; Retrovir®) and ddI (didanosine; Videx®), both nucleoside analogue reverse transcriptase inhibitors (NRTIs) (el Kouni 2002). Ribavirin appears to have a direct but modest inhibitory effect on HCV RNA strand synthesis through chain termination (Maag 2001). Ribavirin may also directly inhibit the HCV NS5B RNA-dependent RNA polymerase (Guo 2003).

Ribavirin may also indirectly inhibit RNA strand synthesis by reducing the intracellular supply of guanosine triphosphate (GTP), one of the four nucleotide building blocks for RNA strand synthesis.
GTP levels depend on the activity of a cellular enzyme, IMPDH (inosine-5'-monophosphate dehydrogenase). Ribavirin functions as an IMPDH inhibitor, thus depleting cells of GTP pools (Sintchak 2000). IMPDH inhibition may account for part of ribavirin's immunomodulatory effects. However, IMPDH inhibition does not appear to account for all of the anti-HCV activity of ribavirin, and IMPDH inhibitors do not always have antiviral effects (Lanford 2001b; Markland 2000; S. Zhou 2003).

A recent theory, which has rapidly gained currency, about ribavirin's mechanism of action postulates that ribavirin induces a state called error catastrophe during HCV replication. Under this model, ribavirin is seen as a mutagen, incorporated into the synthesized negative sense HCV RNA strand and leading to mispaired bases in the complementary strand of genomic RNA. Consequently, the mutations introduced in HCV RNA through nucleotide substitutions result in amino acid changes in the proteins synthesized from the new positive sense mRNA strand. HCV, like other RNA viruses, already has a relatively high mutation rate—viral RNA-dependent RNA polymerase-directed strand synthesis is an inherently error prone process (Steinhauser 1992). HCV replication is relatively tolerant of mutations, which may actually promote viral persistence by enabling the viral population to adapt to host cell environments and resist cellular defenses, immune responses, and antiviral therapy. However, some mutations—individually or in combination—will lead to loss of viral protein function and ultimately abolish the replicative efficiency of HCV. From this perspective, a background level of mutation during HCV replication does not impair viral fitness as long as it does not exceed a certain threshold. A mutation rate that exceeds that threshold will lead to the irretrievable loss, or "melting," of genomic information and viral viability, effectively driving the virus into extinction (Cameron 2001; Domingo 2003).

In this model, termed lethal mutagenesis, ribavirin would increase the mutation rate and push HCV over the threshold and into error catastrophe (Graci 2002). Unlike nucleoside analogue inhibition through chain termination, in lethal mutagenesis, ribavirin is (mis)incorporated into the growing chain without interrupting strand synthesis. This role of ribavirin was first observed through in vitro poliovirus experiments, where the poliovirus polymerase 3Dpol (analogous to HCV's RNA-dependent RNA polymerase) incorporated RTP while continuing chain elongation. The subsequent increase in the poliovirus mutation rate caused by ribavirin was correlated with inhibition of poliovirus replication (Crotty 2000; Crotty 2001). Subsequent research found evidence of a similar mechanism operative in the anti-HCV activity of ribavirin, though ribavirin misincorporation appears to be a relatively infrequent event (Contreras 2002; Maag 2001; Tanabe 2004; S. Zhou 2003). Further support for the lethal mutagenesis theory came from studies of GBV-B infection in tamarin hepatocytes, a surrogate tissue culture model for HCV, where ribavirin increased replication errors and reduced viral infectivity (Lanford 2001b).

Lethal mutagenesis and IMPDH inhibition may work in tandem, as the depletion of GTP could increase the likelihood of RTP misincorporation (Crotty 2001; Lanford 2001b). Indeed, an HCV replicon study examining ribavirin's effects on viral replication found evidence consistent with both lethal mutagenesis and IMPDH inhibition contributing to antiviral activity. The combination of ribavirin and another IMPDH inhibitor (either mycophenolic acid or VX-497; see 'Ribavirin's Successors' later in this chapter) increased inhibition of replication, but without ribavirin, the IMPDH inhibitors had only modest inhibitory effects (S. Zhou 2003). These findings have opened up new possibilities in developing antiviral drugs that promote lethal mutagenesis, and suggest that
IMPDH inhibitors may increase the efficacy of mutagenic compounds such as ribavirin (Crotty 2002; Daifuku 2003; S. Zhou 2003). Interferon may also operate synergistically with ribavirin as an RNA mutagen (Hong 2003). However, the proposed paradigm of error catastrophe as antiviral strategy has not yet been confirmed through in vivo studies of HCV treatment and requires further elaboration to clarify the necessary conditions, constraints, and complexities of these events (Contreras 2002; Eigen 2002; González-López 2004; Grande-Pérez 2002; Pariente 2003; Pfeiffer 2003; Schinkel 2003).

**Interferon alfa and ribavirin as immunomodulators**

Recent microarray research has documented an upregulation in the expression of dozens of genes associated with cellular immune responses within hours of initiating interferon alfa therapy, suggesting the potential importance in HCV treatment of immunomodulatory mechanisms as well as antiviral effects (Ji 2003). Studies into the immunomodulatory role of interferon alfa and its bearing on response to treatment have produced strikingly inconsistent results, in part because they examine different variables and in some cases use different methods. Observed changes in T cell responses and cytokine profiles during therapy suggest that treatment apparently promotes and augments a T\textsubscript{H}1 response to HCV, but studies differ on whether changes in the HCV-specific immune response are sustained or correlate with treatment success (Alvarado Esquivel 2002; Barnes 2002; Cramp 2000; Hempel 2001; Kamal 2002; Sreenarasimhaiah 2003; Z. X. Zhang 1997). Most studies have measured T cells from peripheral blood, though some evidence indicates that robust intrahepatic HCV-specific CD8 cell responses before initiating treatment predict favorable treatment outcomes (Vrolijk 2003).

Ribavirin may also partly function as an immunomodulator, modifying or improving the immune response to HCV (Bergamini 2001b; Cramp 2000; Fang 2001; Tam 1999). The nature of ribavirin’s immunomodulatory effects, particularly in the context of interferon alfa therapy, has not been conclusively determined. Several reports describe a shift to a predominantly T\textsubscript{H}1 response and suppression of T\textsubscript{H}2 responses induced by ribavirin, perhaps mediated by a rise in IL-12 levels or a decrease in IL-4 and/or IL-10 levels (Cramp 2000; Fang 2000; Fang 2001; Hultgren 1998; Ning 1998; Tam 1999). Alternately, some studies indicate that ribavirin counterbalances the immunomodulatory effects of interferon alfa, restoring a proper equilibrium between T\textsubscript{H}1 and T\textsubscript{H}2 responses and increasing the expression of both IFN-\gamma and IL-10 (Amati 2002; J. Martín 1998). IMPDH inhibition may account for part of ribavirin’s immunomodulatory effects. T cells are particularly dependent on IMPDH when they proliferate in response to antigen stimulation (Fairbanks 1995). Ribavirin can reduce T cell proliferation, which may help suppress both inflammation and the development of T\textsubscript{H}2 responses (Heagy 1991; J. Martín 1998).

Overall, research on interferon alfa and ribavirin treatment outcomes tends to support an association between changes in immune dynamics and HCV therapy. Yet studies diverge on the nature and object of the changes in immune parameters induced by interferon alfa, and the relevance of these changes to treatment outcomes. In particular, research has not yet demonstrated that restoration of potent T\textsubscript{H}1-type HCV-specific immune responses is necessary or sufficient for sustained virological responses to treatment. Immunomodulatory mechanisms may have different significance in different individuals, perhaps taking on greater importance when pre-treatment
cytokine profiles are more skewed towards Th2-type responses (Piazzola 2001). However the relationship between cause and effect remains unclear, even in studies documenting an association between response to treatment and improvement in immune responses. Changes in HCV-specific T cell responses, where observed, typically occur during the later stages of treatment, after there have been dramatic reductions in the levels of circulating virus. Perhaps the viral suppression achieved by interferon alfa and ribavirin subsequently enables the re-emergence of HCV-specific immune responses which were previously exhausted by persistent levels of high viral replication.

**Treatment failure and resistance**

Despite the many potential routes through which interferon alfa and ribavirin therapy suppresses HCV, many people do not experience sustained virological responses to treatment. Viral factors—particularly genotype and viral load—play a major role in treatment failure; HCV infection with genotype 1 and a pre-treatment HCV viral load greater than 2 million copies (about 800,000 international units) are both associated with poorer treatment outcomes. The association between genotype and treatment outcome implies that some HCV strains are more resistant to the effects of therapy. Similarly, if a higher viral load predicts treatment failure, then viral replication dynamics should in part determine treatment outcomes.

Several studies have investigated the possibility that HCV viral proteins interfere with interferon alfa’s antiviral activity. The NS5A protein may play a particularly important role in interferon alfa resistance. Some researchers in Japan have described a sequence of the HCV genotype 1b NS5A protein characterized as the interferon sensitivity determining region (ISDR), based on initial studies that found mutations in this region could predict response to interferon alfa treatment (Enomoto 1995; Enomoto 1996). In theory, differences in the genetic sequences encoding NS5A between individuals or across genotypes could therefore account for variations in responsiveness to interferon alfa treatment. While other research in Japan has supported the predictive value of ISDR mutations on treatment outcome, researchers in other countries have been unable to confirm this association, suggesting the existence of subtle strain-specific differences related to geographic distribution of genotype 1b variants (Herion 1997). The role of ISDR mutations in treatment response remains controversial (Schinkel 2004).

Other research has found that the HCV envelope E2 also interacts with and inhibits PKR in vitro, though correlates to interferon alfa treatment outcomes have not been identified (Pavio 2002; Taylor 1999; Taylor 2001a). Similarly, research investigating whether greater pre-treatment complexity and diversity of HCV quasispecies populations predicts treatment failure has been inconclusive. Little is known about potential failure to respond to ribavirin, though a mutation in the NS5B region of the HCV genome conferring in vitro resistance to ribavirin has recently been identified (Young 2003).

In addition to viral factors, host factors also influence treatment outcomes (B. Gao 2004). Several host variables have been proposed, but the extent of their contribution to impaired responses to interferon alfa therapy is unknown. Exagen Diagnostics is developing a genomic marker test to identify individuals most likely to respond to interferon alfa/ribavirin treatment based on gene expression patterns, as well as a prognostic test to assess risk of liver disease progression. Immunologic variables may influence response to interferon alfa. Differences in immunologic
status could account for lower response rates to HCV treatment among individuals coinfected with HIV, though viral factors—specifically the higher HCV viral load seen in HCV/HIV coinfection—could also influence treatment outcomes. Similarly, African-Americans generally have poorer responses to interferon alfa-based therapy than Whites. Differences in cell-mediated immune responses, as seen in the response to acute infection, may account for part of this disparity (K. Sugimoto 2003a).

Individual genetic variations could also affect the response to interferon alfa treatment. A polymorphism in the interferon-stimulated gene MxA has been associated with HCV treatment outcomes (Knapp 2003). In at least some patient groups, high iron levels and a genetic predisposition to iron overload may predict poorer response rates to HCV treatment, though other studies found that hepatic iron concentrations have no impact on treatment outcomes (Coelho-Borges 2002; Distante 2002; Fargion 2002; Pianko 2002; Shedlofsky 2002). In addition, some genetic polymorphisms related to proteins involved in immune responses (e.g., IL-10) may influence the likelihood of response to interferon alfa treatment (Edwards-Smith 1999; Promrat 2003a; H. Saito 2002; Y. Sugimoto 2002; Yee 2001; Yee 2003).

Finally, pharmokinetic parameters affecting the tissue distribution of interferon alfa in the body may also influence treatment outcomes. In some studies, treatment efficacy is reduced among obese patients, potentially implying that overall concentrations of interferon alfa are lower in individuals with high body mass indices (McCullough 2003). Individual variations in ribavirin concentrations have also been linked to differences in HCV treatment outcomes (Larrat 2003).

**Implications for current treatment strategies and future drug development**

Ideally a more refined understanding of the mechanisms of action—and reasons for failure—of interferon alfa and ribavirin could lead to improved treatment outcomes. For instance, minor variations in interferon alfa proteins, as seen with IFN-α subtypes, could hypothetically modify the gene expression profile induced by interferon alfa treatment. If a sustained virological response is associated with a particular profile of ISG expression, then it might be possible to identify or design an interferon alfa protein most likely to induce the desired gene expression profile, perhaps with milder side effects than current treatments. Without information about which interferon-stimulated genes correlate with treatment success, it is difficult to evaluate particular interferon alfa variants in order to optimize HCV treatment response rates. Such data could also help to evaluate proposed strategies to increase the efficacy of current treatment through higher induction doses or longer courses of treatment. The development of alternative, less toxic forms of ribavirin would also benefit from a clearer understanding of the desired effects.

Understanding how the immunomodulatory effects of interferon alfa and ribavirin contribute to HCV treatment success could have particular relevance for individuals coinfected with HIV. If interferon alfa-based treatment succeeds through modulating immune responses, its efficacy may require an intact immune system. This logic underlies the suggestion that people coinfected with HCV and HIV may require antiretroviral therapy aimed at reversing immunodeficiency and immune dysfunction prior to initiating HCV treatment. Similarly, if treatment outcomes depend on the enhancement of immune responses targeting HCV-infected cells, then response rates to interferon alfa-based treatment may be improved by adjunctive therapy with other immuno-
modulatory cytokines.

The broad outlines of the potential mechanisms underlying the success of interferon alfa/ribavirin therapy have largely been established. The specific contributions of the multiple effects of these compounds, the nature of their synergy when used in combination, and mechanisms of resistance all require further investigation (Buckwold 2003; Buckwold 2004; Y. He 2002a; Pawlotsky 2004b; Pfeiffer 2003; Tanabe 2004; Taylor 2001b).

Alternate forms of interferon alfa

Consensus Interferon:

Consensus Interferon (CIFN; Infergen®; interferon alfacon-1) is a synthetic form of interferon alfa, developed by Amgen and licensed to InterMune, that is based on a consensus sequence of all IFN-α subtypes. In contrast, interferon alfa-2a (Roferon®-A; Roche) and interferon alfa-2b (Intron® A; Schering-Plough) are recombinant forms of interferon alfa based on a single subtype. By some measures, CIFN demonstrates higher levels of antiviral activity than interferon alfa-2a and interferon alfa-2b in vitro (Blatt 1996). Studies of CIFN used as monotherapy for chronic HCV showed efficacy and tolerability comparable to or better than interferon alfa-2b (Jensen 1999; Keefe 1997; Tong 1998). A preliminary analysis of a thrice-weekly regimen of CIFN in combination with daily ribavirin showed a sustained virological response of 55%, compared with 31% among patients treated with standard interferon alfa-2b and ribavirin (Sjogren 2002). In small, open-label studies, treatment with CIFN and ribavirin produced sustained virological responses in some individuals who did not respond to or who relapsed after prior interferon alfa treatment, suggesting a role as second-line therapy (Barbaro 2002; da Silva 2002). InterMune announced that in the second quarter of 2004, the company will initiate the DIRECT Trial, a phase III study of daily CIFN and ribavirin in non-responders to prior treatment.

The FDA has approved CIFN for the treatment of chronic HCV, though its use in clinical practice is minimal compared to Roche and Schering’s pegylated interferons. Use of CIFN was initially limited due to the superiority of combination therapy with interferon alfa and ribavirin. When originally approved, ribavirin was only available for use with Schering’s interferon alfa-2b (Intron® A). Schering bundled ribavirin with Intron® A so that ribavirin was not sold separately to be combined with other interferons. While Schering now markets ribavirin separately, standard interferon has been supplanted by more effective pegylated forms (see Chapter V, Hepatitis C Treatment). InterMune initiated a phase I trial of a pegylated version of Infergen, PEG-Alfacon-1, in early 2003. Further development of PEG-Alfacon-1 has been suspended for financial reasons while InterMune seeks a partner to subsidize development costs.

Other interferon alfa variants:

- Albuferon™-alpha, a form of interferon alfa fused to albumin molecules, is in development by Human Genome Sciences. Albumin fusion can extend the half-life of the drug, so that less frequent dosing is possible. Preliminary data from a phase I study indicated that Albuferon has a half-life of up to 157 hours, or nearly a week, suggesting a potential for a once or twice monthly dosing schedule.
The side effect profile was comparable to other forms of interferon alfa (Davis 2002). An on-going phase I/II trial is examining the safety and pharmacologic profile of Albuferon, given as a single dose or in two doses 14 days apart, in 92 people who did not respond to prior HCV treatment.

In May 2004 Human Genome Sciences began a phase II dose-ranging trial in Canada to compare three different doses of Albuferon in people with HCV genotype 1. Study participants will be given Albuferon twice, with 14 days between doses, to evaluate declines in HCV viral load at day 28. The results of this trial will guide the Albuferon dose used in a larger study that will combine Albuferon with ribavirin for 48 weeks in people with genotype 1 who have not had prior HCV treatment.

- Omega interferon, a genetically engineered type I interferon originally discovered by Boehringer Ingelheim and acquired by BioMedicines for development, has entered phase II trials. BioMedicines has announced plans to develop an implantable drug delivery system for omega interferon in partnership with ALZA Corp., as well as an oral prodrug formulation in collaboration with Nobex Corp. An oral drug would provide substantial advantages over currently approved forms of interferon alfa, which require injection, but oral formulations that allow adequate and efficacious drug levels pose significant challenges for drug development. BioMedicines is also researching methods to target omega interferon to the liver, reducing systemic side effects.

- Natural alpha interferon (Multiferon™), developed by Viragen, is a multi-subtype interferon produced from human white blood cells; in contrast, other forms of interferon alfa used to treat HCV are recombinant, synthetic proteins. Multiferon has not been submitted for FDA approval in the United States, though it is marketed in Mexico. Due in large part to its weak financial position, Viragen has no plans to conduct registrational trials that would allow a submission for FDA approval.

Additional forms of interferon alfa are in development. Maxygen is developing an optimized pegylated interferon, which could enter clinical trials for HCV infection in 2005, and recently entered into a partnership with Roche for clinical development and marketing. Amarillo Biosciences is exploring an oral formulation of low-dose interferon alfa, though no trials for HCV are currently planned and the company lacks the resources to conduct clinical research.

Other interferons:

- Interferon beta: Several studies have examined the effects of interferon beta, which might induce an antiviral response similar, though not identical, to the effects of interferon alfa (Cheney 2002). Early studies at low doses showed limited efficacy of interferon beta (Castro 1997; Perez 1995; Villa 1996). More recently, researchers in Japan have used higher doses of interferon beta, administered intravenously—most often as induction therapy before initiating interferon alfa.
treatment. Some studies have shown decent rates of treatment success, though at higher doses interferon beta appears to have a side effect profile similar to that seen with interferon alfa therapy (Horiike 2003; F. Ikeda 2000; Kakizaki 1999; Shiratori 2000; F. Suzuki 2001; Watanabe 2002). Serono has been conducting clinical trials of recombinant interferon beta-1a to treat chronic HCV infection in Asians, and is developing a pegylated formulation of interferon beta. Maxygen, now in partnership with Roche, is also developing an interferon beta compound to treat HCV.

- Interferon gamma: IFN-\(\gamma\) can inhibit HCV replication in a replicon model, and has antiviral effects distinct from but overlapping with those of IFN-\(\alpha\) (Cheney 2002; Frese 2002; Lanford 2003). InterMune has conducted phase II trials of Actimmune\(\textregistered\), a formulation of interferon gamma-1b, as an antifibrotic therapy in people with chronic HCV infection who did not respond to interferon alfa treatment. Results from a 24-week study, which followed twenty patients receiving 200 mcg of interferon gamma subcutaneously thrice weekly, found no overall improvement in fibrosis scores. No serious adverse events were reported, though two study participants dropped out due to side effects (similar to interfer on alfa’s, particularly flu-like symptoms) and ALT elevations. Interferon gamma therapy had no effect on HCV viral load (Muir 2003). Another pilot study of a four week course of interferon gamma treatment administered subcutaneously at 100 or 200 \(\mu\)g thrice weekly found no evidence of antiviral efficacy as measured by changes in HCV viral load and ALT levels (Soza 2003).

InterMune reported in early 2004 that a phase II study of individuals with chronic HCV infection and advanced fibrosis found no evidence of a protective effect of Actimmune on liver histology. Actimmune is being investigated in small studies in combination with Infergen (consensus interferon) to treat non-responders to standard therapy. In 2004 InterMune launched a phase II trial in non-responders that combines daily Infergen with Actimmune three times a week, each given at varying doses. InterMune is also considering conducting studies in 2005 that combine Actimmune with pegylated interferon (Roche’s Pegasys and/or Schering’s Peg-Intron) for people who have not been previously treated for HCV.

Some research has also suggested that sequential interferon alfa and interferon gamma therapy may improve \(T_{H1}\) responses and facilitate viral clearance (Katayama 2001; Kumashiro 2002). InterMune is also collaborating with Maxygen on a more effective, longer-acting form of interferon gamma that is currently in preclinical testing.
Ribavirin’s Successors

The relative success of ribavirin as a component of HCV therapy has led to a search for compounds with similar antiviral effects and fewer toxicities (see Chapter V, Hepatitis C Treatment). Drug development in this area can be seen as a range of hypotheses about the mechanism of action of ribavirin as a component of HCV treatment. Candidate compounds fall under four overlapping categories: next-generation forms of ribavirin, IMPDH inhibitors, agents that induce lethal mutagenesis, and other nucleoside analogues (most of which have been described earlier in this chapter, under ‘NS5B RNA-dependent RNA polymerase and NS3 helicase/NTPase’; see also the entry for isatoribine in ‘General Approaches to HCV treatment: Immune modulators and broad-spectrum antivirals’ later in this chapter):

Next-generation forms of ribavirin. This category includes two compounds, viramidine and levovirin, both discovered by Valeant. Viramidine is a pro-drug of ribavirin that targets the liver, meaning that viramidine enters the body in an inactive form until converted to its active ribavirin form in the liver by the enzyme adenosine deaminase (C. C. Lin 2003; J. Z. Wu 2003). Viramidine, developed by Valeant’s former subsidiary Ribapharm, is anticipated to have effects similar to ribavirin, but with less toxicity, particularly with respect to anemia. A phase II trial of 180 subjects taking viramidine in combination with pegylated interferon for 48 weeks began in December 2002 (Agora 2002; C. C. Lin 2002). Based on favorable 12-week results in an interim analysis of the phase II study, Valeant announced two phase III trials, VISER1 (launched in late 2003) and VISER2 (scheduled to commence in mid-2004). Each trial will enroll about 1,000 patients and compare viramidine to ribavirin, both used in combination with pegylated interferon.

Leovirin is an L-isomer of ribavirin, meaning that its chemical structure is the mirror image of ribavirin’s—the same but reversed; unlike ribavirin, levovirin does not undergo phosphorylation inside cells. Like viramidine, levovirin is thought to have a favorable side effect profile in comparison to ribavirin. Levovirin has no direct antiviral effects against HCV, but shows immunomodulatory properties similar to, and perhaps greater than, ribavirin (Tam 2000). Roche had been investigating levovirin in early phase studies, but discontinued development of levovirin based on unfavorable results from phase I/II trials. Roche also conducted phase I studies of R1518, a pro-drug of levovirin, but further development of R1518 is doubtful.

IMPDH inhibitors. Two IMPDH inhibitors, merimepodib (also denoted VX-497) and mycophenylate mofetil (MMF, marketed by Roche as CellCept®), are being explored as anti-HCV therapies in combination with alpha interferon. Unlike ribavirin, both merimepodib and MMF are non-competitive IMPDH inhibitors—that is, while ribavirin monophosphate mimics inosine 5’-monophosphate and competes with IMP for IMPDH, these new compounds inhibit IMPDH through other mechanisms (Sintchak 2000). In vitro studies suggest that Merimepodib, in combination with alpha interferon, exerts some direct antiviral activity, presumably through depletion of cellular GTP pools (Markland 2000).

Merimepodib, developed by Vertex, entered phase II trials in Europe in 2002 in combination with pegylated interferon and ribavirin. Common side effects attributed to merimepodib in a phase II trial reported at the 2003 HEP DART meeting include diarrhea, abdominal pain, and mild rash, which occurred in up to a quarter of study participants receiving merimepodib, compared to none.
in the control arms. Preliminary unpublished data show that 50 mg of merimepodib taken twice a day, in combination with pegylated interferon and ribavirin, increases the likelihood of prior treatment non-responders reaching undetectable HCV viral loads during re-treatment, though data on sustained virological responses have not been presented.

Based on interim phase II safety and efficacy results, Vertex has announced a phase IIb study, the Merimepodib Triple Combination study (METRO), to begin enrolling in the second half of 2004. METRO will be a randomized, placebo-controlled study of 315 prior non-responders who will receive either merimepodib (at 50 or 100 mg twice-daily) or placebo for six months, in combination with pegylated interferon alfa-2a (Roche’s Pegasys) and ribavirin. Study participants who have undetectable HCV RNA after six months will continue treatment with Pegasys and ribavirin for another 24 weeks.

Research using an HCV replicon model suggests that merimepodib and MMF, at least in the absence of alpha interferon treatment, may have little antiviral activity on their own but could potentiate the mutagenic effects of ribavirin (S. Zhou 2003). Merimepodib and MMF also have immunosuppressive properties, with MMF already approved for use as part of combination therapy to prevent organ rejection following heart, kidney, and liver transplants (J. Jain 2001; Tossing 2003). T cells and B cells involved in the immune response are particularly dependent on the availability of GTP, so merimepodib and MMF effectively suppress immune responses by inhibiting cell division and proliferation (see Chapter IX, Immune Response, Persistence, and Pathogenesis). Despite initial promising findings, recent studies of HCV recurrence following liver transplantation and MMF as monotherapy tend to indicate that MMF itself has no direct antiviral effect on HCV post-transplant recurrence or viremia (Charlton 2002; Firpi 2003). MMF is currently being studied in combination with alpha interferon in patients who did not respond to prior HCV treatment following favorable preliminary clinical data (Afdahl 2001).

Lethal mutagens. The putative role of ribavirin as an inducer of error catastrophe has prompted a search for nucleoside analogues that may have similar effects on HCV; this approach has also been proposed for HIV drug discovery (Crotty 2002; Daikufu 2003; Loeb 1999). At least one company, Koronis Pharmaceuticals, has made the development of lethal mutagens—which they describe as “stealth nucleosides”—the centerpiece of its drug development efforts, focusing on HCV, HBV, and HIV. No candidates have entered preclinical development yet.

General Approaches to HCV treatment: Immune modulators and broad-spectrum antivirals

The search for more effective HCV therapies and better options for interferon alfa-based treatment non-responders and relapers has generated a number of candidates with antiviral and immunomodulatory properties. These agents do not specifically target HCV, and their discovery and synthesis typically predate the identification of the hepatitis C virus. In many cases, these compounds have been investigated or even approved and marketed for the treatment of other conditions. Some, such as the immunomodulatory cytokines and amantadine, have yet to prove their value in the treatment of HCV, despite initial promise. Still other agents are at early stages of development. A few drugs, such as thymosin alfa-1 (Zadaxin®) and histamine dihydrochloride (Ceplene™), have entered large, late-stage efficacy studies to determine whether they benefit prior
non-responders or relapers to interferon alfa-based treatment. For both practical and statistical reasons, trials in non-responders are typically easier to design and less expensive than research in treatment-naïve populations. For smaller companies in particular, this decreases the risk that a drug will fail to reach FDA approval.

**Immunomodulatory cytokines:**

Several studies have attempted to identify the therapeutic potential of several cytokines involved in mediating Th1 and Th2 responses, including IL-2, IL-10, and IL-12. Th1 cytokine therapy may augment cell-mediated immune responses and facilitate clearance of HCV; Th2 cytokine therapy may suppress inflammatory responses and reduce fibrosis. As with interferon alfa, all these cytokines are administered subcutaneously and can have systemic side effects. Due to disappointing results in monotherapy studies, none of these cytokines are currently under active investigation for HCV treatment.

- **IL-2:** A pilot study of 33 individuals with chronic HCV examined the effects of a 12-week course of varying doses of subcutaneous recombinant interleukin-2 (IL-2), a Th1-type cytokine involved in T cell proliferation. While 4 of 33 (8%) experienced a sustained biochemical response, indicated by normalized ALT levels, decreases in HCV RNA during treatment were transient (Pardo 1997). Research on HCV/HIV coinfected patients receiving IL-2 treatment generally indicate that IL-2 does not significantly affect HCV viral load, though some small studies report that IL-2 therapy may suppress HCV replication in some individuals (Hengge 2000; Schlaak 2002; Thibault 2002; Uberti-Foppa 1999; Valdez 2001). A study combining low-dose IL-2 with pegylated interferon and ribavirin in people with HCV/HIV coinfection found that adding IL-2 to standard combination therapy for HCV provided no benefit in response to treatment and resulted in high drop-out rates (Glesby 2004).

- **IL-10:** As a Th2-type cytokine, IL-10 has been investigated for potential antifibrotic activity. A pilot study of 16 patients receiving a 30-day course of IL-10 found that 50% experienced transient normalization of ALT levels during treatment (McHutchison 1999). Another study of a three-month course of IL-10 at different doses in 22 non-responders to interferon alfa treatment found substantial decreases in inflammation (experienced in 19 of 22 subjects, or 86%) and fibrosis (14 of 22 subjects, or 63%) with no elevation in HCV viral load (Nelson 2000). A subsequent study following 28 subjects treated for at least 12 months found decreases in inflammation and fibrosis scores in less than half the treated subjects (13 out of 28 or 46%, and 11 out of 28 or 39%, respectively). HCV viral load increases during therapy averaged 0.5 log, and IFN-γ-producing HCV-specific CD4 and CD8 T cells decreased, with a shift towards a Th2-type immune response (Nelson 2003).

- **IL-12:** Endogenous IL-12 promotes Th1 responses, augments natural killer (NK) cell cytotoxicity, and increases the secretion of IFN-γ. A phase I/II study showed that a ten-week course of IL-12 in 60 patients failed to reduce HCV RNA to
undetectable levels (Zeuzem 1999). A smaller dose-ranging study showed that HCV viral load became undetectable in a few patients receiving the highest dose of IL-12 at the end of twelve weeks of treatment, but all relapsed after therapy (O’Brien 2001). A more recent study of a 48-week course of IL-12 in 225 non-responders to interferon alfa-based treatment was terminated when the proportion of subjects discontinuing treatment due to severe adverse events (7 of 225 or 3%) exceeded the number experiencing a sustained virological response (2 of 160 subjects completing at least 8 weeks of therapy, or 1% [Pockros 2003a]).

Other immunomodulators:

- Thymosin alfa-1 (thymalfasin; Zadaxin®): Thymalfasin is a synthetic peptide derived from human thymus gland extracts (α-thymosins) believed to promote and enhance Th1 responses. Thymalfasin may also have additional antiviral effects through upregulating MHC class I molecule expression on virus-infected cells, and through reducing oxidative stress. Thymosin alfa-1 was originally developed at George Washington University and licensed to Hoffman-La Roche, which in turn licensed the compound to Alpha 1 Biomedicals. Alpha 1 Biomedicals subsequently licensed thymalfasin to SciClone, which is currently studying the agent as a treatment for HCV in non-responders to prior interferon alfa therapy.

Like interferon alfa, thymalfasin is administered subcutaneously; standard dosing is twice weekly. Preliminary data from a dose-ranging study of 31 non-responders to prior interferon-based treatment showed that thymalfasin in combination with Roche’s pegylated interferon alfa-2a produced an early (12-week) virological response ranging from 20% to 36%, depending on thymalfasin dosage (Iftikar 2002). Two randomized, phase III trials, each enrolling 500 patients in the United States, will study the effects of treatment with pegylated interferon alfa-2a, with or without thymalfasin, in non-responders to prior interferon treatment. One study recruited people with mild cirrhosis, and the other will examine people with no cirrhosis. SciClone expects that these studies will be completed by the end of 2005, with data available in 2006.

A third European phase III study, announced in May 2004 and conducted by Sigma-Tau (SciClone’s European partner) will enroll 550 non-responders to prior pegylated interferon/ribavirin therapy. All study participants will receive Roche’s pegylated interferon alfa-2a with ribavirin; in addition, half will receive thymalfasin (1.6 mg twice a week by subcutaneous injection) and half will receive placebo. Study enrollment will commence in late 2004.

SciClone also announced in May 2004 plans to develop a pegylated version of thymalfasin using pegylation technology from Nektar Therapeutics (the same technology used for Roche’s Pegasys). SciClone also has another compound, SCV-07, in preclinical development as a potential oral therapy for infectious diseases with immunomodulatory effects similar to those of thymalfasin.
Histamine dihydrochloride (HDC; Ceplene™): Developed by Maxim Pharmaceuticals, histamine dihydrochloride binds to receptors on intrahepatic monocytes/macrophages, blocking the release of reactive oxygen species. This reportedly reduces oxidative stress associated with viral infection, protecting NK and T cells and facilitating their activation, possibly in synergy with INF-α and mediated by INF-γ. Like interferon alfa, HDC is administered subcutaneously, though an oral formulation is in development. Clinical development of HDC has been relatively slow; after a brief partnership with Roche, Maxim entered into an agreement with Schering-Plough to study HDC in combination with Schering's interferon alfa-2b. A phase II dose-ranging study of 129 individuals with untreated HCV showed that HDC treatment combined with interferon alfa-2b yielded sustained virological responses between 31% and 38%, depending on HDC dosing regimen (Lurie 2002).

In July 2003, Maxim completed enrollment of 302 non-responders to prior interferon alfa/ribavirin therapy in a randomized phase II efficacy trial conducted at European and Canadian sites. This study will compare responses to pegylated interferon alfa-2b and ribavirin with or without HDC. In March 2004, Maxim completed an initial phase Ia of an oral formulation of HDC (dubbed HD-O) and plans to focus further clinical development of histamine dihydrochloride on the oral version.

Isatoribine (ANA245): Anadys has licensed from Valeant two nucleoside analogues, ANA245 (isatoribine) and ANA246, with immunomodulatory properties. Isatoribine reportedly stimulates innate immune responses through interactions with a receptor found on white blood cells called Toll-like receptor 7 (TLR7), thereby increasing INF-α and TNF-α levels and activating NK cells. Isatoribine, administered intravenously, has entered phase I trials, and preliminary data found that isatoribine can reduce HCV viral loads by nearly 1 log (10-fold).

ANA971, an oral prodrug of isatoribine, began phase I testing in healthy volunteers in early 2004. Anadys has also conducted preclinical research on another oral prodrug of isatoribine, ANA975. Anadys announced in May 2004 that ANA975 will be its lead candidate for HCV treatment. ANA246 appears to promote a TH1 response, but Anadys is not currently pursuing development of this compound.

Actilon™ (CPG 10101): Actilon, developed by Coley Pharmaceuticals, is an agonist of Toll-like receptor 9 (TLR9). TLR9 is a pattern recognition receptor found in dendritic cells and B cells. TLR9 recognizes a particular molecular pattern commonly found in bacterial and viral pathogens (CpG motifs, or cytosine-guanine sequences), prompting dendritic cells to release IFN-α and IL-12, promoting a Th1-type T cell response (Kapsenberg 2003; Vollmer 2004). Actilon is a compound that interacts TLR9 and triggers IFN-α secretion and Th1 responses. Coley began two phase I studies of Actilon, administered by subcutaneous injection, in early 2004. Coley expects data from a phase I/II study
of Actilon in people with hepatitis C to be available in late 2004, and plans to begin phase II trials in the second half of 2004.

- Imiquimod and resiquimod: Imiquimod and resiquimod are imidazoquinolinamines, a class of non-nucleoside drugs that stimulates the secretion of IFN-α and various T_H1-type cytokines (Dockrell 2001). Imiquimod has been approved as a topical cream to treat genital warts; a pilot study of oral imiquimod as an HIV treatment found a range of toxicities similar to those associated with other cytokine therapies, including fatigue, fever, malaise, and depression (Goldstein 1998). Preliminary data from a phase II study of resiquimod for chronic viral hepatitis failed to demonstrate antiviral activity (Pawlotsky 2004a).

**Antivirals:**

- Amantadine and rimantadine: Amantadine was the very first antiviral drug, approved by the FDA to prevent influenza in 1966 and marketed as Symmetrel® by Endo Pharmaceuticals. Amantadine has been under investigation for several years as an HCV therapy, having shown some anti-HCV activity in vitro, though no specific inhibition of viral protein synthesis, polyprotein processing, or RdRp-directed strand synthesis was observed (Jubin 2000; J. Martín 1999). Despite some initially promising results for amantadine in clinical trials, several studies have shown little or no antiviral effect alone or in combination with alpha interferon or with alpha interferon and ribavirin (Berg 2003; J. Chan 2002; Craxi 2001; Helbling 2002; Mangia 2001; Thuluvath 2004; Zeuzem 2000). Rimantadine, another drug closely related to amantadine and also used to treat influenza A (Flumadine®, Forest Labs), has shown no benefit as monotherapy for HCV treatment and is not currently under investigation as a component of combination therapy (Fong 1999; Sherman 1999).

- Kemin Pharma compounds: Kemin Pharma, a Belgian company, announced in early 2004 that it was evaluating two compounds for anti-HCV activity. One compound, KPE02003002, is a synthetic molecule derived from a plant chemical. KPE02003002 has advanced to phase II trials. Another compound, KPE00001133, is under preclinical investigation. Their mechanisms of action have not been reported.

**Anti-inflammatory and anti-fibrotic agents:**

- Idun Pharmaceuticals has developed an oral apoptosis inhibitor, IDN-6556, which acts against cellular caspases (proteases that trigger cell death). IDN-6556 has been shown to reduce ALT levels in initial studies of individuals with mild liver disease. In a 14-day study of 40 subjects with HCV, individuals were randomized to receive IDN-6556 at five different doses (ranging from 25 mg once daily to 100 mg twice daily) or to placebo. Compared to placebo, all treatment groups experienced significant transient decreases in ALT and AST levels. Most ALT levels...
remained above the upper limit of normal, except in the 100 mg twice-daily group. Mild side effects included headache, dry mouth, and stomach ache; IDN-6556 did not have significant effects on HCV viral load (Pockros 2003b). Animal toxicology studies lasting up to one year have not found evidence that IDN-6556 increases the risk of hepatocellular carcinoma (a potential concern for apoptosis inhibitors, since the pathogenesis of cancer typically involves overriding signals leading to cell death—see Chapter IX, Immune Response, Persistence, and Pathogenesis). Further phase II studies are planned.

- Pirfenidone: Pirfenidone is an oral compound under investigation by InterMune for the treatment of idiopathic pulmonary fibrosis. A pilot study treated 26 cirrhotics, 15 of whom had chronic hepatitis C infection, with pirfenidone for one year. Pirfenidone treatment reduced inflammation, steatosis, and HCV viral load in a subset of study participants, but results were highly variable and no declines in fibrosis scores were observed (Armendariz-Borunda 2003).

- Enzo Biochem has conducted a phase I study of EHC18, an oral compound thought to modulate immune responses to HCV by inducing tolerance, or non-responsiveness to viral antigens, thus potentially reducing inflammation. No further development plans for EHC18 have been announced, though additional studies are under consideration.

- Several complementary and alternative therapies, particular herbal remedies including milk thistle (active agent: silymarin) and licorice root (active agent: glycyrrhizin) have also been proposed to have beneficial effects in chronic HCV infection and other liver diseases, though data on the safety and efficacy of these agents is limited and often ambiguous (Coon 2004; J. Liu 2003; NCCAM 2003).

Other compounds already in clinical use may also have activity against HCV, directly or indirectly. Recent reports suggest that cyclosporin A (CsA), an immunosuppressant used in liver transplant recipients, can inhibit HCV replication in vitro through a mechanism apparently unrelated to its immunosuppressive properties (Nakagawa 2004; Watashi 2003a). Though CsA does not appear to control HCV effectively in liver transplant recipients, presumably due to immunosuppressive effects, a study in Japan found that a six-month course of HCV treatment with a combination of CsA and alpha interferon was more effective at achieving sustained virological responses than interferon alone (42/76 [55%] vs. 14/44 [32%]; p=0.01) (K. Inoue 2003). Further research is focused on NIM811, a CsA analogue without immunosuppressive activity. In vitro research also shows that sodium stibogluconate, an injectable drug used to treat the parasitic disease leishmaniasis, also inhibits HCV replication through an unknown mechanism (Yeh 2003). Etanercept (Enbrel®), an injectable TNF-α antagonist used to treat rheumatoid arthritis, showed promise in combination with standard interferon and ribavirin in one small study (Zein 2002).
Vaccine Development

With an estimated 170 million hepatitis C infections globally, the development of a vaccine to prevent HCV infection is an urgent priority. Ideally, a prophylactic (preventive) vaccine could block HCV as soon as it enters the body, before it has a chance to establish infection—an immune response called sterilizing immunity. Alternately, a prophylactic vaccine might not fully prevent HCV infection, could facilitate clearance during acute infection, or else attenuate the effects of chronic infection, stimulating protective immunity, so that the virus persisted at low and ultimately harmless levels. Chronically infected individuals could also benefit from a therapeutic vaccine, which could similarly facilitate the suppression of HCV replication (Moingeon 2003).

Vaccines work by stimulating virus-specific humoral (antibody) and/or cell-mediated (cytotoxic T lymphocyte) immune responses (see Chapter IX, Immune Response, Persistence, and Pathogenesis). Effective antibody responses enable sterilizing immunity, since virus-specific antibodies can intercept virions before they are able to infect cells. If a vaccine does not induce antibody responses, cell-mediated immune responses may provide protection against the effects of viral infection, but on their own would not be expected to block infection completely. HCV vaccine research relies on an understanding of the correlates of immunity that enable viral clearance during acute infection. Viral diversity is also an important consideration for vaccine design, since potential antibody and cytotoxic T lymphocyte (CTL) epitopes may vary between genotypes and subtypes, and the quasispecies nature of HCV may allow the virus to escape from vaccine-induced immune responses.

HCV vaccine research has been constrained by its reliance on chimpanzees as the only established model of HCV infection, given the substantial expenses and limited availability of these animals for research (Bukh 2001a). To date, only a handful of published research has studied vaccine efficacy in chimpanzees (Q. L. Choo 1994; Esumi 1999; Forns 2000; Goto 2001; Rollier 2004; Weiner 2001). Mice have been used to study the nature and potency of immune responses to various HCV antigens and prospective vaccine candidates. Rhesus macaques, though not susceptible to HCV infection, have also been used as a primate model to study immunogenicity of potential vaccines (Forns 1999; Q. Li 2003; Polakos 2001).

Researchers have nevertheless made some progress in vaccine design and development, and conducted studies establishing a number of important considerations:

- Prior resolved HCV infection in chimpanzees does not confer sterilizing immunity that prevents reinfection (Farci 1992; Prince 1992).

- Previously infected chimpanzees who resolved prior HCV infection retain HCV-specific memory T cell responses that facilitate viral clearance and/or confine viral replication to low levels, indicative of long-lasting protective immunity (Bassett 2001; Major 2002; Nascimbeni 2003; Shoukry 2003).

- Resolution of HCV infection in vaccinated chimpanzees is associated with the quality of HCV-specific immune responses—specifically a T_{H1}-type response to E1 and NS3—rather than the quantity, or magnitude, of the response (Rollier 2004).
Sterilizing immunity to HCV has occasionally been induced in chimpanzees following immunization, but generally does not protect against other strains of HCV; protective immunity is more frequently observed (Abrignani 1998; Q. L. Choo 1994; Esumi 1999; Esumi 2002; Farci 1994; Farci 1996b; Forns 2000).

Clearance of acute HCV infection can be achieved without the induction of a significant antibody response to HCV. Four cases of resolved HCV infection were documented in an Australian cohort of prisoners; all mounted detectable HCV-specific T cell responses, without conversion to HCV-antibody seropositivity, though weak antibody responses were detected (Post 2004).

Prior resolved HCV infection may confer some protection against viral persistence following re-infection. A group of injection drug users (IDUs) who previously cleared HCV were less likely to develop chronic HCV infection when reinfected than a comparison group of IDUs with no prior history of HCV clearance who became infected (S. H. Mehta 2002).

Prior resolved HCV infection may confer protective immunity against other strains of HCV. Four chimpanzees who had previously cleared infection with HCV genotype 1 were each rechallenged with inocula containing HCV genotype 1, a mixture of genotypes 2 and 3, genotype 4, or a mixture of genotypes 1, 2, 3, and 4. All animals again cleared infection, more rapidly and with lower viral loads than observed during their initial infections (Lanford 2004).

Collectively, these findings support the viability of developing vaccines that elicit protective immune responses—particularly cell-mediated immunity—that facilitate viral control and clearance. However, sterilizing immunity may be difficult to induce, especially against multiple strains (Burton 2002). At the same time, inducing cell-mediated immune responses through vaccination poses considerable scientific challenges in the vaccine field as a whole (Esser 2003; Zinkernagel 2002; Zinkernagel 2003).

In spite of substantial evidence questioning both the relevance of antibody responses to HCV and the prospects of an HCV vaccine inducing sterilizing immunity, the HCV envelope proteins E1 and E2 have been a main focus of HCV vaccine research (Beyene 2002). Attempts to identify and induce effective HCV-specific immune responses directed at E1 and E2 regions, using both in vitro methods and in vivo mice and chimpanzees models, have yielded promising leads but mixed results (Bichr 2002; Esumi 1999; Esumi 2002; Forns 2000; Heile 2000; Lucas 2003; X Ma 2002; Rosa 1996; Satoi 2001; Seong 2001; Tedeschi 1997; Y. H. Zhou 1999; Y. H. Zhou 2002a; J. Zhu 2002). Future efforts should benefit from new techniques to identify neutralizing antibodies using HCV pseudo-particles (Bartosch 2003c).

Some research has attempted to elicit both antibody and T cell responses, using mimotopes (synthetic peptides mimicking naturally-occurring viral epitopes) for the hypervariable region 1 (HVR1) of E2. Immunization with HVR1 mimotopes elicited broad antibody responses in rabbits (Roccasecca 2001). A study of the immunogenicity of HVR1 mimotopes in blood samples from 40 subjects, half of whom were chronically infected with HCV, demonstrated the induction of
cross-reactive CD4 T cell responses recognizing multiple HVR1 variants (Frasca 2003). Ideally, such mimotopes could offer a potential vaccine strategy to prevent the emergence of viral escape mutations by inducing broad, cross-reactive antibody and T cell responses.

Other HCV proteins, including core and NS3, have also received attention as potential immunogens, particularly for vaccines attempting to induce HCV-specific T cell responses. Vaccine approaches under preliminary investigation in mice include DNA vaccines, subunit vaccines, viral vectors, prime/boost combinations, hepatitis C virus-like particles (HCV-LPs), lipopeptides, and dendritic cell vaccines. While current small-animal models cannot adequately reproduce conditions of natural infection, some of these approaches have displayed impressive immunogenicity (Arribillaga 2002; Brinster 2002; Jiao 2003; K. Murata 2003; Pancholi 2003; Qiao 2003; Racanelli 2004; Youn 2003). Various adjuvants, including IL-12 and GM-CSF (granulocyte/macrophage colony stimulating factor) genes, IL-23, CpG motifs, and ISCOMs (immune-stimulating complexes) are also being examined for their potential to enhance vaccine-induced immune responses (Ha 2004; X Ma 2002; Matsui 2003; Ou-Yang 2002; Polakos 2001; Qiao 2003).

Most current vaccine development efforts are in pre-clinical testing, with only a few candidates in human trials. Chiron, having abandoned an early effort in the late 1990s to develop an HCV envelope-based vaccine, has begun clinical testing of a new vaccine candidate. The compound, developed with researchers at St. Louis University’s Center of Vaccine Development, is designed to induce an immune response directed towards envelope proteins for use in preventing HCV infection. Data from a chimpanzee study showed that vaccinated chimpanzees were not protected from infection, but were able to clear HCV. A phase I study currently underway will test the safety and immunogenicity of the vaccine in 45 healthy volunteers. Chiron also has a therapeutic vaccine candidate, administered with the Australian company CSL Limited’s ISCOM® adjuvant and designed to stimulate cell-mediate immune responses. Phase I clinical trials were conducted in Australia in 2002, and further testing in people with chronic HCV infection is planned.

Another therapeutic vaccine, based on a purified version of the HCV E1 protein, has been developed by the Belgian-based Innogenetics. Innogenetics has conducted small phase I and II trials among patients with chronic HCV infection, where its vaccine did not show an effect on HCV viral load. However, some improvements in biochemical and histological markers of disease progression were observed, including reductions in ALT levels, in a study of thirty-five subjects. Twenty-six subjects received vaccinations given intramuscularly at weeks 0, 4, 8, 12, and 24; the remaining nine subjects initially received placebo. After nearly a year, thirty-four subjects from both arms received a round of six vaccinations given every three weeks. Twenty-four subjects who received both rounds of vaccination underwent biopsies before vaccination and seventeen months later. Nine subjects experienced modest histological improvements (37%), with stable histology in ten other subjects (42%); biopsy scores worsened in five subjects (21%) (Nevens 2003). These results certainly warrant further study, but are difficult to interpret due to the lack of effect on HCV viral load and the absence of an unvaccinated control group (Ghany 2003). A larger placebo-controlled phase II trial is planned for 2003-4, enrolling 150 patients for whom current HCV treatment is contraindicated or previous treatment was unsuccessful. Innogenetics is also conducting testing in animals of a prophylactic HCV E1 vaccine.

Intercell, an Austrian company, is developing a therapeutic vaccine that incorporates five HCV
peptides primarily targeting non-structural proteins, for use with a proprietary adjuvant. These peptides were selected to present T cell epitopes associated with immune responses that succeed in clearing HCV during acute infection. A phase II dose-escalation study in non-responders to prior interferon-based treatment was initiated in late 2002, with completion expected by the end of 2003. The company has indicated that it hopes to file for approval in 2007; a second-generation HCV vaccine using other epitopes is in preclinical development. Epimmune and Genencor are also collaborating on the development of prophylactic and therapeutic vaccines for HCV, with a focus on stimulating HCV-specific CD4 T cell responses; Genencor transferred its rights under the collaboration agreement to Innogenetics in 2004. The Swedish company Tripep is planning clinical trials for its therapeutic vaccine candidate Chron-VacC™, and is developing a prophylactic vaccine candidate with the Vaccine Research Institute of San Diego. The Canadian firm ViRexx is pursuing preclinical development of HepaVaxx C, a therapeutic vaccine comprised of viral antigen fused to foreign antibodies; clinical studies could begin in late 2005.

HCV vaccine development will require further exploration of the correlates of immunity and the mechanisms of viral persistence. The capability of some people to clear acute infection through successful immune responses certainly supports the concept of prophylactic vaccination. Therapeutic vaccine development may pose different challenges, since HCV-specific immune responses observed during chronic infection shown signs of functional deficits. For example, dendritic cell impairments could prevent the immune system from mounting the desired responses following vaccination. Finally, viral diversity among genotypes and the quasispecies nature of HCV suggest that vaccine development will require careful targeting of highly conserved epitopes common across strains, lest escape mutations emerge.

Vaccine development efforts would no doubt benefit from greater investment and coordination. In addition to scientific challenges, several practical issues will need to be addressed. For example, testing a prophylactic vaccine will require the recruitment and retention of sizeable cohorts of uninfected individuals at elevated risk for HCV infection, such as young injection drug users. Licensure of a vaccine will require outlining clear standards for proof of efficacy by regulatory agencies, primarily the United States’ Food and Drug Administration (FDA) and the European Agency for the Evaluation of Medical Products (EMEA). If a prophylactic HCV vaccine does not fully prevent all infections in all vaccinated subjects, how effective does it need to be for approval? Similarly, what parameters—virologic, histologic, immunologic, biochemical, and clinical—would need to change, by how much, and over what amount of time to consider a therapeutic HCV vaccine effective? The answers to these questions will determine not only clinical trial design, but also the willingness of drug companies to invest in vaccine development.
Research Recommendations

Over 14 years after the identification of HCV, treatment options rely exclusively on interferon alfa and ribavirin. By comparison, within 14 years of the identification of HIV, fifteen antiretroviral drugs—six nucleoside analogues, four protease inhibitors, and three non-nucleoside reverse transcriptase inhibitors—had been approved by the FDA. HCV and HIV are different viruses, and as such pose different challenges for drug development, but the disparity in progress is striking. Federal funding for HIV research dwarfs the amounts allocated for HCV; increased and targeted funding to investigate critical research areas in HCV would be welcome. At the same time, HCV research is divided across several institutes within the NIH, and has arguably suffered from a lack of consistent coordination and effective mechanisms for prioritizing and supporting scientific goals and cross-disciplinary research.

Increase funding and coordination of research

The recently established Liver Disease Research Branch within the NIDDK (National Institute of Diabetes and Digestive and Kidney Diseases) should develop into a strong, responsive, and accountable mechanism for advancing basic and clinical research on HCV. Indeed, this development heralds a renewed emphasis on coordination of efforts within the NIH and in collaboration with other Federal agencies (such as the Centers for Disease Control and the Veteran's Administration). The announced preparation of an Action Plan for Liver Disease Research, due in April 2004, will also enable a more thoughtful and thorough assessment of HCV research needs, and should allow for the meaningful involvement of the broadest possible range of stakeholders. Ultimately, the intensification of both basic and clinical research activities will be necessary for further progress in HCV treatment. The six NIH-supported Hepatitis C Cooperative Research Centers (HC CRCs) provide a compelling model for combining basic and clinical research programs. The HC CRCs are jointly funded by the National Institute of Allergy and Infectious Diseases (NIAID), the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK), and the National Institute of Drug Abuse (NIDA). Additional resources should be directed to this innovative funding initiative.

Support refinement of in vitro and animal models

HCV drug development efforts will also depend on the quality and accessibility of in vitro and animal models for HCV infection. The lack of reliable, efficient cell cultures supporting HCV replication, the absence of a small animal model for HCV infection, and the expense of chimpanzee research pose considerable constraints to HCV drug and vaccine development. HCV replicon systems have revolutionized drug screening by offering a viable model for gauging the inhibitory effects of compounds on viral replication, though they do not fully reproduce the dynamics of in vivo infection. Further refinements in these models will be crucial for the development of antiviral agents targeting early or late stages of the HCV replication cycle, including attachment, entry, and uncoating as well as virion assembly and release. XTL Biopharmaceuticals in Israel has developed a proprietary mouse model carrying transplanted human liver tissue infected with HCV (Ilan 2002). A Canadian group of academic researchers has been pursuing a similar strategy, producing chimeric mice, transplanted with human hepatocytes, that are susceptible to infection with HCV (Fausto 2001; Mercer 2001). Further work will be
necessary to increase the utility of mouse models. Researchers at GlaxoSmithKline have reported success in using a marmoset model of GB virus B to screen HCV protease inhibitors for antiviral efficacy (Bright 2004). Biomedical research funders, including government and industry, should launch a concerted effort to develop, refine, and validate in vitro and animal models for assessing candidate antiviral compounds against HCV.

**Promote drug development efforts that study safety and efficacy in real-world populations**

Current efforts at antiviral drug development targeting HCV appear promising, but should be complemented by expanded research programs directed at prophylactic and therapeutic vaccine development, the identification of antifibrotic agents, and strategies to prevent and treat hepatocellular carcinoma. The advent of new classes of medications for HCV—in particular the NS3 serine protease inhibitors in clinical and preclinical development—will hopefully usher in a new era for individuals chronically infected with hepatitis C. At the same time, clinical trial design must consider the particular care and research issues of patient populations with high HCV prevalence rates and urgent needs for more effective and better-tolerated HCV treatment regimens. People coinfected with HIV will make up a substantial proportion of candidates for new treatments, as will current and former injection drug users; combined, these groups likely constitute at least half of the domestic market for HCV treatment. If phase II and III studies completely exclude these groups, they decrease their relevance to real-world clinical care and limit the information patients and health care providers receive to make treatment decisions. Drug interaction studies will also be particularly important for these groups. Current interferon alfa and ribavirin therapies have reached the market before the results of studies in HIV coinfected individuals were available; virtually no data exists on the use of these treatments in drug users. This pattern cannot be repeated for future HCV medications.

**Initiate partnerships between industry, government, academia, and community**

Speeding the development of new therapeutic strategies for HCV will require a coordinated effort involving government, industry, research institutions, private foundations supporting biomedical research, and HCV advocates, especially people infected with hepatitis C. Some models for such efforts already exist; NIAID’s Partnerships for Novel Approaches to Controlling Infectious Diseases, a collaboration between government, industry, and academia, has begun to focus on hepatitis B and could be expanded to address HCV. A strategic partnership between public and private sectors could support exploration of new targets and a better understanding of viral-host interactions through techniques such as microarray analysis (Aizaki 2002; Bigger 2001). An intensive research and development program would speed and expand the refinement of tools for rapid and high-throughput screening of candidate compounds, preclinical research, and the establishment of appropriate research infrastructure to facilitate the recruitment of diverse patient groups into clinical trials.
List of Terms Used in This Chapter

3' UTR (3-prime untranslated region): non-coding region of HCV RNA; site of initiation of negative-sense strand synthesis.

5' UTR (5-prime untranslated region): non-coding region of HCV RNA; contains the internal ribosomal entry site (IRES); site of initiation of translation.

Adenosine deaminase: a cellular enzyme that converts viramidine to ribavirin.

Alkavirs: a class of imino sugar derivatives containing alkyl side chains.

Alkyl side chains: a chemical modification made to some imino sugar derivatives.

α-glucosidase inhibitor (alpha-glucosidase inhibitor): an antiviral agent that inhibits α-glucosidase, an enzyme involved in the glycosylation of HCV envelope proteins E1 and E2.

Antisense oligonucleotide: short sequences of RNA or DNA that are complementary to a sequence of viral RNA. Antisense oligonucleotides can bind to HCV RNA inside infected cells and block viral replication.

Apoptosis: programmed cell death.

BH3 interacting domain death agonist (BID): a cellular protein involved in apoptosis (programmed cell death).

Calnexin: a chaperone protein involved in the heterodimerization of HCV envelope proteins E1 and E2.

Calreticulin: a chaperone protein involved in the heterodimerization of HCV envelope proteins E1 and E2.

Cap-independent: a form of translation (protein synthesis) using an internal ribosomal entry site (IRES); the method used by HCV to synthesize viral proteins.

Caspases: cellular enzymes involved in apoptosis (programmed cell death).

Castanospermine: an imino sugar that inhibits glycosylation.

Chaperone proteins: cellular proteins (e.g., calnexin and calreticulin) that help HCV envelope proteins E1 and E2 fold into heterodimers.

Chimeric mouse model: mice that have human liver cells transplanted into them. A possible small animal model for testing HCV drugs.

Complementary: an RNA sequence that is the mirror image of a section of HCV RNA; siRNA and antisense oligonucleotides are designed to be complementary to sections of HCV RNA, allowing them to bind to viral RNA, targeting it for destruction.

CpG motifs (cytosine-guanine sequences): molecular patterns commonly found in bacterial and viruses that trigger toll-like receptor 9 and stimulate innate immune responses, including IFN-α production.

Cytokine: secreted proteins that function as chemical messengers between cells to influence (e.g., stimulate, inhibit) immune responses. Cytokines include chemokines, interferons, and interleukins.

Dipeptides: short peptides composed of two linked amino acids.

Endoplasmic reticulum (ER): a membrane within cells; the site of HCV translation (protein synthesis) and strand synthesis.

Glycans: sugar molecules added to HCV envelope proteins E1 and E2 during glycosylation.
**Glycosylation**: a chemical modification that adds sugar molecules to proteins. HCV envelope proteins E1 and E2 must undergo glycosylation in order to function properly.

**Guanosine triphosphate (GTP)**: one of the four nucleotide building blocks for RNA strand synthesis. GTP levels depend on a cellular enzyme, IMPDH. Ribavirin and merimepodib may work by inhibiting IMPDH, thereby lowering GTP levels and preventing HCV replication.

**Helicase**: an HCV enzyme, contained within NS3, which unwinds and separates RNA strands during strand synthesis.

**Heterodimerization**: the formation of complexes joining two different proteins; the HCV envelope proteins E1 and E2 must form heterodimers in order to function properly.

**Hexapeptide**: short peptides composed of six linked amino acids.

**Hybridize**: bind to; as in, an RNA molecule (e.g., siRNA or antisense oligonucleotide) hybridizes to the complementary sequence on HCV RNA.

**Hyperimmune serum**: blood containing highly reactive antibodies to HCV.

**Imidazoquinolinamines**: a class of drugs that stimulates the secretion of IFN-α and various cytokines.

**Imino sugar derivatives**: antiviral agents that chemically resemble sugars (monosaccharides).

**Immunoglobulin**: antibodies.

**IMPDH (inosine-5'-monophosphate dehydrogenase)**: an enzyme that controls guanosine triphosphate levels. Merimepodib (and possibly ribavirin) may work as IMPDH inhibitors.

**INF-α subtype**: one of the forms of naturally occurring IFN-α molecules produced by the body. Different subtypes may have slightly different antiviral activity. The two FDA-approved forms of pegylated interferon alfa, Pegasys and Peg-Intron, are synthetic molecules derived from IFN-α subtype 2.

**INFAR (INF-α receptor)**: a cell surface receptor that binds IFN-α; composed of two subunits, INFAR1 and INFAR2.

**Ion channels**: a gateway, composed of one or more proteins, that allows ions (charged atoms or molecules such as calcium) to pass through cell membranes.

**IRF-3 (interferon regulatory factor 3)**: a protein that regulates INF-α-stimulated gene expression and cellular defenses against HCV; the HCV NS3 serine protease blocks the action of IRF-3.

**IRF-7 (interferon regulatory factor 7)**: a protein that regulates INF-α-stimulated gene expression and cellular defenses against HCV.

**ISCOMs**: immunostimulatory complexes used as vaccine adjuvants.

**ISDR (interferon sensitivity determining region)**: a region of NS5A possibly involved in resistance to interferon alfa treatment.

**ISGs (interferon-stimulated genes)**: genes involved in the interferon response and cellular defense against viruses.

**JAK/STAT pathway**: a signaling pathway within cells that directs gene expression and cellular functions, including the interferon response.

**JNK pathway**: a signaling pathway within cells that directs gene expression and cellular functions, including the interferon response.
**La protein (La antigen)**: a cellular protein that binds to the HCV internal ribosomal entry site (IRES) and increases the efficiency of translation (protein synthesis). The La antigen also binds to the HCV 3’ UTR. Possible target for antiviral drugs.

**Lipopeptide**: a fat molecule (lipid) bound to a peptide (linked amino acids). Lipopeptides are under investigation as possible HCV vaccines.

**Luciferase**: a gene used in cell culture studies; luciferase lights up and is useful for studies of translation and gene expression.

**Mimotopes**: synthetic peptides mimicking naturally-occurring HCV epitopes.

**Monoclonal antibody (mAb)**: antibodies derived from a B cell line (sometimes taken from individuals infected with HCV) that is engineered to produce identical antibodies, all targeting the same epitope. Monoclonal antibodies to HCV are under investigation for HCV treatment.

**Monosaccharides**: sugar molecules. Imino sugar derivatives are synthetic versions of monosaccharides.

**NF-κB pathway**: a signaling pathway within cells that directs gene expression and cellular functions, including the interferon response.

**NN-DGJ**: an imino sugar derivative being studied as an HCV drug.

**NTPase (nucleotide triphosphatase)**: an HCV enzyme, contained within NS3, which catalyzes chemical reactions that support the movements and RNA binding of the HCV NS3 helicase.

**p7**: an HCV protein of unknown function; may act as a viroporin to create ion channels.

**Peptide**: two or more linked amino acids; proteins are formed from multiple peptides joined together.

**Peptidomimetic**: a compound that mimics the form of a peptide.

**Pharmacodynamics**: the study of the effects of a drug on the body, i.e., antiviral efficacy and toxicity.

**Pharmacokinetics**: the study of the effects of the body on drug levels, i.e., absorption and metabolism.

**Poliovirus 3Dpol**: the poliovirus polymerase enzyme.

**Polymerase**: an enzyme that synthesizes new DNA or RNA strands. HCV NS5B contains a polymerase enzyme, the RNA-dependent RNA polymerase, which synthesizes new HCV RNA.

**Prodrug**: an inactive form of a drug that gets converted inside the body to its active form.

**Protease**: an enzyme that breaks down proteins. HCV contains two viral protease enzymes: the NS2-NS3 protease, and the NS3 serine protease.

**PSMA 7 (proteasome α-subunit 7)**: a cellular protein that may be involved in HCV translation (protein synthesis); a possible target for antiviral drugs.

**RdRp (RNA-dependent RNA polymerase)**: the HCV polymerase enzyme, contained within HCV NS5B and responsible for synthesizing new HCV RNA strands during viral replicaton.

**Ribosome**: cellular machinery responsible for translation (protein synthesis); the ribosome “reads” HCV RNA and translates it into HCV proteins.
**Ribozymes:** RNA molecules that can bind to and cleave (split) HCV RNA inside infected cells.

**RNA aptamers:** RNA sequences that can bind to HCV RNA inside infected cells, blocking viral replication.

**RNase H:** a cellular enzyme that degrades (destroys) RNA after antisense oligonucleotides bind to RNA.

**Saccharomyces cerevisiae:** a species of yeast.

**siRNA:** small RNA molecules that can bind to HCV RNA inside infected cells, targeting it for destruction.

**Substrate:** the region of a protein that binds to an enzyme; e.g., the substrates of the HCV NS3 serine protease are the areas that NS3 binds to and cleaves (splits). Protease inhibitors can be designed to mimic the structure and composition of the HCV NS3 serine protease substrates.

**Toll-like receptor 7 (TLR7):** a pattern-recognition receptor involved in innate immune responses, including IFN-α production.

**Toll-like receptor 9 (TLR9):** a pattern-recognition receptor involved in innate immune responses, including IFN-α production.

**Viroporin:** a viral protein that creates ion channels; HCV p7 appears to function as a viroporin.
## Appendix A: Chart of Drugs in Development

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Developer</th>
<th>Compound</th>
<th>Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HCV NS3 serine protease inhibitors</strong></td>
<td>Boehringer Ingelheim</td>
<td>BILN 2061</td>
<td>Completed phase I; planned phase II on hold pending investigation of toxicities in animals</td>
</tr>
<tr>
<td></td>
<td>Vertex</td>
<td>VX-950</td>
<td>Phase I to begin in 2004</td>
</tr>
<tr>
<td></td>
<td>Schering-Plough</td>
<td>SCH7</td>
<td>Phase I</td>
</tr>
<tr>
<td><strong>HCV NS5B RNA-dependent RNA polymerase inhibitors</strong></td>
<td>Idenix</td>
<td>NM283</td>
<td>Phase II in combination with pegylated interferon to begin in 2004</td>
</tr>
<tr>
<td></td>
<td>Japan Tobacco</td>
<td>JTK-003</td>
<td>Phase II</td>
</tr>
<tr>
<td></td>
<td>Japan Tobacco</td>
<td>JTK-109</td>
<td>Phase I</td>
</tr>
<tr>
<td></td>
<td>Rigel</td>
<td>R803</td>
<td>Phase III</td>
</tr>
<tr>
<td></td>
<td>Roche</td>
<td>R1479</td>
<td>Phase I</td>
</tr>
<tr>
<td></td>
<td>ViroPharma</td>
<td>HCV-086</td>
<td>Phase I</td>
</tr>
<tr>
<td><strong>Imino sugar derivatives</strong></td>
<td>Micrologix</td>
<td>MBI-3253 (celgosivir)</td>
<td>Phase II to begin in 2004</td>
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<tr>
<td></td>
<td>United Therapeutics</td>
<td>UT231B</td>
<td>Phase II</td>
</tr>
<tr>
<td><strong>Monoclonal antibodies</strong></td>
<td>XTL</td>
<td>HepeX-C</td>
<td>Phase II</td>
</tr>
<tr>
<td><strong>Antisense oligonucleotides</strong></td>
<td>Isis</td>
<td>ISIS-14803</td>
<td>Phase III in combination with pegylated interferon and ribavirin</td>
</tr>
<tr>
<td><strong>Interferon variants and alternates</strong></td>
<td>InterMune</td>
<td>Interfergen</td>
<td>Phase III study in combination with ribavirin initiated in 2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(interferon alfacon-1; consensus interferon)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>InterMune</td>
<td>pegylated Interfergen</td>
<td>Phase I conducted in 2003; further development on hold for financial reasons</td>
</tr>
<tr>
<td></td>
<td>Human Genome Sciences</td>
<td>Albuferon-alpha</td>
<td>Phase II dose-ranging study underway</td>
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<tr>
<td></td>
<td>BioMedicines</td>
<td>omega interferon</td>
<td>Phase II underway</td>
</tr>
<tr>
<td></td>
<td>InterMune</td>
<td>interferon gamma-1b (Actimmune)</td>
<td>Phase II study in combination with Interferon initiated in 2004</td>
</tr>
<tr>
<td><strong>Next-generation ribavirin</strong></td>
<td>Valeant</td>
<td>Viramidine</td>
<td>Phase III studies in combination with pegylated interferon</td>
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<tr>
<td></td>
<td></td>
<td>(ribavirin prodrug)</td>
<td></td>
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<tr>
<td></td>
<td>Vertex</td>
<td>Merimepodib</td>
<td>Phase IIb study in combination with pegylated interferon and ribavirin to begin in 2004</td>
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<tr>
<td></td>
<td></td>
<td>(VX-497, an IMPDH inhibitor)</td>
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<tr>
<td>Drug Class</td>
<td>Developer</td>
<td>Compound</td>
<td>Stage</td>
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<td>------------------------------------------</td>
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<td>-----------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Other immuno-modulators, broad antivirals, and non-specific therapies</td>
<td>SciClone</td>
<td>Zadaxin (thymosin alfa-1; thymalfasin)</td>
<td>Two phase III studies in combination with pegylated interferon; another phase III study in combination with pegylated interferon and ribavirin to begin in 2004</td>
</tr>
<tr>
<td>Maxim</td>
<td>Ceplene (histamine dihydrochloride)</td>
<td>Phase II study with pegylated interferon and ribavirin; phase Ia study of oral formulation completed</td>
<td></td>
</tr>
<tr>
<td>Anadys</td>
<td>isatoribine (ANA 245, a TLR7 agonist)</td>
<td>Phase I; ANA 971 (an oral prodrug of isatoribine) entered phase I in 2004</td>
<td></td>
</tr>
<tr>
<td>Coley</td>
<td>Actilon (CPG 10101, a TLR9 agonist)</td>
<td>Phase III underway; phase II to begin in 2004</td>
<td></td>
</tr>
<tr>
<td>Kemin Pharma</td>
<td>KPE02003002</td>
<td>Phase II</td>
<td></td>
</tr>
<tr>
<td>Idun</td>
<td>IDN-6556 (apoptosis inhibitor)</td>
<td>Phase II planned</td>
<td></td>
</tr>
<tr>
<td>Vaccines</td>
<td>Innogenetics</td>
<td>therapeutic vaccine</td>
<td>Phase II</td>
</tr>
<tr>
<td></td>
<td>Intercell</td>
<td>therapeutic vaccine</td>
<td>Phase II</td>
</tr>
<tr>
<td></td>
<td>Chiron</td>
<td>therapeutic vaccine</td>
<td>Phase I</td>
</tr>
<tr>
<td></td>
<td>Chiron</td>
<td>preventative vaccine</td>
<td>Phase I</td>
</tr>
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</table>
Appendix B: Overview of the Drug Development Process

Drug development is a lengthy and uncertain process. The discovery, development, and testing of a new compound can last over a decade from initial concept to widespread clinical use, and is rife with potential for failure. A number of drugs currently in development for HCV may not succeed in demonstrating efficacy, or may show unacceptably high levels of toxicity. In some cases, the development of antiviral agents is halted or delayed due to financial considerations. Pharmaceutical companies may opt to terminate a development program that seems unlikely to result in a product generating sufficient revenue, while small biotechs may be unable to raise the financing necessary to support large clinical trials. Promising compounds identified by government or academic research may never find a commercial sponsor to conduct the preclinical and clinical research necessary for approval. Despite these odds, the outlook for new HCV therapeutics remains encouraging, with a pipeline of compounds at various stages of development.

Drug development can be roughly divided into three stages: drug discovery, preclinical development, and clinical development. New drugs are only tested in humans during the clinical development stage.

Drug Discovery

This stage of research focuses on identifying compounds that may be active against HCV. Several processes may be involved:

- Target validation: confirming that the target—for example, a particular site on the HCV NS3 serine protease—is appropriate for developing inhibitory strategies;

- Screening assay development: establishing in vitro models suitable for testing potential compounds for activity against a target (i.e., that a compound can inhibit NS3 serine protease activity);

- Lead identification: selecting a candidate compound for further preclinical development;

- Lead optimization: examining and potentially improving on the physical and chemical properties of the lead compound with respect to areas that can include potency, toxicity, and pharmacology (drug bioavailability—adsorption, distribution, metabolism, and excretion).

Researchers have a number of methods to pursue these processes, including high throughput screening of compound libraries and structure-based design. Pharmaceutical companies have vast libraries of compounds potentially active against a given target. Automated high throughput screening techniques can rapidly and efficiently identify lead compounds. Rational or structure-based drug design attempts to produce molecules with antiviral potential based on the three-dimensional structure of the target. In HCV drug discovery, the determination of crystal structures of the HCV serine protease, RNA-dependent RNA polymerase, and helicase enzymes has enabled the design of molecules that can bind to active sites on these enzymes. A host of related compounds
with similar targets can be synthesized through combinatorial chemistry techniques, which tweak the chemical structure of a compound to improve its antiviral and pharmacologic properties. All of these methods have been applied to HCV research, identifying and generating lead compounds for targets such as the HCV NS3 serine protease and HCV RNA strand synthesis.

**Preclinical Development**

Pharmacology and toxicity studies of lead compounds are extended in the preclinical phase to *in vitro* studies and animal models, prior to human testing in the clinical development stage. *In vitro* models, such as HCV replicons, can help to characterize the antiviral activity of a lead compound. Other *in vitro* systems can help to predict the specificity, toxicity, and pharmacologic profile of a lead compound. Chimpanzees remain the only established animal model for HCV infection, and therefore the best *in vivo* model for drug efficacy prior to human trials. Some recent work has explored the value of mouse models, tamarin hepatocytes, and GB virus B-infected marmosets in validating the antiviral activity of new compounds.

Other animals not susceptible to HCV infection can be used to study the pharmacokinetics (how the body processes a drug) and pharmacodynamics (how a drug affects the body) of a compound. These studies help to establish potential dosing ranges and frequencies, and can define the relative value of different formulations (oral, infusion, etc.) of a compound. Manufacturing processes with appropriate quality control procedures are also developed during this stage. Animal research can also define the safety issues surrounding a drug candidate, and some drug development efforts are halted at this stage due to unacceptable toxicity in animals. Toxicity can arise for various reasons, but particularly when a compound is not highly selective against its target—for instance, if the compound also acts against cellular proteins in a potentially harmful way.

**Clinical Development**

When an agent’s sponsor (the pharmaceutical company) has compiled all of the necessary preclinical data, particularly on safety, they can submit an investigational new drug application (IND) to the FDA outlining plans for safety and efficacy testing in humans. If approved, research advances in phases. Development can be halted at any phase if study results are unfavorable due to high toxicity and/or poor efficacy, poor pharmacologic properties, or other negative safety data emerges from additional animal studies. Clinical development programs can also be stalled or terminated due to economic factors and business decisions.

- **Phase I**: Small studies of healthy volunteers or, in some cases, individuals with stable HCV infection that determine short-term safety, explore dosing, and determine pharmacokinetics in humans. Phase I trials are not designed to establish the efficacy of a drug, though some information on the drug's activity may be collected. Phase I studies enroll up to a few dozen participants and can be conducted within a year’s time.

- **Phase II**: Medium-sized trials examining longer-term safety and efficacy in the target population—i.e., individuals with chronic HCV infection. Some aspects of phase I and II trials may be combined and are used to identify the optimal dose
of the drug. Phase II studies may enroll up to a few hundred participants and can last for up to two years. The majority of development failures for compounds that have entered human studies occur during this phase.

- **Phase III**: Longer, large-scale randomized clinical trials determining safety and efficacy. These studies can enroll hundreds to thousands of participants and may last for several years. Phase III research is designed to prove that a drug works and is relatively safe, by comparing it to standard of care or placebo. FDA approval is contingent on the results of these trials.

- **Phase IV**: Post-marketing studies conducted after a drug's approval by the FDA. Intended to gather data about long-term or rare toxicities, and may collect information about the use and efficacy of the drug in various patient groups.

The discovery, preclinical, and clinical stages of drug development increasingly overlap. Companies also increasingly describe their clinical trials by dividing early phases into loosely (and often, inconsistently) defined subcategories. Phase Ia may be used to describe initial tests in healthy (e.g., uninfected) volunteers, while phase Ib sometimes refers to early short-term tests in people with HCV. Phase IIa studies compare the pharmacokinetics of various doses for longer periods, and phase IIb studies may generate initial data about the use of a new agent in combination (e.g., with interferon).

Many drugs that are currently in early (phase I and II) clinical development may not reach the market for several years. Compounds currently in preclinical development may not become available until the next decade. These timetables have particular relevance to people with hepatitis C and their doctors who are currently considering whether to treat now with pegylated interferon/ribavirin, or defer treatment until better drugs become available.

The development of a prophylactic vaccine could take even longer. Even if a strong candidate vaccine was entering clinical trials, testing for efficacy—the ability of the vaccine to prevent new HCV infections—could take several years and thousands of subjects. A preventative vaccine must be tested in uninfected persons, with an endpoint of whether people receiving the vaccine are less likely to develop infection than people who do not receive the vaccine. The amount of people necessary to test vaccine efficacy depends on the rate of new infections in a population; a high-risk population (such as injection drug users) with a high annual incidence of new HCV infections would be a logical group for vaccine efficacy trials. But even among injection drug users, not everyone will become infected with HCV in a given period of time, regardless of whether they receive the vaccine under investigation. Also, ethics dictate that vaccine trial participants receive information, counseling, and tools to reduce their risk of HCV infection—for example, information on the risk of needle-sharing and referral to a syringe exchange program or drug treatment. If people participating in a vaccine trial reduce their overall risk of HCV infection as a result of these interventions, sample size and/or duration of observation will necessarily increase.

For both drug and vaccine development, Phase III trials are enormously costly undertakings, and generally require the direct involvement or financing by a large and established pharmaceutical company. Frequently cited (albeit controversial) estimates of the total cost of developing a new
drug from discovery through to FDA approval exceed $800 million (Rawlins 2004). The necessary resources and, to varying degrees, expertise involved in clinical testing and drug approval often exceed the capacities of smaller, start-up biotech firms, which typically operate for several years without substantial revenue until their first products reach the market. As a result, the numerous biotechs with candidate HCV compounds generally have to form partnerships with larger pharmaceuticals to raise the capital for Phase II and III clinical trials.

Many biotechs have particular expertise in a specialized field of drug development (e.g., compound screening; design of prodrugs), and pharmaceutical companies often enter into partnerships with them aimed at HCV drug discovery and lead identification. Companies may also enter partnerships with academic research groups, and compounds and techniques identified through academic research are commonly licensed to industry or spun off into new biotechs.

Many of these partnerships and discovery programs are referenced in chapter X (The Future of HCV Therapy) to provide a glimpse at the scope and extent of HCV research. However, the history of drug development suggests that few of these discovery and preclinical research programs will result in new drugs reaching the market. According to current estimates, only an estimated one in 5,000-10,000 compounds evaluated in initial screening during the discovery and preclinical stages will reach the market. Based on industry averages, only one in three compounds being evaluated in clinical trials will ultimately receive FDA approval (Preziosi 2004).