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HEPATITIS C

Overview

An estimated 3 percent of the world's population — more than 170 million people — carry a mysterious virus that silently attacks their livers, often without their knowledge. That's because up to 80 percent of those infected with the hepatitis C virus (HCV) have no symptoms at all. In fact, most people don't know they have the disease until decades later when liver damage shows up during routine medical tests.

Hepatitis C is one of six identified hepatitis viruses — the others are A, B, D, E and G. All cause the liver to become inflamed, which interferes with its ability to function. Hepatitis C is generally considered to be among the most serious of these viruses.

Over time, HCV infection can lead to liver cancer, liver failure or cirrhosis — irreversible and potentially fatal scarring of the liver. It ranks second only to alcoholism as a cause of liver disease and is the leading reason for liver transplants in the United States. Unlike HIV, the virus that causes AIDS, HCV usually isn't transmitted through sexual contact. Instead, its primary mode of transmission is contaminated blood — through needles shared by drug users or through blood transfusions. Nearly 4 million Americans have been infected at one time with HCV and close to 3 million are chronically infected.

The encouraging news is that new cases of HCV have declined 80 percent since blood banks began screening for the virus in 1992. At the same time, because standard drug treatments are effective in only about half the people treated, the annual death toll from the disease is expected to triple in the next 10 years.

Although vaccines exist for hepatitis A and B, no vaccine for hepatitis C has been developed, primarily because the virus has many subtypes that change rapidly. Researchers hope to find a medication that will inhibit the growth of the virus and prevent long-term complications, such as cirrhosis and cancer, from developing.

Trends in Hepatitis C Prevalence in the United States

The Centers for Disease Control and Prevention (CDC) reported on the prevalence of hepatitis C infection derived from data in the National Health and Nutrition Examination Survey (NHANES), which was conducted during 1999-2002 in 15,079 noninstitutionalized, nonmilitary personnel. The results were then compared with those from a similar survey performed 10 years ago.

They found the prevalence of anti-hepatitis C virus (HCV) antibody to be 1.6%, corresponding to 3.8 million people. The prevalence was 2.1% in men and 1.1% in women. Black individuals had an anti-HCV prevalence rate of 3.0%, which was higher than the rates seen in white individuals (1.5%) and Mexican-American individuals (1.3%). Of all individuals infected with HCV, 69.9% were between the ages of 35 and 54 years. Prevalence was highest among those aged 45-49 years, with 7.1% of men and 2.3% of women infected in this group. Black men aged 45-49 years had an astounding prevalence rate (anti-HCV-positive) of 17.9%. Individuals with a history of injection drug use had a disease prevalence rate of 57.3%.

The study authors compared these data with data from the NHANES III survey performed 10 years ago. They found that the overall incidence of hepatitis C antibody prevalence has not changed over the past 10 years, but that the peak age-specific disease prevalence has increased from the group aged 35-39 years as found a decade ago, to 45-49 years in the current NHANES. These data are consistent with a past epidemic of acute hepatitis C that affected a large cohort of people. One of the major shortcomings of this study is that it reports on the prevalence of the hepatitis C antibody, which may indicate previous exposure or active disease. This study does not report on disease prevalence, as prevalence of hepatitis C infection is defined by the presence of hepatitis C viral RNA in serum. Only 148 of the 15,079 participants had serum available for hepatitis C viral RNA testing, making any statements regarding disease prevalence, and not potential exposure to the disease, invalid.

Signs and symptoms

Normally, HCV produces no signs or symptoms in its earliest stages. When it does, they're generally mild and flu-like and may include:

- Slight fatigue
- Nausea or poor appetite
- Muscle and joint pains
- Tenderness in the area of your liver

Even if you develop chronic hepatitis from the hepatitis C virus, you may have few, if any, symptoms. In many cases, symptoms may not appear for up to 30 years. Sometimes, though, you may experience one or more of the following:

- Fatigue
- Lack of appetite
- Nausea and vomiting
- Persistent or recurring yellowing of your skin and eyes (jaundice)
- Low-grade fever

Hepatitis C can cause damage to your liver even if you don't have symptoms. You're also able to pass the virus to others without having any symptoms yourself. That's why it's

important to be tested if you think you've been exposed to hepatitis C or you engage in behavior that puts you at risk.

Causes

In general, you contract hepatitis C by coming in contact with blood contaminated with the virus. Most people with HCV became infected through blood transfusions received before 1992; the year improved blood-screening tests became available. You can also contract the virus by injecting drugs with contaminated needles, and, less commonly, from contaminated needles used in tattooing and body piercing. In rare cases, HCV may be transmitted sexually.

Risk factors

Effective blood-screening procedures have greatly reduced the chances of HCV infection from transfusions. But if you received a blood transfusion before 1992, you're at risk of hepatitis C.

You are also at risk if you:

- Used illegal intravenous (IV) or intranasal (such as cocaine) drugs even once

- Received an organ transplant before 1992

- Are a health care worker who was exposed to infected blood

- Received clotting factor concentrates before 1987 or have the clotting disease hemophilia and received blood before 1992

When to seek medical advice

See your doctor if you think you may have been exposed to the hepatitis C virus, if you notice your skin or eyes turning yellow or if you have any other symptoms of hepatitis. Don't let concerns about what others may think keep you from getting medical care.

If you're being treated for hepatitis, see your doctor right away if you develop any of the following symptoms:

- Increased drowsiness, confusion or irritability
- Vomiting, diarrhea or abdominal pain
- Increased jaundice
- Skin rash
- Fever
- Loss of appetite

Screening and diagnosis

Ask your doctor to screen you for HCV if you think you've been exposed to the virus or if you're at risk of the disease. If you received a blood transfusion before 1992 from a donor who later tested positive for HCV, you may have received a letter from your hospital or blood bank recommending that you be screened.

Hepatitis C can be diagnosed with a blood test. In addition to having you undergo the blood test; your doctor will take a complete medical history and perform a physical exam. He or she may also recommend a liver biopsy, a procedure in which a small sample of liver tissue is removed for microscopic analysis.

Before the biopsy, you'll receive a local anesthetic to keep you comfortable. Your doctor then inserts a thin needle into your liver to remove the tissue sample. Liver biopsy is unlikely to have any complications, although you may have some pain or bleeding afterward.

Although a biopsy isn't necessary to confirm the diagnosis, it can help determine the severity of the disease. It may also help rule out other causes for your liver problem, such as alcoholic or drug-induced hepatitis, autoimmune hepatitis or excess iron (hereditary hemochromatosis).

Lab Studies

- Alanine aminotransferase

    o Determining the alanine aminotransferase (ALT) level is useful for monitoring the effectiveness of therapy for HCV infection.
    o Because ALT levels can fluctuate, a single value in the reference range does not rule out active infection, progressive liver disease, or cirrhosis. ALT normalization with therapy is not proof of cure.

- Hepatitis C antibody test

    o Anti-HCV serologic screening involves an enzyme immunoassay (EIA), including second- and third-generation EIAs. These assays are 97% specific but cannot distinguish acute from chronic infection.
    o The most recent third-generation EIA involves detecting antibodies against core protein and nonstructural proteins 3, 4, and 5 and can yield positive results an average of 8 weeks after the onset of infection.
    o False-negative results for the presence of HCV antibody can occur in persons with compromised immune systems, such as those with HIV type 1 infection, renal failure, or HCV-associated essential mixed cryoglobulinemia. False-positive EIA results can occur in persons without risk factors and in those without signs of liver disease, such as blood donors or health care workers.

- Recombinant immunoblot assay [RIBA]

    o The recombinant immunoblot assay is used to confirm HCV infection. A positive immunoblot assay result is defined as the detection of antibodies against 2 or more antigens and an indeterminate assay result defined as the detection of antibodies against a single antigen.

    o A positive immunoblot assay result followed by 2 or more instances of undetectable HCV RNA suggests HCV infection has resolved. A positive anti-HCV immunoassay result followed by a negative immunoblot assay result represents a false-positive immunoassay, and no further testing is required.

    o The recombinant immunoblot assay has limited usefulness in clinical practice.

- Qualitative and quantitative assays for HCV RNA

    o Qualitative assays can be used to test for HCV RNA. HCV RNA can be detected in blood using amplification techniques such as PCR or transcription-mediated amplification (TMA). The US Food and Drug Administration (FDA) have approved 2 PCR-based tests for qualitative HCV RNA detection.
        - Amplicor Hepatitis C Virus Test, version 2.0 (Roche Molecular Systems; Pleasanton, Calif) - PCR with a lower limit of detection of 50 IU/mL
        - Cobas Amplicor Hepatitis C Virus Test, version 2.0 (Roche Molecular Systems; Branchburg, NJ) - PCR with a lower limit of detection of 50 IU/mL
        - Versant HCV RNA Qualitative Assay, (Bayer HealthCare; Tarrytown, NY) - TMA with a lower limit of detection of 9.6 IU/mL

    o Quantitative assays ascertain HCV RNA quantity on blood using signal amplification (branched DNA assay) or target amplification techniques (PCR, TMA). The HCV RNA level in blood helps predict the likelihood of a response to treatment, and the change in HCV RNA level can also be used to monitor response. The same quantitative test should be used throughout therapy to avoid confusion, and results should be reported in international units to standardize data. The only FDA-approved quantitative test is Versant HCV RNA, version 3.0 (Bayer HealthCare; Tarrytown, NY). It is based on branched DNA technology and has a dynamic range of 615-7,700,000 IU/mL.

- HCV genotyping

  - Genotyping is helpful for predicting the likelihood of response and duration of treatment. Patients with genotypes 1 and 4 are generally treated for 12 months, whereas 6 months of treatment is sufficient for other genotypes.

  - Genotyping can be performed by direct sequence analysis, reverse hybridization to genotype-specific oligonucleotide probes, or restriction fragment length polymorphisms.

  - Two tests are available; however, neither has been approved by the FDA. They are as follows:
    - Trugene HCV 5'NC Genotyping kit (Visible Genetics; Toronto, Canada) - Based on direct sequencing followed by comparison with a reference sequence database
    - Line Probe Assay (Inno LiPA HCVII, Innogenetics; Ghent, Belgium) - Based on reverse hybridization of PCR amplicon on a nitrocellulose strip coated with genotype-specific oligonucleotide probes

- Baseline investigations

  - Perform a CBC count with differential.
  - Perform liver function tests, including an ALT level determination.
  - Obtain the patient's thyrotropin level.
  - Order an anti-HCV antibody EIA.

  - Genotyping should be performed as an aid for guiding treatment.
  - Order a quantitative HCV RNA assay. Reverse transcriptase PCR is more sensitive than bDNA testing.
  - Stress testing may be necessary in appropriate patients.
  - Screen the patient for co-infection with HIV or hepatitis B virus (HBV).
  - Screen the patient for alcohol abuse, drug abuse, and/or depression.
  - An ophthalmological examination may be necessary.

- Treatment toxicity

  - Patients should be closely monitored for treatment toxicity.
  - Tests to help monitor drug toxicity include CBC count with differential, renal function testing, liver function tests (including ALT level), and TSH level.

Imaging Studies:

- Ultrasonography of the liver

Procedures:

- Liver biopsy: Liver biopsy is not considered mandatory before the initiation of treatment, but this may be helpful for assessing the activity and severity of HCV-related liver disease. However, some experts recommend biopsy only in the following situations:

    o The diagnosis is uncertain.

    o Other co-infections or disease may be present.

    o The patient being considered for treatment has normal liver enzyme levels and no extrahepatic manifestations.

    o The patient is immunocompromised.

Histologic Findings: Lymphocytic infiltration, moderate degrees of inflammation and necrosis, and portal or bridging fibrosis are noted. Regenerative nodules are seen in patients with cirrhosis. Some patients also may have findings indicative of HCC.

Most pathologists give separate measurements of disease activity (grade) and fibrosis (stage). Many trials use the Ishak (6-point scale) and Knodell histological activity index (18-point score); both are useful for assessing improvements in histology findings in studies but are impractical for clinical use because of interobserver disagreement. The METAVIR score was developed by the French METAVIR Cooperative Study Group and reported by Bedossa and Poynard in 1996; it is frequently used in European trials. It consists of a 3-point activity scale and 4-point fibrosis score, with good agreement among pathologists. In the United States, many pathologists use a scale described by Batts and Ludwig in 1995, which consists of an activity grade (0-4) and a fibrosis stage (0-4).

Noninvasive methods of assessing hepatic fibrosis are in development. Current serum assays are directed at measuring breakdown products of extracellular matrix constituents (eg, glycoproteins, propeptides) and their regulatory enzymes (eg, lysyl oxidase, lysyl hydroxylase, propyl hydroxylase).

Natural History

As indicated previously, hepatitis C is a major public health problem. It is currently both the leading cause of liver disease-related death and the most common indication for liver transplantation in the United States. Hepatitis C is also one of the leading causes of hepatocellular carcinoma (HCC). A population-based study of cancer epidemiology (the Surveillance, Epidemiology and End-Results [SEER] program) has revealed that over the last 10 years in the United States, the incidence of liver cancer has increased more than that of any other malignancy, with HCV directly responsible for the majority of cases. This increase is also expected to continue to rise as the burden of HCV-related disease increases.

HCC risk is highest in individuals with HCV infection and cirrhosis, with estimates of annual incidence ranging between 1% and 7%. However, not every patient chronically infected with HCV develops significant fibrosis. A retrospective study of chronic hepatitis C acquired through blood transfusion found that the mean time to the onset of cirrhosis was 18 years after exposure. It has been postulated that approximately 20% of HCV-infected patients develop cirrhosis after 20 years. Male sex, older age at acquisition (> 40 years), concomitant daily alcohol consumption (≥ 50 g per day), and HIV or HBV coinfection independently increase the risk for disease progression. Studies also suggest that the degree of hepatic inflammation determines the development of fibrosis. One study found that up to one third of HCV-infected patients had severe hepatic inflammation on biopsy and thus risk rapid progression to cirrhosis in 20-30 years, whereas those with mild disease on liver biopsy did not develop the same degree of fibrosis over the same time interval. Another study by Yano and colleagues found that aggressive histology was associated with a 50% chance of progression to cirrhosis in less than 10 years.

The rate of fibrosis progression is not necessarily linear, with documented cases of mild stable histology followed by rapid accelerated progression. Poynard and colleagues applied mathematic modeling to a large cross-sectional study of HCV-infected patients with known histology. They found that after acquiring disease, fibrosis did progress linearly but at different rates over different time periods. There was minimal progression during the first 10 years after infection, although disease progression accelerated during each subsequent decade, with the most rapid phase occurring in the last 5 of 40 years. Disease was significantly more virulent in those patients who acquired hepatitis C after the age of 50. Once acquired, cirrhosis is generally well tolerated, with excellent survival until symptoms of decompensation begin to occur. This may include the onset of ascites, muscle wasting, variceal bleeding, cholestasis, coagulopathy, and encephalopathy. Approximately 4% of compensated cirrhotic patients decompensate per year, and this drastically affects survival. Once complications arise there is more than a 50% chance of succumbing to disease manifestations within 5 years.

Clinical decisions, including the selection of patients to receive therapy for viral hepatitis, require appraisal of both therapeutic risk and benefit. Current American Association for the Study of Liver Diseases guidelines state that treatment is widely accepted for adults (at least 18 years of age) with an abnormal ALT and well-compensated liver disease in whom liver biopsy demonstrates significant fibrosis, defined as more than portal fibrosis (Metavir score ≥ 2; Ishak score ≥ 3). Uncontrolled depression, organ transplantation, autoimmune disease including autoimmune hepatitis, severe concurrent disease, pregnancy, and untreated hyperthyroidism are therapeutic contraindications. Individuals falling outside of these parameters should be considered for treatment on a case-by-case basis.

Treatment

Since the first reported use of interferon α (IFN-α) for the treatment of chronic hepatitis C more than 20 years ago, IFN-based therapy has become the cornerstone of treatment for

this disease. Pegylated IFN-α (peginterferon α), which was developed to ensure sustained exposure with once-weekly dosing, offers improved convenience, a better adverse effect profile and, above all, superior clinical efficacy when compared with IFN-α. For these reasons, peginterferon α has replaced conventional IFN-α for the treatment of chronic hepatitis C. Today, the combination of peginterferon α2a or peginterferon α2b plus ribavirin (RBV) is the standard of care for chronic hepatitis C.

The primary goal of treatment for chronic HCV infection is a sustained virological response (SVR), which is defined clinically as HCV RNA levels undetectable with a sensitive molecular assay 24 weeks after cessation of therapy. Patients who achieve an SVR have a greater than 95% chance of still being virus-free 5 years later. This end point is associated with regression of fibrosis, decreased incidence of hepatocellular carcinoma, and overall reduced morbidity and mortality.

Currently, around 50% of patients infected with HCV genotype 1, 80–93% of those infected with HCV genotype 2 and 66–80% of those infected with HCV genotype 3 achieve an SVR with peginterferon plus RBV treatment, which is a major improvement compared with the SVR associated with conventional IFN-α therapy. A substantial proportion of patients, however, do not have an optimum response to current treatment regimens. Individualization of therapy offers the possibility of tailoring treatment to particular patients and selecting the treatment duration that ensures the best chance of achieving an SVR while preventing over treatment.
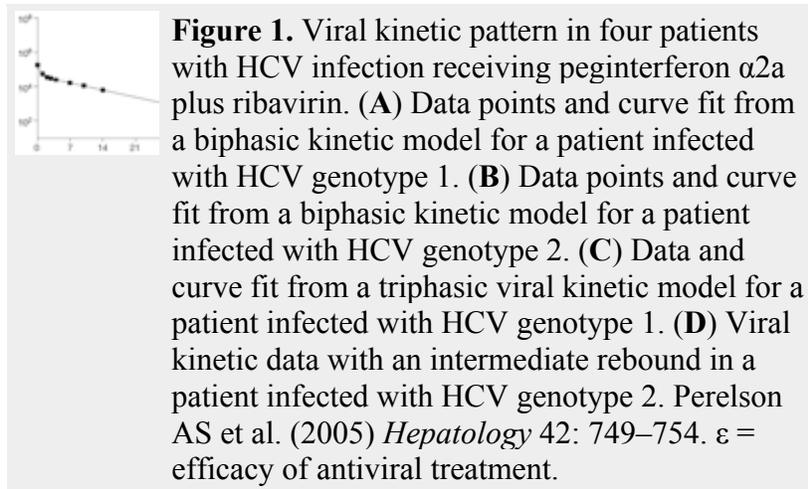
The development of new anti-HCV agents might also help improve treatment outcome. The study of viral kinetics offers a means of comparing different treatment regimens and assessing response to new agents, a number of which have shown promise in preliminary studies. Although novel anti-HCV drugs are still in the early stages of development, it is hoped that these agents might not just increase SVR rates, but also reduce treatment duration and improve tolerability.

Viral Kinetics of Interferon-Based Therapy

IFN-α has potent antiviral properties. Treatment with IFN-α induces the expression of a range of antiviral effector proteins, of which the best known include 2',5'-oligoadenylate synthetase, double-stranded RNA-activated protein kinase, and the myxovirus proteins. In addition to its direct antiviral properties, IFN-α has immunomodulatory properties that might contribute to its antiviral efficacy by activating cells and molecules involved in the host antiviral response. Although the exact mechanisms contributing to the clinical efficacy of IFN-α are not completely understood, several indirect antiviral functions have been demonstrated. For example, IFN-α stimulates the effector function of natural killer cells, cytotoxic T lymphocytes and macrophages, upregulates the expression of major histocompatibility complex class I and class II molecules, induces immunoglobulin synthesis by B cells, and stimulates the proliferation of memory T cells.

Treatment with IFN-based therapy typically produces a biphasic decrease in serum HCV RNA levels: a rapid first phase of decline lasting approximately 1–2 days is followed by

a slower second phase of decline (Figure 1A,B). During the initial rapid first phase, HCV RNA levels can fall by 1–2 $\log_{10}$ IU/ml in genotype 1 infected patients and by as much as 3–4 log10 IU/ml in genotype 2 infected patients. Some patients have a more-complex viral kinetic pattern, for example an intermediate increase in HCV RNA levels after an initial decline. Other patients show further accelerations of the decline after some time or even a rebound in HCV RNA levels (Figure 1C & D). Nonresponders to IFN-based therapy can have no first-phase or second-phase decline (null response), or alternatively can have a first-phase decline followed by little or no second-phase decline (flat response).

Figure 1. Viral kinetic pattern in four patients with HCV infection receiving peginterferon α2a plus ribavirin. (**A**) Data points and curve fit from a biphasic kinetic model for a patient infected with HCV genotype 1. (**B**) Data points and curve fit from a biphasic kinetic model for a patient infected with HCV genotype 2. (**C**) Data and curve fit from a triphasic viral kinetic model for a patient infected with HCV genotype 1. (**D**) Viral kinetic data with an intermediate rebound in a patient infected with HCV genotype 2. Perelson AS et al. (2005) *Hepatology* 42: 749–754. ε = efficacy of antiviral treatment.

Overall, biphasic patterns of decline in HCV RNA levels are seen when high daily doses of IFN-α are used, with more-complex patterns tending to arise when standard three times-weekly doses of conventional IFN-α are administered. The initial rapid phase of viral decline in response to treatment corresponds to the blocking of viral replication in infected cells by IFN-α, and its efficacy depends not only on the treatment regimen but also on other factors, such as HCV genotype. The second phase of decline in HCV RNA levels, which occurs in patients who respond well to therapy, is thought to reflect the clearance and net loss of infected cells. According to models of viral kinetics, the lower viral load caused by the first phase decline results in less *de novo* infection of hepatocytes, which ultimately leads to a net loss of infected cells and further reduction in overall viral production and RNA levels.

A triphasic decline in HCV RNA levels has been reported that consists of a rapid first phase, followed by a 'shoulder phase' of slow decline lasting for around 7–28 days, and finally a third phase of more-rapid decline (Figure 1C). The reason for this pattern is unclear; the pattern could reflect either a delayed effect of IFN-α therapy on cell clearance or an effect of RBV on the second-phase slope.

The overall pattern of viral response to IFN-based therapy can be used to determine the likelihood of treatment success and guide treatment duration in patients with chronic hepatitis C. The primary goal of treatment for chronic HCV infection is an SVR. Patients

who fail to achieve an early virological response (EVR), which is defined as either an undetectable level of HCV RNA or a drop in HCV RNA levels of at least 2 $\log_{10}$ IU/ml after 12 weeks of therapy, are highly unlikely to go on to achieve an SVR–the negative predictive value in this setting is around 97%. These findings form the basis of the week 12 stopping rule for HCV genotype 1 infected patients. Testing for rapid virological response (RVR), which is defined as an undetectable level of HCV RNA (< 50 IU/ml) at 4 weeks of treatment, has been shown to offer further prospects for the individualization of therapy according to treatment-related viral kinetics.

Current Guidelines for the Treatment of Patients with HCV

HCV Genotype and Treatment Duration

The main baseline predictor of response to therapy is HCV genotype, and genotype is consequently the primary determinant of treatment duration and response monitoring procedures in current treatment recommendations. Patients infected with HCV genotype 1 or 4 should receive 48 weeks of peginterferon α plus RBV, while 24 weeks of treatment is recommended for patients with an HCV genotype 2 or 3 infection.

Data for patients infected with HCV genotypes other than 1–4 were limited or lacking when current treatment guidelines were developed; thus, it is recommended that such individuals are treated in the same way as patients with HCV genotype 1 infections. Data now indicate that this is an appropriate approach; for example, patients infected with HCV genotype 6 have a higher rate of SVR with 48 weeks of treatment than with 24 weeks. The response to treatment in patients infected with HCV genotype 4 seems to be at an intermediate level compared with that of patients infected with HCV genotype 1 or 3.

The indicated doses for the two approved peginterferons, peginterferon α2a (180 μg once weekly) and peginterferon α2b (1.5 μg/kg once weekly), are independent of HCV genotype, but there are different recommendations for RBV dose depending on genotype and body weight. For patients with an HCV genotype 1 or 4 infection, weight-based RBV doses of 800–1,200 mg per day (1,400 mg per day for patients who weigh > 105 kg receiving peginterferon α2b) are recommended. For patients with an HCV genotype 2 or 3 infection the recommended dose of RBV is 800 mg per day, and there is no additional benefit associated with higher doses (at least for the 24-week standard treatment duration).

On-treatment Response and Treatment Duration

Current recommendations for patients infected with HCV genotype 1 or 4 include the week 12 stopping rule. This rule states that if a patient fails to achieve an EVR, consideration should be given to stopping treatment as achieving an SVR is unlikely. Almost all patients with an HCV genotype 2 or 3 infection have an EVR; therefore, recommendations do not suggest measuring HCV RNA at week 12 in these patients but simply treating them for 24 weeks.

Further Individualization of Therapy–The Role of Viral Response

There is increasing evidence to suggest that current dosing regimens for peginterferon α could potentially result in the over treatment of some patients who respond well to treatment and are more likely to achieve an SVR or, conversely, the under treatment of those patients who respond less well. Evidence is growing to support the taking of additional measurements of viral response to facilitate individualization of therapy for such patients.

Rapid Virological Response and Shorter Treatment Duration. The presence of an RVR is the strongest independent positive predictor of the likelihood of achieving an SVR for all HCV genotypes. The rapid response seen in some patients has given rise to the question as to whether such individuals might respond equally well, in terms of SVR, to a shorter treatment duration.

Early studies using conventional IFN-α, such as the study by Poynard and co-workers, indicated that patients infected with HCV genotype 1 who had low pretreatment viral loads (≤ 2,000,000 copies/ml; ~800,000 IU/ml) could be treated for 24 weeks without compromising SVR rates. In a study by Zeuzem and colleagues, response rates at the end of treatment with peginterferon α2b plus RBV were similar among HCV genotype 1 infected patients with low baseline viral load (≤ 600,000 IU/ml); however, overall SVR rates achieved with 24-week treatment were significantly lower than those observed in historical controls treated for 48 weeks, owing to a high virologic relapse rate in patients treated for 24 weeks.

The study by Zeuzem *et al.* found that a subset of HCV genotype 1 infected patients with baseline HCV RNA levels below 600,000 IU/ml plus undetectable serum levels of HCV RNA at week 4 of treatment (RVR) had a similar rate of SVR after 24 weeks of therapy to the historical control group treated for 48 weeks (89% and 85%, respectively). The importance of an RVR in predicting an SVR was confirmed in a retrospective analysis, which showed that HCV genotype 1 infected patients who achieved an RVR when treated with a standard regimen of peginterferon α2a plus RBV (around 24% of patients) were highly likely to achieve an SVR (89% vs 19% for patients with and without an RVR, respectively). Baseline viral load was shown to be predictive of an RVR, and patients with baseline HCV RNA levels of 800,000 IU/ml or lower were more likely to achieve an RVR than were those with baseline HCV RNA levels greater than 800,000 IU/ml.

Additional evidence supporting the shortening of treatment duration to 24 weeks in patients with low viral loads and an RVR has accumulated not only from studies in patients infected with HCV genotype 1, but also from those in patients infected with HCV genotype 4. As a result, both peginterferon α2a and peginterferon α2b have been approved in the European Union for a shortened treatment duration of 24 weeks in HCV genotype 1 patients with a low viral load (defined as < 800,000 IU/ml for peginterferon α2a and < 600,000 IU/ml for peginterferon α2b) and an RVR.
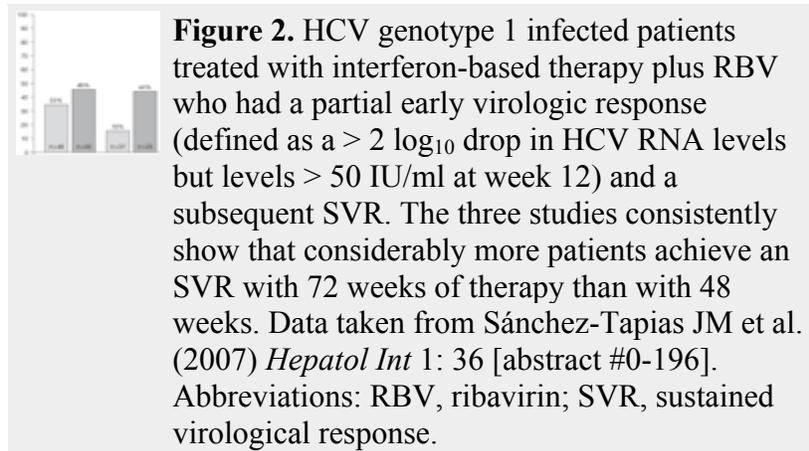
For patients infected with HCV genotype 2 or 3, the results of several studies have indicated that individuals who achieve an RVR could be candidates for treatment duration of less than 24 weeks. Indeed, a number of studies have demonstrated comparable SVR rates with 16 weeks and 24 weeks of treatment in patients who achieve an RVR. Among patients who had an RVR in the large-scale, randomized, multinational ACCELERATE study, however, the SVR rate was significantly higher in the 24-week treatment group than in the 16-week treatment group (85% vs 79%; $P < 0.001$), although patients who achieved an RVR were more likely to achieve an SVR overall. The difference in SVR rates reflects a significantly higher relapse rate in the 16-week treatment group than in the 24-week treatment group (31% vs 18%; $P < 0.001$). This difference was seen in both patients infected with HCV genotype 2 and those infected with genotype 3.

It has been suggested that the discrepancy between the results of the ACCELERATE study and those of the other studies might be due to the fact that the ACCELERATE study used a standard 800 mg dose of RBV as opposed to a weight-based RBV dosing regime, which was used in the other studies. On the other hand, in a trial by Dalgard and colleagues that used higher doses of RBV (800–1,400 mg per day) in HCV genotype 2 or 3 infected patients, the SVR rate was lower among patients who achieved an RVR with 14 weeks of treatment than among those who achieved an RVR with 24 weeks of treatment (81% vs 91%, respectively).
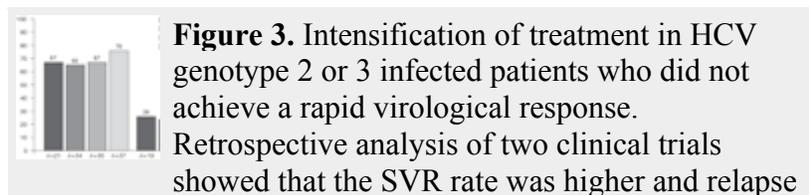
Baseline HCV RNA level has also been shown to influence SVR rates in patients with an HCV genotype 2 or 3 infection. Patients with low pretreatment serum HCV RNA levels who achieve an RVR have been reported to respond equally well to both 16 weeks and 24 weeks of therapy (SVR rates of 82–100% and 81–100%, respectively).Given the high SVR rates achievable, the potential to reduce adverse effects, and the economic benefits, shorter treatment might be appropriate for HCV genotype 2 or 3 infected patients who have low baseline HCV RNA levels and achieve an RVR.

Slow Virological Response and Longer Treatment Duration. There is increasing evidence to support extending the duration of treatment beyond 48 weeks in patients with an HCV genotype 1 infection who have a slow virological response (i.e. HCV RNA levels > 50 IU/ml at week 12, but undetectable [< 50 IU/ml] at week 24). In a study of HCV genotype 1 infected patients treated with peginterferon α2a (180 μg once weekly) plus RBV (800 mg per day), extending treatment duration to 72 weeks did not increase the SVR rate in the intention to treat population. Patients who still had detectable levels of HCV RNA (≥ 50 IU/ml) at week 12 according to the results of a sensitive molecular test, however, had a significantly higher SVR rate when treated for 72 weeks than for 48 weeks (29% vs 17%; $P = 0.04$), with the greatest benefit observed in patients who had HCV RNA levels below 6,000 IU/ml at week 12. These findings were subsequently confirmed in a study in HCV genotype 1 infected patients who met the criteria for an EVR and had detectable levels of HCV RNA at week 12, but had undetectable levels at week 24. In this trial, 72 weeks of treatment with pegylated interferon α2b plus weight-based dosing of RBV resulted in a better SVR rate than the same treatment for 48 weeks (39% vs 18%).

In HCV genotype 1 infected patients who do not achieve an RVR, extending treatment to 72 weeks also significantly increases the SVR rate compared with 48 weeks of therapy; for example, Sánchez-Tapias *et al.* reported SVR rates of 44% and 28% with 72 weeks and 48 weeks of treatment, respectively ($P = 0.003$). In an analysis of three European studies, 72 weeks of treatment was found to consistently improve the rates of SVR in patients who had a decline in HCV RNA levels of more than 2 $\log_{10}$ IU/ml but still had detectable levels of HCV RNA at week 12 of treatment (Figure 2). Taken together, the available data show that longer duration of therapy improves rates of SVR in 'slow' virological responders infected with HCV genotype 1.

Figure 2. HCV genotype 1 infected patients treated with interferon-based therapy plus RBV who had a partial early virologic response (defined as a > 2 $\log_{10}$ drop in HCV RNA levels but levels > 50 IU/ml at week 12) and a subsequent SVR. The three studies consistently show that considerably more patients achieve an SVR with 72 weeks of therapy than with 48 weeks. Data taken from Sánchez-Tapias JM et al. (2007) *Hepatol Int* 1: 36 [abstract #0-196]. Abbreviations: RBV, ribavirin; SVR, sustained virological response.

HCV genotype 2 or 3 infected patients who have a high baseline viral load and/or do not achieve an RVR have low SVR rates after peginterferon α plus RBV therapy. High baseline HCV RNA levels (> 600,000 IU/ml) are associated with a high rate of virological relapse (23%) in HCV genotype 3 infected patients, and data from the ACCELERATE study showed that patients infected with HCV genotype 2 or 3 who did not achieve an RVR had only a 49% probability of achieving an SVR. These data raise the question of whether such patients might benefit from more-intensive treatment than is currently used. In a retrospective analysis of data from two large clinical trials, most HCV genotype 2 or 3 infected patients were found to have achieved an RVR; however, among patients without an RVR, the SVR rate was higher and relapse rate lower for those receiving 48-week treatment with higher doses of RBV (1,000–1,200 mg per day) than for those receiving 24-week treatment with a lower dose of RBV (800 mg per day; Figure 3). These results need to be confirmed in a prospective controlled study, but it is possible that patients with an HCV genotype 2 or 3 infection who do not achieve an RVR could benefit from longer treatment with peginterferon α and higher doses of RBV (> 800 mg per day).

Figure 3. Intensification of treatment in HCV genotype 2 or 3 infected patients who did not achieve a rapid virological response. Retrospective analysis of two clinical trials showed that the SVR rate was higher and relapse

A final consideration concerns the assumption that HCV genotype 2 and genotype 3 infections require a similar duration of treatment. Evidence now indicates that this might not be the case. HCV genotype 2 infected patients seem to respond better to therapy and have consistently higher SVR rates than do HCV genotype 3 infected patients, with an overall SVR rate of 80–93% compared with 66–80%, respectively, after treatment for up to 24 weeks. These differences are also seen following the same duration of treatment in patients who achieved an RVR (SVR rate 87–95% for genotype 2 vs 76–89% for genotype 3). These findings indicate that separate management algorithms, possibly with a longer treatment duration for HCV genotype 3 infected patients, could be appropriate, and further studies are required to confirm whether this is the case.

Re-treatment of Patients

The management of patients with chronic hepatitis C who relapse after treatment (i.e. those who achieve an end of treatment virological response but not an SVR) or who fail to respond to current standard IFN-based therapy presents a particular problem. In patients who relapse after a first treatment course of IFN-α alone, combination therapy with IFN-α plus RBV has been shown to lead to substantially higher SVR rates than an additional course of IFN-α monotherapy. In the Hepatitis C Antiviral Long-Term Treatment Against Cirrhosis (HALT-C) trial, 18% of patients who did not respond to or had relapsed after treatment with conventional IFN-α or conventional IFN-α plus RBV had an SVR in response to re-treatment with 48 weeks of peginterferon α2a plus RBV. Factors associated with an SVR included previous treatment with IFN-α monotherapy, infection with HCV genotype 2 or 3, a low serum aspartate aminotransferase to serum alanine aminotransferase (ALT) ratio, and the absence of cirrhosis. Similar findings were reported in the Evaluation of PEG Intron in Control of Hepatitis C Cirrhosis (EPIC-3) trial, with 23% of patients who did not respond or who had relapsed after previous IFN-based treatment achieving an SVR following re-treatment with peginterferon α2b plus RBV.

Patients who relapse after treatment with conventional IFN-based regimens often respond to re-treatment with peginterferon α plus RBV, with SVR rates of 41–59% being reported. Peginterferon α plus RBV re-treatment should, therefore, be considered for all patients who have previously responded to a conventional IFN-based regimen and subsequently relapsed.

Re-treatment of nonresponders to IFN-α is generally associated with poor SVR rates, especially in HCV genotype 1 infected patients or patients with cirrhosis. Evidence now

suggests, however, that prolonged re-treatment of nonresponders significantly improves SVR rates. In the Retreatment with Pegasys in Patients Not Responding to Peg-Intron Therapy (REPEAT) study, 72 weeks of treatment produced an overall SVR rate of 16% compared with 8% after 48 weeks of treatment ($P = 0.006$). In this study, patients who had undetectable levels of HCV RNA after 12 weeks of treatment were more likely to achieve an SVR after 72 weeks treatment than were those who had detectable levels of HCV RNA (57% vs 4%). These findings indicate that duration of therapy could be pivotal to improving the outcome in nonresponders to IFN-α.

Another potential strategy to achieve a response in patients who are unresponsive to the standard of care is to use increased doses of RBV, as described by Lindahl *et al.* in a small study of previously untreated patients. In their study, the authors used high doses of RBV (1,600–3,600 mg per day), tailored to each patient according to an individualized schedule. Although 9 out of 10 patients achieved an SVR, suggesting that this approach is feasible, the use of such high RBV doses was associated with more-frequent and more-serious adverse effects such as anemia.

Even in patients who do not achieve an SVR, IFN-based regimens can reduce hepatic inflammation. Given that progression of fibrosis to cirrhosis is a function of hepatic inflammation, it has been suggested that IFN-based maintenance therapy might slow disease progression. In addition, although some patients are classified as virological relapsers and/or nonresponders, they might have a biochemical response to treatment (i.e. reduction or normalization of ALT levels). Results from the NIH-sponsored HALT-C trial showed that peginterferon α2a maintenance therapy improved ALT level, HCV viral load, and necroinflammation. Despite these results, however, there was no long-term effect on the rate of disease progression. In a similar study that compared the effects of low dose peginterferon α2b with those of low-dose colchicine (Colchicine Versus PEG-Intron Long Term [COPILOT] study), the rate of bleeding from esophageal varices observed in patients treated with peginterferon α2b for up to 4 years was lower than that in patients who received colchicines.

Guidelines recommend that decisions regarding re-treatment should include consideration of the severity of the underlying liver disease, adherence and/or compliance, tolerance issues, the previous therapy and type of response to it, viral genotype, and other predictive factors for response.

New Therapeutics Approaches

New Interferons

Consensus Interferon. Consensus interferon (CIFN) is a recombinant, non-naturally, 166-amino acid IFN, containing the most frequently observed amino acids from various natural IFNs in each corresponding position. Some studies suggested that CIFN in combination with RBV may not be superior to the combination of PEG-IFNα-2b plus

RBV in naïve CHC patients with genotype 2/3 or genotype 1. CIFN is associated with SVR rates of 26-30% in non-responders and rates as high as 58% in relapsers. Cornberg *et al.* conducted an open-label pilot study of CIFN and RBV in 77 patients who did not respond to standard IFN regimens (90% of patients had HCV genotype 1). CIFN was given in an induction dose of 18μg/day for 8 weeks, followed by 9 μg/day for 40 weeks or as a standard dose of 9 μg/day for the full 48 weeks. RBV dose was weight based: 1000 mg/day for < 75 kg or 1200 mg/day for > 75 kg. The SVR rate was 30% (23/77) of the entire population and 22% (9/41) of prior non-responders with HCV genotype 1. Surprisingly, the SVR rate was 28% with the 18/9 μg/day induction regimen but 32% with the 9 μg/day regimen. In subset analyses, investigators noted the greatest response among patients previously treated with IFN monotherapy and the poorest response among those with liver cirrhosis.

The DIRECT trial (Daily-Dose Consensus Interferon and Ribavirin: Efficacy of Combined Therapy trial) was a phase 3, open-label, multicenter study investigating daily CIFN in 343 previous non-responders to peg-IFN and RBV. Patients were randomized to receive either CIFN 9 μg/day and RBV (1000-1200 mg/day) or CIFN 15 μg/day and RBV. The majority of patients had evidence of bridging fibrosis or cirrhosis on biopsy, and the mean washout period was 485-535 days. Viral response rates at week 48 were 16% [transcription-mediated amplification (TMA) assay] and 22% [branched DNA (bDNA) assay] for patients receiving CIFN 9 μg, and 19% (TMA) and 25% (bDNA) for those receiving CIFN 15 μg. Viral response was lower in patients with higher fibrosis scores. Among patients receiving CIFN 9 μg, end-of-treatment responses were noted in 19% with fibrosis scores of F0-F2 (TMA), 16% with F3 and 8% with F4. Among patients receiving CIFN 15 μg/day, end-of-treatment responses were noted in 28, 19 and 6% of patients respectively. The end-of-treatment response rate was lower for patients who underwent longer washout periods. The effect of fibrosis score and washout period in this study may require further investigation.

A major drawback of CIFN is the need for daily dosage because of its short half-life. It should be also noted that most of the data on CIFN-based therapies have not been published as full papers to date.

Albuferon. Albuferon (alb-IFN) is an 86 kDa novel recombinant protein consisting of IFNα genetically fused to human albumin extending its serum half-life. Recent studies have shown that its prolonged half-life (6 days) supports dosing at 2- to 4-week intervals, regardless of other factors, such as age, gender, race or stage of liver fibrosis. An ongoing phase IIb trial in 458 IFN-naïve CHC patients with genotype 1 showed that the efficacy of alb-IFN (900-1200 μg every 2 or 4 weeks) in combination with RBV for 48 weeks exhibits efficacy and safety at least comparable to PEG-IFNα-2a plus RBV with similar frequency in adverse events, significantly less frequent development of antibodies against IFNα (3% vs. 19%, $P < 0.0001$), and better quality of life because of its improved dosing schedule. In this study, the efficacy of alb-IFN and RBV appeared to be similar with PEG-IFNα-2a and RBV (SVR rates: 51-59% vs. 58%) and greater in heavier (≥75 kg) patients (SVR: 61-74% vs. 53%). A phase III trial of alb-IFN in naïve CHC patients started in late 2006.

The efficacy of alb-IFN in difficult-to-treat patients has been evaluated in 115 nonresponders to previous IFNα-based therapies. This study showed that alb-IFN (900-1800 μg every 2 or 4 weeks) in combination with weight-based RBV (1000-1200 mg daily) for 48 weeks was effective [the overall end-of-treatment response (ETR) in the alb-IFN cohorts was on average 31% (range: 25-44) and the SVR rate 20% (range: 9-30)] and safe at doses up to 1800 μg every 2 weeks. The HCV-RNA undetectability rates at week 24 were comparable across the 900-1800 μg cohorts, whereas they ranged from 15% to 27% in genotype 1 patients with the highest rates achieved in the 1500 and 1800 μg cohorts. Most patients with undetectable HCV-RNA at week 24 maintained the virological response until the end-of-treatment (week 48), while the virological response at week 12 and week 24 predicted 48-week responses in the 900-1200 μg cohorts. Interestingly, in the 1800 μg group, six (50%) of the 12 CHC genotype 1 patients 'null-responders' to prior PEG-IFNα/RBV combination, achieved early virological response (undetectable HCV-RNA at week 12).

Omega Interferon. Omega IFN is a new type-1 IFN, homologous to IFNα in 60% and homologous to IFNβ in 30%, that has been designed for continuous delivery by an implantable device. In 102 IFN-naïve CHC patients with genotype 1, 48 weeks of treatment with omega IFN 25 μg daily in combination with RBV achieved undetectable HCV-RNA in 36% of cases at 12 weeks after the end-of-treatment, compared to only 6% of patients with omega IFN monotherapy. Omega IFN was well tolerated in both groups with discontinuation because of adverse events in only two patients.

Other Interferons. R7025 is a novel human pegylated IFNα-2a molecule generated using DNA shuffling technology. R7025 has the same (40 kDa) PEG molecule used in PEG-IFNα-2a, but it has 50-fold higher *in vitro* anti-viral activity. Single ascending doses of R7025 in 72 healthy volunteers gave promising results with mild flu-like symptoms and reversible drop in neutrophils and without serious adverse events. Locteron is another controlled release recombinant IFNα-2a. In a phase Ib trial, combination of Locteron (at doses 160, 320, 480 and 640 μg every 2 weeks) with RBV was given in 32 naive CHC genotype 1 patients for 12 weeks. At the end-of-treatment, the mean HCV-RNA reduction was 1.8, 4.5, 4.2 and 4.7 $\log_{10}$ IU/mL respectively, while arthralgia, weakness, myalgia and headache were the most common adverse events.

Ribavirin Analogues - Inosine Monophosphate Dehydrogenase Inhibitors

Ribavirin acts via inhibition of inosine monophosphate dehydrogenase (IMPDH), a cellular enzyme implicated in the production of guanine nucleotides. RBV treatment alone does not produce a significant anti-viral effect, but its addition to IFNα therapy significantly increases the rate of complete viral eradication, compared to IFNα monotherapies, possibly because of the production of viruses, which are more sensitive to IFNα. However, its tolerability is frequently limited because of RBV induced hemolytic anemia. Thus, RBV analogues lacking a hemolytic effect are needed, but their development is difficult, as the exact anti-viral mechanisms of RBV remain unknown.

Viramidine or Taribavirin. Taribavirin hydrochloride or Viramidine is a RBV prodrug that is metabolized preferentially in the liver by adenosine deaminase and thus it does not accumulate in erythrocytes. In a phase II trial, Viramidine (400-800 mg daily) combined with PEG-IFNα-2a, achieved a relatively lower SVR rate, but significantly less frequently hemolytic anemia, compared with RBV. In this study, the daily dose of 600 mg of Viramidine achieved the highest SVR rate.

A recent phase III trial revealed that Viramidine combined with PEG IFNα-2b, compared with PEG IFNα-2b plus RBV combination, exhibited significantly lower anemia rates (5% vs. 24%, $P < 0.001$), but again lower virological response rates (SVR: 38% vs. 52%). Viramidine at doses >18 mg/kg yielded response rates similar to the current standard combination therapy without increasing the rates of anemia or other side effects. In addition, 970 patients with slow virological response (particularly those with HCV-RNA drop <2 log within the first 4 weeks of treatment) had higher SVR when treated with Viramidine, compared with RBV, attributable to improved tolerability of Viramidine over RBV (63% vs. 36% respectively). Interestingly, pre-treatment with Viramidine monotherapy (perhaps with RBV as well) for 4 weeks prior to PEG-IFNα-2b therapy resulted in a steeper decline of HCV-RNA levels after PEG-IFNα-2b introduction, compared with Viramidine and PEG-IFNα-2b simultaneous therapy, but this difference was not significant. Thus, further studies are needed to assess the impact of predosing of Viramidine (or RBV) on viral response, as well as the development of weight-based dosing of Viramidine. For the time being, Viramidine seems to be a potential substitute of RBV only for cases who develop severe anemia under RBV but not for all CHC patients.

VX-497 or Merimepodip. VX-497 (Merimepodip) is a potent, specific and orally taken inhibitor of IMPDH. Although it has been found that VX-497 monotherapy may increase viral replication because of T-cells inactivation and IFNα plus VX-497 combination did not have stronger anti-viral activity than IFNα monotherapy in naïve CHC patients, the addition of VX-497 to PEG-IFNα and RBV was reported to improve significantly the virological response at week 24 in nonresponders CHC patients.

Amantadine

Amantadine is a tricyclic amine, which has been used to treat and limit influenza A infection. Amantadine monotherapy has little activity against HCV, but its combination with PEG-IFNα and RBV has been shown to increase anti-viral response in patients with CHC. The results of a phase IV trial aiming to compare the efficacy of the triple PEG-IFNα-2a/RBV/amantadine (400 mg/day) combination for 48 weeks against the standard combination of PEG-IFNα-2a/RBV in 703 naïve genotype 1 CHC patients were recently presented. SVR rates were similar between the two groups (49% vs. 53%), while the drop-out rate was significantly higher in the amantadine group (32% vs. 23%, $P = 0.01$). Although, amantadine seems to have no impact on virological response in naïve CHC patients, a recent meta-analysis of 31 randomized-controlled trials suggested that the triple therapy (PEG-IFNα/RBV/amantadine) may significantly improve the SVR rates (23%) in nonresponders to previous standard therapy.

Inhibition of RNA Replication

NS5B Polymerase Inhibitors. The NS5B RdRp is an attractive target for anti-viral therapy. Several research groups have discovered nucleoside analogues, which are converted to nucleotides and terminate the RNA chain. Laboratory efforts have also uncovered a variety of structurally unrelated non-nucleoside inhibitors (NNIs) of RNA polymerase, which target the allosteric sites of the RdRp. Interestingly, the different classes of NS5B polymerase inhibitors elicit diverse patterns of resistance, whereas several different binding sites for NNIs exist on the HCV polymerase. Thus, although these agents target the same viral enzyme, they offer the potential to be used in combination. Further studies are needed to clarify their exact anti-viral effect in the clinical setting after mid- and long term administration, but generally monotherapies must be avoided in favor of combination strategies because of the susceptibility of these agents for development of viral resistance.

Nucleoside inhibitors. NM283 or Valopicitabine is the oral prodrug of the nucleoside analogue of 2'-*C*-methyl-cytidine. Pharmacokinetic studies have supported its co-administration with PEG-IFNα. NM283 has less potent anti-viral efficacy, compared with BILN 2061 or VX-950, but it has demonstrated anti-viral activity at tolerated doses. In an ongoing phase IIb trial including 173 naïve genotype 1 CHC patients, combination of NM283 (200-800 mg daily) and PEG-IFNα-2a achieved significant dose-dependent HCV-RNA reductions with high undetectability rates of HCV-RNA at weeks 24 and 36 by the sensitive TaqMan assay (range: 49-68% of patients at both time points). Gastrointestinal side effects were common and occasionally severe at the NM283 daily dose of 800 mg, leading to a protocol amendment with reduction in the 800 mg dose after 14-22 weeks of therapy.

In another phase IIb trial, 178 genotype 1 nonresponders to PEG-IFNα and RBV, received NM283 (400-800 mg daily) plus PEG-IFNα-2a. At 48 weeks, HCV-RNA undetectability by the Amplicor and TaqMan assay was achieved in 40% and 28% of patients treated with NM283 plus PEG-IFNα-2a compared to 27% and 24% of those treated with PEG-IFNα-2a plus RBV re-treatment. HCV-RNA levels reduction was sixfold greater in the NM283/PEG-IFNα-2a than in the PEG-IFNα-2a/RBV group ($P =$ 0.06). Again, the high NM283 dose (800 mg daily) was reduced to 400 mg daily after 40 weeks of therapy because of gastrointestinal side effects. Recently, however, further development of the agent was stopped before the onset of phase III trials because of low efficacy and poor tolerability.

R1626, a prodrug of R1479 (4'-azido-cytidine), is a potent, specific inhibitor of HCV polymerase-mediated RNA synthesis. *In vitro* experimental studies have shown that R1479 has additive anti-viral effect combined with other HCV replication inhibitors, such as IFNα, RBV, NM107 and BILN2061, without any antagonistic effects and without any effect on stability of human erythrocytes membrane when combined with RBV, R1626 gave promising results in initial clinical trials. In particular, in a phase Ib trial including 47 naïve genotype 1 CHC patients, R1626 monotherapy at doses of 1500-4500 mg twice daily achieved 1.2-3.7 $\log_{10}$ IU/mL reductions in HCV-RNA levels at 14 days. The drug

was generally well tolerated, but with increasing rates of adverse events at the highest dose (e.g. significant drop in hemoglobin at doses of 6000-9000 mg daily). In a phase II study, the combination of R1626 (1500-3000 mg twice daily) plus PEG-IFNα-2a with or without RBV was compared to standard therapy in 104 genotype 1 naïve CHC patients. At 4 weeks, HCV-RNA was undetectable in 81% of patients treated with triple therapy (mean reduction of 5.2 $\log_{10}$ IU/mL), compared to only 5% of patients treated with standard therapy (mean reduction of 2.4 $\log_{10}$ IU/mL). There was no evidence of resistance to R1626 during this 4-week period. Gastrointestinal adverse events were commonly observed with higher doses of R1626, while neutropenia was not associated with increased incidence of infection. Further studies of different dosages of R1626 in combination with PEG-IFNα-2a and RBV are in progress.

R7128 is a new oral nucleoside HCV-RNA polymerase inhibitor. The anti-viral efficacy and tolerability of ascending dose of R7128 (750-3000 mg/day) were evaluated in 40 genotype 1 CHC patients. At 2 weeks, a significant dose-dependent reduction in serum HCV-RNA was observed (mean: 0.3-2.1 $\log_{10}$ IU/mL, range: 0.9-2.7). No evidence of viral rebound was detected and no serious adverse events were recorded (headache, dry mouth, nausea and upper respiratory infection were the most frequent adverse events).

A-837093 is a new potent HCV polymerase inhibitor, with a strong anti-viral efficacy in the chimpanzee model, which may be useful in combination with other small-molecules (such as NS3/4A protease inhibitors) to treat HCV infection effectively. Its oral bioavailability was very good in animals.

Non-nucleoside Inhibitors [NNIs]. JTK-109 and JTK-003 were the first NNIs of NS5B polymerase that entered clinical trials, but further clinical development is still awaited. Subsequently, several other HCV polymerase NNIs have been developed and evaluated in clinical trials, such as R803, HCV-371, HCV-086 and HCV-796. Further clinical investigation, however, has been stopped because of insufficient anti-viral activity of the first three and elevations of liver enzymes of the latter.

HCV-796 is an oral NNI of HCV-RNA polymerase, which belongs to the benzofuran family and has demonstrated potent anti-viral activity in both *in vitro* and *in vivo* studies. In a clinical dose-escalation study including 102 treatment-naive CHC patients (72% with genotype 1), HCV-796 administered orally at doses of 50-1500 mg twice daily for 14 days demonstrated an initial rapid anti-viral activity without significant side effects apart from mild-to-moderate headache. In particular, the highest dose of HCV-796 achieved 1.4 $\log_{10}$ IU/mL mean reduction in HCV-RNA levels at day 4. However, selection of viral variants (Cys316Tyr) was observed with subsequent increase in viremia, which returned to nearly baseline levels (the mean reduction of HCV-RNA at day 14 was only 0.7 $\log_{10}$ IU/mL). In a phase II clinical trial, the combination of HCV-796 and PEG-IFNα-2b was evaluated in patients with CHC. At 14 days, the combination therapy achieved greater reduction in HCV-RNA levels, compared with PEG-IFNα-2b monotherapy (3.5 vs. 1.6 $\log_{10}$ IU/mL). However, the combination of HCV-796 with PEG-IFNa-2b/RBV was halted because of significant elevation of liver enzymes.

Bilb1941 is another specific NNI of HCV polymerase, which, at an oral dose of 10-450 mg thrice daily, was found to have potent anti-viral activity at 5 days in 96 males, genotype 1, CHC patients. Although serious adverse events were not recorded, Bilb1941 was associated with frequent gastrointestinal intolerance (particularly at higher doses) and exacerbations of aminotransferases levels.

AG-021541 is a representative compound from a novel series of HCV polymerase inhibitors characterized by a dihydropyrone core. AG-021541 has exhibited satisfactory *in vitro* anti-viral activity against HCV, but the development of resistance because of mutations in the inhibitor-binding region needs further evaluation.

Thiophene derivatives and benzothiadiazines are other two classes of allosteric inhibitors of the HCV polymerase, but clinical trials are missing. The latter class of inhibitors showed anti-viral synergy with IFN in the HCV replicon, but a mutant replicon resistant to benzothiadiazines has been identified (M414T within NS5B).

PSI-6130 and GSK625433 are also potent and selective HCV NS5B inhibitors. PSI-6130 seems to be compatible with IFN, RBV, NNIs and BILN-2061. GSK625433 belongs to a novel series of acyl-pyrolidine, which binds to the palm region of HCV polymerase and its combination with other polymerase/protease inhibitors seems to be promising. VCH-759, a new oral HCV-RNA polymerase inhibitor given at ascending doses (1200-2400 mg/day) for 10 days in 32 naïve genotype 1 CHC patients, achieved a significant (≥ 2 $\log_{10}$ IU/mL) decline in HCV-RNA at doses of 1600-2400 mg/day (e.g. 22% of patients under VCH-759 2400 mg/day had ≥ 3 $\log_{10}$ IU/mL reduction in viral load) with only mild gastrointestinal adverse events. GS-9190, a novel HCV NS5B inhibitor, was also recently evaluated in 31 genotype 1 CHC patients. In this study, a single dose of GS-9190 (40-480 mg) showed a potent dose-dependent reduction in serum HCV-RNA levels (range: 0.19-2.5 $\log_{10}$ IU/mL) without serious adverse events. Recent data indicated that GS9190 remains active against known resistant HCV drug-resistant mutants. Finally, the pharmacokinetic properties of ANA598, a potent NNI of NS5B polymerase, were evaluated in a recent preclinical trial; ANA598 had high oral bioavailability and was suggested to achieve higher concentration in the liver compared with other compounds of the same class.

Prevention of Functional Replication Complexes. ACH-806 is a new potent inhibitor of HCV replication, which has been shown to act synergistically in combination with different classes of HCV inhibitors, such as VX-950 and NM283. Its exact mechanism of anti-viral action remains unclear, but it seems to prevent the development of functional replication complexes via inhibition of NS3-NS4A interaction. However, further evaluation was stopped because of possible development of renal dysfunction.

Inhibition of Protein Translation

Antisense Oligonucleotides. Antisense oligonucleotides are short synthetic nucleic acids (usually with <25 nucleotides) that bind an RNA target forming RNA-RNA (antisense RNA) or RNA-DNA (antisense DNA) hybrids resulting in inhibition of RNA translation

of viral proteins and/or replication. Several oligonucleotides targeting the 5'-UTR, the most conserved region of the HCV genome, have been reported to inhibit HCV gene expression *in vitro*. ISIS 14803 is a 20-base antisense oligonucleotide, which is complementary to the HCV translation initiation region within the IRES. ISIS 14803 gave promising results in early phase II clinical trials, but subsequent aminotransferases flares and poor anti-viral efficacy led to discontinuation of further studies. AVI-4065 has been shown to inhibit HCV protein translation *in vitro* and in animal models, whereas a phase II study is under progress.

RNA Interference. RNA interference is a method of specific degradation of messenger RNA leading to RNA silencing. Small interfering RNA or short hairpin RNA are used, but both approaches currently are not orally bioavailable and require parenteral administration. SirnaAV34 is planned for further evaluation following preclinical animal studies. BLT-HCV (Benitec), the first clinical candidate to treat HCV infection through RNA interference, consists of three components targeting different HCV sequences, underlining the importance of a multi-targeting approach to prevent resistance development.

Ribozymes. Ribozymes are synthetic nuclease-resistant catalytic RNA molecules acting by cleavage of specific HCV-RNA sequences. *In vitro* studies have demonstrated the potent anti-viral activity of various ribozymes to inhibit HCV polyprotein translation. Heptazyme is a ribozyme against the HCV IRES, which had progressed to early phase clinical studies in patients with CHC. It showed moderate anti-viral efficacy, but further development was stopped because of toxicity in animal models.

Post-translational Modification

NS3/4A Protease Inhibitors. NS3 serine protease, with the cofactor NS4A, forms a heterodimeric protease, which acts as a serine protease and plays an essential role for the generation of components of the viral RNA replication complex. In addition, it has been implicated in the initiation of the cellular anti-viral response. Although it was difficult to design potent and high-affinity inhibitors because of the characteristics of the protease-binding pocket which is wide and shallow, the NS3-4A protease has emerged as one of the most popular targets of many novel small-molecule inhibitors. There is, however, great concern regarding the development of viral resistance to this group of anti-HCV agents.

BILN 2061 or Ciluprevir, a noncovalent inhibitor of NS3-4A protease, was the first compound of this class tested in clinical trials. Although the compound demonstrated dramatic reductions in serum HCV-RNA levels in genotype 1 patients within 48 h, further development was halted because of cardiotoxocity.

VX-950 or Telaprevir is another peptidomimetic inhibitor of the viral NS3-4A serine protease, which, in contrast to BILN 2061, forms a covalent but reversible complex with the target enzyme through the inclusion of an α-ketoamide in the active site of the enzyme. Recent studies have shown that VX-950 is capable of reducing serum levels of

neopterin (a marker of inflammatory activity), but its *in vitro* activity against genotype non-1 HCV genotypes has not been elucidated yet. In a 14-day dose-defining study including eight healthy volunteers and 36 CHC genotype 1 patients, the VX-950 dose of 750 mg every 8 h achieved the greatest effect with 4.4 $\log_{10}$ IU/mL median HCV-RNA reduction. However, viral breakthroughs were observed during the second week of treatment because of selection of telaprevir-resistant variants associated with substitution of alanine to serine at position 156, whereas replacement of the same residue with threonine or valine conferred cross-resistance to both VX-950 and BILN 2061. After treatment discontinuation, the sensitive wild-type virus slowly replaced the resistant variants. The most commonly reported drug-related adverse events were headache and diarrhea.

In a subsequent 14-day randomized trial, the efficacy of VX-950 and PEG-IFNα-2a combination was compared to VX-950 or PEG-IFNα-2a monotherapy in 20 naive CHC genotype 1 patients, while all patients received standard PEG-IFNα-2a and RBV combination for 24 or 48 weeks within 5 days after completing the 14-day dosing period. The VX-950/PEG-IFNα-2a combination was well tolerated and achieved greater median decline in viral load, compared with VX-950 or PEG-IFNα-2a monotherapy (5.5 vs. 4.0 or 1.0 $\log_{10}$ IU/mL respectively). Additionally, four of eight patients treated with the VX-950/PEG-IFNα-2a combination had undetectable HCV-RNA at day 14, while no patient had an increase in HCV-RNA levels during treatment. Interestingly, SVR was achieved in nine of 15 patients from the VX-950 monotherapy and VX-950/PEG-IFNα-2a combination groups, who continued with PEG-IFNα-2a/RBV therapy for 24 or 48 weeks, suggesting that VX-950-based regimens may increase SVR rates. However, three of the five patients who relapsed after the end of the standard therapy had developed known VX-950 resistance mutations within the NS3 protease gene (V36/A156, V36/R155 and T54). Nevertheless, combination therapy seems to be a superior therapeutic option, without the disadvantage of VX-950 monotherapy, which frequently leads to viral rebound because of presence of uncovered viral variants with low-level (V36M/A, T54A or R155K/T) or high-level (A156V/T and 36/155) of resistance to telaprevir after wild-type virus clearance. These variants have decreased replication capacity and are fully sensitive to PEG-IFNα/RBV therapy.

In another study, VX-950 (750 mg thrice daily) and PEG-IFNα-2a/RBV for 4 weeks followed by standard PEG-IFNα-2a/RBV combination for 44 weeks were given in 12 naive genotype 1 patients. The triple therapy was well tolerated and achieved undetectable HCV-RNA (<10 IU/mL) within 4 weeks in all patients without any breakthrough. Serum HCV-RNA remained undetectable in all but one patient after 12 weeks of follow-on therapy with PEG-IFNα-2a/RBV, while SVR was observed in eight (67%) patients. In a phase II trial (PROVE-1), 250 naïve genotype 1 patients received VX-950 and PEG-IFNα-2a/RBV for 12 weeks followed by 0, 12 or 36 weeks of PEG-IFNα-2a/RBV or standard PEG-IFNα-2a/RBV combination for 48 weeks. At 12 weeks, HCV-RNA remained undetectable (<10 IU/mL) in 88% and 52% of patients treated with triple and double therapy respectively ($P = 0.0001$). The rate of adverse events was similar but discontinuations were more common in the triple combination arm (11% vs. 3%). SVR rate was higher after the 12-week triple followed by 12-week standard double

combination than after the 12-week triple combination only (61% or 35%), while end-of-therapy response rate was higher after the 12-week triple followed by 36-week standard double combination than after the 48-week standard double combination (65% vs. 45%). In another trial (PROVE 2), 323 naïve genotype 1 patients received VX-950 and PEG-IFNα-2a ± RBV for 12 weeks or VX-950 and PEG-IFNα-2a/RBV for 12 weeks followed by 12 weeks of PEG-IFNα-2a/RBV or standard PEG-IFNα-2a/RBV therapy for 48 weeks. Triple combination achieved greater 4- and 12-week responses than any double combination, while VX-950/PEG-IFNα-2a achieved greater on-therapy responses than PEG-IFNα-2a/RBV. SVR rates were higher in the two triple combination arms (59-65%) than in the VX-950/PEG-IFNα-2a arm (29%). Viral breakthroughs usually because of selection of VX-950-resistant variants developed less frequently in the triple than in the VX-950/PEG-IFNα-2a combination arm (at 12 weeks: 2% vs. 24%). Rash, pruritus, nausea and diarrhea were the most common adverse events in the VX950 arms of the latter two trials.

SCH 503034 is an oral well-tolerated protease inhibitor of NS3/4A protease. In a phase IIa, placebo-controlled trial, SCH 503034 monotherapy (at a dose ranging from 100 mg twice daily to 400 mg thrice daily for 14 days), showed potent anti-viral efficacy (2.1 $\log_{10}$ IU/mL mean reduction of viral load at a dose of 400 mg thrice daily) in 61 genotype 1 nonresponders to previous PEG-IFNα-based therapy. The most common side effect of SCH 503034 was headache, but the overall side effects did not significantly differ from those observed in patients taking placebo. Similar to the other compounds of the same class, combination of SCH 503034 with IFNα was considered as a strategy for protection against the development of resistant variants leading to enhanced anti-viral activity.[98] In a recent 14-day study, SCH 503034 (400 mg thrice daily) and PEG-IFNα-2b combination achieved greater reduction in viral load, compared with PEG-IFNa-2b or SCH 503034 monotherapy (2.9 $\log_{10}$ IU/mL for SCH 503034 plus PEG-IFNα-2b vs. 1.1 $\log_{10}$ for PEG-IFNα-2b alone vs. 0.5-2.5 $\log_{10}$ for SCH 503034 alone) in genotype 1 nonresponders to previous PEG-IFNα-2b and RBV therapy. Further phase II trials assessing 24 and 48 weeks of combination treatment are under progress.

ITMN-191 and TMC435350 are new potent oral inhibitors of the NS3/4A protease. Pharmacokinetic properties of ITMN-191 have been evaluated in animal models (rats and cynomolgus monkeys). ITMN-191 has a favorable cross-resistance profile with VX-950 and other related linear tetrapeptides, while recent data support its use against multiple HCV genotypes. In addition, recent *in vitro* studies suggested synergistic anti-viral activity of ITMN-191 and PEG-IFNα-2a, but further clinical studies are required. TMC435350 has been evaluated in HCV-negative volunteers at single and multiple ascending doses with good safety profile. This compound will be further investigated at once daily administration in CHC patients.

NS5A and Helicase Inhibitors. New potent anti-viral agents, such as NS5A (A-831) and helicase (QU663) inhibitors have been evaluated *in vitro* with encouraging results, but further clinical evaluation is needed.

Inhibitors of Viral Assembly and Release

Inhibitors of cellular glycosidases are potential candidates of anti-viral therapy, inhibiting viral assembly and release. Celgosivir or MX-3253 is a novel potent inhibitor of the host enzyme α-glucosidase I, which is involved in HCV assembly and release. In an open-label phase II trial, 43 naive or IFNα-intolerant genotype 1 CHC patients were randomized to receive celgosivir 200 mg daily, 400 mg daily or 200 mg twice daily for 12 weeks. During the study period, celgosivir was well tolerated with only mild gastrointestinal side effects and asymptomatic dose-related elevation of creatine phosphokinase, while 5% of patients had peak on-treatment viral load reductions of ≥ 1 $\log_{10}$ IU/mL. Although celgosivir monotherapy had moderate anti-viral activity, further studies of combination with PEG-IFNα plus RBV are ongoing.

Prevention of Binding

Similar to many other viruses, HCV entry into the cells is based on different steps, i.e. binding, internalization and cell penetration. The E1 and E2 are type I transmembrane highly glycosylated proteins of the HCV envelope, and E2 is considered to play an important role in viral attachment interacting with one or more components of the cell membrane, such as low-density lipoprotein receptor, glycosaminoglycans and CD81. Thus, inhibition of HCV entry can be based on the development of specific inhibitor molecules, which act at the receptor(s)-binding site(s) or specific antibodies that neutralize infectious particles. Recently, the anti-viral activity of specific E2-derived peptides, such as GNS-037, have been evaluated *in vitro* with encouraging results.[107] Polyclonal immune globulins and monoclonal antibodies, such as HCV-AB 68 and HCV-AB 65 have been evaluated in early clinical trials including CHC patients who underwent liver transplantation.[70] Only HCV-AB 68 has been evaluated in the non transplant setting. In particular, HCV-AB 68 given intravenously in 40 CHC patients was well tolerated and resulted in transient reduction of viremia following single or multiple doses up to 120 mg without serious adverse events.

Immunomodulators

Toll-like receptor agonists. Toll-like receptors (TLR) are molecules on the cells surface that recognize the presence of invading pathogens, such as bacteria and viruses. TLRs are expressed by immune cells and their activation leads to acute inflammatory response by inducing the expression of proinflammatory cytokines, such as IFN. There are 10 human TLRs, each recognizing molecular signatures associated with specific class of microbial species.

ANA245 or isatoribine, a selective agonist of TLR7, was found to reduce viremia in 12 naïve CHC patients after 7 days of intravenous administration with some patients achieving >90% reduction in serum HCV-RNA levels. The drug had relatively mild side effects. Following these encouraging results, ANA975, an oral pro-drug of ANA245 was evaluated. ANA975 was found to have comparable anti-viral efficacy with ANA245 and to be well tolerated after a single dose in 36 healthy volunteers, but it was suspended

following intense immune stimulation in animal models. The safety, pharmacokinetics and pharmacodynamic properties of resiquimod, a new oral TLR7-8 agonist, were analysed in two recent randomized phase II trials. Resiquimod was given at 0.01 and 0.02 mg/kg twice per week for 1 month. Although the high dose had greater anti-viral efficacy than the low dose, it was associated with intense adverse events because of induction of IFNα.[111] SM360320 is another TLR7 agonist, which has been found to reduce *in vitro* HCV-RNA levels by induction of type I IFN.

CPG 10101 (Actilon) is a synthetic TLR-9 agonist. In a phase I study, 74 genotype 1 patients, who had relapsed after PEG-IFNα and RBV therapy, were randomized to receive GPG10101 subcutaneously, alone or in combination with PEG-IFNα and/or RBV. Triple CPG 10101/PEG-IFNα/RIB combination, compared with PEG-IFNα/RBV, was well tolerated and achieved greater reduction in serum HCV-RNA levels at week 12 (50% vs. 13%, $P < 0.05$), whereas end-of-therapy response was achieved in 45% of patients who continued the triple therapy for 48 weeks. However, further development of CPG 10101 was stopped because of lack of efficacy.

Histamine Dihydrochloride. Histamine dihydrochloride (HID) is considered to have both immunomodulatory and antioxidant properties. Initial studies supported the co-administration of HID with IFNα. In a phase II trial including 129 naïve CHC patients, this combination given for 48 weeks achieved SVR rates ranging between 30% and 40%, which seem to be somewhat inferior to those reported with the combination of IFNα and RBV. The triple therapy (HID/IFNα/RBV) was also tried in 18 CHC patients without SVR after IFNα monotherapy, in who 50% virological response was achieved at the end of 48 weeks of therapy, but SVR rates were not reported. The trials with HID in combination with PEG-IFNα and RBV were recently discontinued.

Thymalfasin. Thymalfasin or Zadaxin is a synthetic analogue of the natural immunomodulatory peptide thymosin-α1, which promotes T lymphocyte and natural killer cell activation. Zadaxin has been evaluated in 25 nonresponders to IFNα and RBV combination. The triple combination of Thymalfasin (1.6 mg twice per week), PEG-IFNα-2a and RBV given for 48 weeks achieved end-of-therapy responses in 12 (48%) of 25 patients, but further investigation of Zadaxin was halted because the combination of Zadaxin and PEG-IFNα showed no benefit over PEG-IFNα alone.

Interleukins. Interleukin (IL)-10 given in nonresponders to IFNα-based therapies has been found to improve serum aminotransferases levels and liver histology. IL-12 is unlikely to become an alternative to conventional IFNα-based therapy because of its poor anti-viral efficacy and substantial severe adverse events. IL-29 binding to a heterodimeric IL-28R/IL-10R receptor activates, similar to IFNα, the JAK/STAT pathway. Recently, the activity of a pegylated form of IL-29 (PEG-IL-29) in different doses (0.03-3 mg/kg) intravenously was evaluated in animals. This study showed that PEG-IL-29 is pharmacologically active inducing the expression of known IFN serum biomarkers (e.g. neopterin and β-2 microglobulin) and genes (e.g. MxA and PkR) in liver biopsy specimens, to an extent similar to IFNα. Interestingly, in contrast to IFNα, PEG-IL-29 is active in liver, but not in circulating white cells.

Therapeutic Vaccines. Although no prophylactic vaccine is available for prevention of HCV infection, several therapeutic vaccines have been developed to induce HCV-specific immune responses. A recombinant E1 vaccine against the E1 envelope glycoprotein has been found to stimulate both humoral and cellular immune responses. This vaccine has yielded encouraging results in preliminary clinical trials. In particular, E1 vaccine was associated with improvement in serum ALT levels and liver histology, but with no effect on HCV-RNA levels in 35 genotype 1 nonresponders to previous standard therapy.

IC-41 is another vaccine containing several epitopes of the HCV genome and polyarginine as an adjuvant. In a phase II trial, IC-41 was able to induce significant immune responses and transient reduction of HCV-RNA levels in 60 nonresponders.

T-cell vaccine based on the HLA and HCV genotype cross-reactivity and dendritic cell-based vaccines are novel and very promising approaches for therapeutic immunization in patients with CHC. In particular, dendritic cell vaccination is able to elicit potent activation of antigen-specific cellular immunity against HCV proteins, which is a more physiological process of capturing, internalization and presentation of HCV antigens. In recent studies, immunization of mice with beads coated with NS5 protein and anti-DEC205-endocytosis receptor of dendritic cell was able to induce a significant cellular immune response.

Cyclosporin A and its Analogues. Cyclophilins (CyP), a family of peptidyl-propyl *cis*/*trans* isomerases (PPIase), seem to have a significant role in HCV replication. Based on findings in laboratory animal models (chimpanzees) and small study in CHC patients, the immunosuppressant drug cyclosporin may suppress HCV-RNA levels via inhibition of PPIase. Interestingly, the anti-viral effect of cyclosporin was related to its blood concentration being greater in combination with IFNα. DEBIO-025 is a novel non-immunosuppressive form of cyclosporin. *In vitro* studies have shown that DEBIO-025 has a 10-fold greater anti-viral activity on HCV replication, compared with cyclosporin, independently of HCV genotype. DEBIO-025 is also attractive for HCV patients co-infected with human immunodeficiency virus as well as for combination approaches with NS5B and/or NS3-4A inhibitors.

NIM811 and SCY-635 are new cyclosporin analogues, which exhibit stronger suppression of HCV-RNA *in vitro*, compared with cyclosporin itself. Again, their anti-viral activity was greater in combination with IFNα. Based on *in vitro* studies, NIM811 exerts its anti-viral activity through a CyP-B-dependent mechanism (CyP-B is an important host factor for HCV replication) and it can be combined with other anti-viral agents. Thus, although further clinical studies are needed, cyclosporin and its analogues might provide a new strategy for anti-HCV treatment, particularly in combination with standard or other new anti-HCV treatments (polymerase or protease inhibitors).

GI-5005. GI-5005 is a whole, heat-inactivated recombinant yeast genetically modified to express HCV-specific (HCV NS3 and core) protein targets, which acts via immune elimination of infected hepatocytes. In a recent 1B trial, 71 CHC patients received 0.05-

40 yeast units/week subcutaneously for 5 weeks followed by monthly dosing for seven additional weeks. There was no serious adverse event, while ALT normalization was observed in a dose-dependent manner and mild HCV-RNA reduction (0.75-1.4 $\log_{10}$ IU/mL) was detected in six patients. A multicenter phase II trial comparing GI-5005 plus PEG-IFNα/RBV vs. PEG-IFNα/RBV is in progress.

Other Agents

Agents Implicated in Lipid Biosynthesis. NA-255 is a novel serine palmitoyltransferase inhibitor, which exhibits HCV anti-viral activity via disrupting of HCV assembly and/or NS5B and lipid rafts interaction. Bezafibrate, a lipid lowering agent acting via peroxisome proliferator-activated receptor-alpha, was found to reduce serum gamma-glutamyl-transpeptidase and ALT levels in 34 CHC patients, nonresponders to previous PEG-IFNα/RBV therapy. Statins [3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors] have also been shown inhibitory effects on HCV replication. In a genome-length HCV-RNA replication system (OR6), atorvastatin, fluvastatin, simvastatin and lovastatin, but not pravastatin, were found to have anti-HCV activity and to have a synergistic effect with PEG-IFNα. However, the anti-HCV activity of atorvastatin given at standard doses, and perhaps of all HMG-CoA reductase inhibitors, was not confirmed *in vivo* in a pilot clinical trial including 10 CHC patients. Thus, further clinical studies are required for these agents. As the anti-viral activity of Statins is possibly mediated via inhibition of geranylgeranylation of host cell proteins, which is required for HCV assembly rather than the reduction of cholesterol synthesis itself, the selective inhibition of geranylgeranylated proteins, such as FBL2, might be a safer approach for HCV-RNA inhibition in the future.

S-Adenosyl-methionine and Oxidative Stress. S-Adenosyl-methionine (SAMe) and betaine (thimethyl-glycine) were shown to increase the anti-viral effect of IFN *in vitro*. They may act via methylation of important enzymes implicated in the anti-viral function of IFN, such as STAT1, and may improve IFN signaling and thus virological responses. Interestingly, SAMe may act also synergistically with IFN via reduction of oxidative stress in hepatocytes induced by HCV genes expression. Phase II studies of SAMe in combination with PEG-IFNα-2a and RIB are in progress.

Herbs and other hepatoprotectants. Hepatoprotectans, a large family of agents with antihepatotoxic activity, are given in various acute and chronic liver diseases. They are made mostly from traditional remedies, but their mode of action is more or less unknown. Several herbs, such as silymarin and glycyrrhizin, multivitamins, methionine, oleanolic acid and thioctic acid are considered the most frequently prescribed hepatoprotectants.

Although herbs are widely used for the treatment of CHC in Asia, their efficacy and safety remain controversial. Silymarin, the seed extract of milk thistle (*Silybum marianum*), is an ancient herbal remedy used to treat various liver and gall-bladder diseases. In a recent study, MK-001, a standardized extract of Silymarin was evaluated *in vitro*. MK-001 was found to have anti-inflammatory properties with inhibition of tumour necrosis factor-α expression in human peripheral blood mononuclear cells and nuclear

factor kβ in human hepatoma Huh7 cells, while its anti-viral effects were partly related to induction of Stat1 phosphorylation.

Glycyrrhizin, a natural compound extracted from the roots of *Glycyrrhiza glabra*, has been evaluated in CHC nonresponders with or with contraindications to IFNα. Glycyrrhizin, given by six infusions weekly for 4 weeks, was able to induce only biochemical responses in 72 (60%) of 121 patients, whereas at the end-of-treatment (22 weeks) there were no significant changes in necroinflammation. Glycyrrhizin was also evaluated in CHC patients, nonresponders to previous PEG-IFNα/RBV therapy. Glycyrrhizin (200 mg) given three or five times a week intravenously for 52 weeks was associated with reduction of aminotransferases and 45% improvement of ≥1 point in the necroinflammation score.

Nitazoxanide. Nitazoxanide (NTZ), an antiprotozoan agent, has also anti-viral efficacy blocking the viral protein synthesis via inhibition of eukaryotic initiation factor 2. NTZ monotherapy for 12 weeks followed by combination of NTZ/PEG-IFNα-2a (with or without RBV) for 36 weeks was compared to standard therapy (PEG-IFNα-2a/RBV) in 120 CHC genotype 4 patients. Triple compared to standard double therapy was reported to achieve higher ETR and SVR rates (in naïve patients: 79% vs. 43%, *P* = 0.006) without significant difference in adverse events.

Antiapoptotic Agents. As hepatocyte apoptosis plays an important role in development of liver injury in CHC, antiapoptotic agents have been investigated in such patients. In a recent double-blind, placebo-controlled trial, IDN-6556, a potent reversible pan-caspase inhibitor, was evaluated in 105 patients with chronic liver diseases (80 with CHC). IDN-6556 (5-40 mg/day) for 14 days was well tolerated and achieved significant ALT reduction, compared to placebo, but no effect on viremia. PF-03491390, another oral pan-caspase inhibitor, was also tried in CHC (5-50 mg/day for 12 weeks) achieving greater reduction in serum markers of inflammation and fibrosis, compared with placebo.

Evaluation for the Treatment against HCV

Anti-HCV agent	Phase of development	Comments
New IFN		
Consensus IFN, albuferon, omega IFN, PEG-IFNβ, R7025, Locteron	II-III	Albuferon: better dosing schedule (every 2-4 weeks) Omega IFN: continuous infusion
RIB analogues - IMPDH inhibitors		
Viramidine or taribavirin	III	Less frequently anemia but lower response rates than RBV*
VX-497 or Merimepodip	II	Preliminary data in combination with PEG-IFNα + RBV

Amantadine	IV	Combination with PEG-IFNα + RBV may improve SVR in NRs
Inhibition of RNA replication		
NS5B Polymerase inhibitors	Preclinical-III	
Nucleoside inhibitors (NM283, R1626, R7128, A837093)		NM283: stopped because of low efficacy and poor tolerability R1626: no evidence for resistance after 4 weeks
Non-nucleoside inhibitors (R803, HCV-371, HCV-086, HCV-796, Bilb1941, AG021541, PSI6130, CSK625433, GS9190, VCH-759, ANA598)		R803, HCV-371, HCV-086: stopped due to insufficient anti-viral activity, HCV-796: stopped due to elevations of liver enzymes
Prevention of functional replication complexes (ACH806)	I	ACH806: stopped due to renal dysfunction
Inhibition of protein translation	Preclinical-II	
Antisense oligonucleoside (ISIS 14803, AVI-4065)		ISIS 14803: stopped because of aminotransferase flares and poor anti-viral efficacy
RNA interference (SirnaAV34, BLT-HCV)		BLT-HCV: multi-targeting approach to prevent resistance
Ribozymes (Heptazyme)		Heptazyme: stopped because of toxicity in animal models
Post-translational modification		
NS3/4A serine proteinase inhibitors (BILN2061, VX950, SCH503034, ITMN191, TMC435350)	I-III	BILN2061: stopped due to cardiotoxicity Addition of VX-950 seems to improve the efficacy of PEG-IFNα or PEG-IFNα/RBV
NS5A (A831) and helicase inhibitors (QU663)	Preclinical-I	
Inhibitors of viral assembly/release (Celgosivir)	II	
Prevention of binding		
Polyclonal or monoclonal antibodies, GNS-037	Preclinical-II	Most studies in transplant CHC patients
Other immunomodulators		
Toll-like receptors agonists	I	Need further evaluation

(resiquimod, ANA245, ANA975, SM360320, CPG10101)		CPG10101 was suspended
Histamine dihydrochloride	II	Studies with PEG-IFNα/RBV were stopped
Thymalfasin	II-III	Stopped because of insufficient efficacy
Interleukin (IL-10, IL-12, IL-29)	II-III	IL-12: poor anti-viral efficacy and severe adverse events, PEG-IL-29: active in liver but not circulating white cells
Vaccines (recombinant E1, TG4040, IC-41, T cell, dendritic cell)	Preclinical-II	Induction of cytotoxic and T-helper cells response
Ciclosporin A analogues (DEBIO025, NIM811, SCY635)	I-II	DEBIO-025: 10-fold greater anti-HCV activity than cyclosporin
GI-5005		Monotherapy gave promising results. Trial of GI-5005 plus PEG-IFNα/RBV in progress
Others	Preclinical-II	
Agents implicated in lipid biosynthesis (NA255, Bezafibrate, statins)		Statins: inhibition of geranylgeranylation of host cell proteins
S-Adenosylmethionine, Betaine		*In vitro*: increase of IFNα anti-viral effect, reduce oxidative stress
Hepatoprotectans and other herbs (Silymarin, MK-001, Glycyrrhizin)	-	Controversial efficacy
Nitazoxanide	-	Nitazoxanide/PEG-IFNa-2a/RBV: higher end-of-therapy response rate compared to standard therapy
Antiapoptotic agents (IDN-6556, PF-03491390)	-	Pan-caspase inhibitors: promising results

IMPDH = inosine monophosphate dehydrogenase; NR = nonresponder; SVR = sustained virological response; CHC = chronic hepatitis C; RBV = ribavirin; IFN = interferon; RIB = ribavirin; HCV = hepatitis C virus.
* Perhaps higher responses at Viramidine doses >18 mg/kg daily.

Evolution of the Treatment Paradigm

To date, every STAT-C agent performs best when given in combination with *both* PEG and RBV, confirming that these agents will continue to be the backbone of HCV therapy for many years. A new dimension, drug resistance, is rapidly becoming part of the therapeutic dialog. Similar to the case for hepatitis B and HIV, concerns now center on not just resistance to a single agent, but also on whether these mutations will lead to cross-resistance, thus compromising several drugs and potentially drug classes. This is especially important for the first phases of STAT-C integration, which will likely involve the addition of a single agent to the standard of care. The studies discussed in the previous section suggest that mutated virus remains sensitive to interferon-based therapy, but only if a person is innately responsive to interferon. This "safety net" does not exist for interferon nonresponders, and resistant virus may persist for months despite treatment discontinuation. This is a concern for the roughly 20% of genotype 1 HCV-infected patients who are nonresponders to standard of care.

The anticipation of newer agents has created debate regarding which patients should be treated with current standard-of-care therapy and which should be "held" for upcoming compounds. Whereas most clinicians agree that patients with advanced HCV disease should be treated immediately, some advocate delaying treatment for patients with minimal histologic injury. Certainly, the rationale behind this is that HCV is, on average, slowly progressive, and this patient subset has time to wait for a more effective therapy. This approach must be carefully weighed against the disadvantages of delaying treatment. In addition to the underlying risk for disease progression, age is associated with other medical conditions, such as obesity, diabetes, heart disease, anemia, depression, alcohol abuse, and malignancies, all of which have a negative impact on treatment response or indeed on the ability to treat at all. The immune system becomes less responsive to immune manipulation, likely as a result of the blunted immune response that occurs with aging.[81] Patients younger than 40 years are approximately 2.5 times more likely to respond to interferon-based therapies as those over age 40. Not only does older age affect response to therapy, but it also results in an immune system less capable of controlling hepatitis C. This may explain in part why persons who acquire HCV infection at an older age statistically have more progressive disease, as well as why an increase in fibrosis rate occurs over the age of 50.

Certain subsets of patients have good baseline prognostic factors, with anticipated SVR rates greater than 80%.Some clinicians advocate treating only those patients who have good expected outcomes, yet it is important to remember that on-treatment response (RVR and EVR) is the strongest predictor of SVR, especially for a patient infected with HCV genotype 1. Conversely, others would advocate treating only those persons at very high risk for progression, assuming that this subset has the most to gain from therapy. Certainly, as more effective medications come to market, the pool of patients eligible for treatment should also increase, including those patients with minimal fibrosis. It is this group that has the highest potential for achieving SVR, and thus, from an efficacy standpoint, may be the most appealing population to whom therapy could be offered. The goal of therapy will change as the treatment paradigm evolves, namely transitioning from

treating only those patients with significant liver disease to also treating those with viremia who have not yet developed liver disease or comorbidities that negatively affect treatment success

Liver Transplantation

Liver transplantation is the best option for an individual with hepatic decompensation due to end-stage liver disease or early HCC. Currently there are 17,227 people actively waiting for approximately 7000 expected deceased liver donations per year.[42] Hepatitis C is already the most common indication for liver transplantation. If projections are correct, the number of new patients with cirrhosis may double by the year 2020. Obviously, this increased burden will be impossible to meet given an already limited supply of organs. In addition, hepatitis C universally recurs post transplant for HCV infection, with reinfection at the time of hepatic reperfusion. By the fourth day post transplant, the viral load has reached pretransplant levels, peaking 1-3 months after surgery at levels that are frequently 10-100 times greater than the original baseline.

Although not predictable, post transplant hepatitis C is generally more cytotoxic, with the median interval from hepatic replacement to development of cirrhosis of only 10 years. Thirty percent of patients will develop cirrhosis 5 years after transplantation, and the time to manifesting symptoms of decompensation and death is significantly shorter than in immunocompetent hosts. Up to one fourth of patients transplanted with hepatitis C will die or require retransplantation within 5 years. Ideally, HCV should be eradicated prior to transplantation. However, very few patients are able to tolerate the therapy without dose reductions or interruptions, and therefore few clear virus or achieve SVR. By carefully selecting a subset of pretransplant patients with stable cirrhosis and initiating low-dose therapy with subsequent dose escalation, investigators from the University of Colorado were able to achieve SVR in only 22% of a heterogeneous group of treated individuals. HCV genotype 2/3 and the ability to tolerate full-dose therapy were associated with a more favorable response. These patients also remained virus-free after transplant.

Statistically, the majority of individuals with HCV infection awaiting orthotopic liver transplantation will not tolerate therapy with the current standard of care or achieve SVR if treated. As expected, HCV treatment after liver replacement is just as challenging as pretransplant treatment. Several strategies exist for management of HCV post transplantation. Some advocate for preemptive HCV therapy as soon as the patient is clinically able to tolerate treatment. Ideally, this would eliminate disease while the viral load is still low and prior to histologic damage. Unfortunately, therapy immediately post liver transplant is poorly tolerated, with only 10% to 25% achieving SVR. Few would debate that those patients with recurrent disease and evidence of at least stage 2 fibrosis should be considered for combination PEG-RBV therapy. This approach post transplant is particularly challenging because it requires serial protocol liver biopsies to identify patients with progressive fibrosis, and results remain suboptimal, with SVR rates ranging between 9% and 45% in case series. Although therapy was tolerated poorly, these investigations failed to demonstrate any increased risk for cellular rejection, which is a theoretical concern with immune-modulating interferon-based therapy.

HCV treatment in patients actively engaging in alcohol and injection drug use

In 1998, Wiley et al reported that because of the strong association between alcohol use and rapid liver fibrosis, hepatoma, and deleterious effects on treatment response, complete alcohol abstinence is recommended during treatment.

As reported by Strader et al in 2004, the practice guidelines of the American Association for the Study of Liver Diseases recommend that HCV treatment should not be withheld from persons who use illicit drugs or are on a methadone maintenance program, provided they are willing to maintain close monitoring, including practicing contraception. The complexity of HCV treatment in these patients is aided by a multidisciplinary team approach composed of physicians and drug abuse and psychiatric counselors.

Effect of IFN on HCC recurrence

In 2002, Hayashi and Kasahara noted that exposure to IFN, irrespective of HCV eradication status, was associated with a reduced incidence of HCC. An important randomized study by Kubo et al, first conducted in 1996 with follow-up in 2002, also demonstrated that the administration of IFN to patients undergoing liver resection for HCC was associated with reduced tumor recurrence and improved survival. Although IFN may have a role in reducing the incidence of HCC, which subgroup of HCV patients are most likely to benefit remains unclear?

Prevention

Because no effective vaccine for hepatitis C exists, the only way to protect you is to avoid becoming infected. That means taking the following precautions:

- Avoid unprotected sex with multiple partners or with one partner whose health status is uncertain.

- Don't share needles or other drug paraphernalia. Contaminated drug paraphernalia is responsible for about half of all new hepatitis C cases.

- Avoid nasal use of cocaine.

- Avoid body piercing and tattooing unless you're absolutely certain the equipment is sterile.

Self-care

If you receive a diagnosis of hepatitis C, your doctor will likely recommend certain lifestyle changes. These simple measures will help keep you healthy longer and protect the health of others as well:

Eliminate alcohol consumption. Alcohol speeds the progression of liver disease.

Avoid medications that may cause liver damage. Your doctor can advise you about these medications, which may include over-the-counter (OTC) medications such as acetaminophen (Tylenol, others) as well as prescription drugs.

Maintain a healthy lifestyle. Be sure you exercise regularly, get plenty of rest and eat a healthy diet that emphasizes fresh fruits, vegetables and whole grains.

Help prevent others from coming in contact with your blood. Cover any wounds you may have and don't share razors or toothbrushes. Don't donate blood, body organs or semen, and advise health care workers that you have the virus.

Complications

- Chronic infection develops in 70-80% of patients infected with HCV.

- Cirrhosis develops within 20 years of disease onset in 20% of those with chronic infection.

- HCC develops in 1-4% of patients with cirrhosis each year. HCC may develop at an average of 30 years after the onset of infection and is more common in the presence of cirrhosis, alcoholism, and HBV co-infection.

- HCV is associated with many extrahepatic manifestations. Among the most common are the following:

  - Cryoglobulinemia: In 2001, Della Rossa et al reported that cryoglobulins are found in as many as half the persons with HCV infection. HCV is the primary cause of essential mixed cryoglobulinemia (ie, type 2 cryoglobulinemia); as many as 90% of affected persons have HCV viremia. Cryoprecipitates usually contain large amounts of HCV antigens and antibodies. Approximately 10-15% of affected patients have symptoms such as weakness, arthralgias, and purpura; these are often related to vasculitis.

  - Membranoproliferative glomerulonephritis

  - Idiopathic thrombocytopenic purpura
  - Lichen planus
  - Keratoconjunctivitis sicca
  - Raynaud syndrome
  - Sjögren syndrome
  - Porphyria cutanea tarda

  - Necrotizing cutaneous vasculitis

  - Non-Hodgkin lymphoma

- The precise pathogenesis of these extrahepatic complications has not been determined, although most are the clinical expression of autoimmune phenomena.

Prognosis

- Infection with HCV is self-limited in only a small minority of infected persons. Chronic infection develops in 70-80% of patients infected with HCV.

- Cirrhosis develops within 20 years of disease onset in 20% of persons with chronic infection.

- HCC develops in 1-4% of patients with cirrhosis each year after an average of 30 years. HCC is more common in the presence of cirrhosis, alcoholism, and HBV co-infection.

- With the currently recommended therapy for chronic hepatitis C, which includes PEG-IFN and ribavirin, cure rates are as high as 60% [genotype 1] and 80% [non-genotype 1].

HCV and HIV Co-infection

Introduction

It is estimated that 170 million people are infected with HCV worldwide, 10 million of who are co-infected with HIV. In the HIV-infected population of the developed world, liver disease is the primary cause of non-AIDS-related deaths and is almost always due to chronic HCV infection. Prevalence of anti-HCV antibodies varies widely depending upon the transmission route, the country and the population under study. Between 7 and 85% of HIV-positive individuals are co-infected with HCV (approximately 25–30% in the USA). The natural history of HCV infection in the HIV co-infected individual is characterized by an accelerated rate of liver fibrosis, cirrhosis and end-stage liver disease (ESLD) compared with HCV mono-infection. Co-infected patients have a lower chance of survival than HCV mono-infected individuals once they present with decompensated liver disease. Hepatocellular carcinoma is also more likely to develop and after a shorter interval of time from acquisition of HCV infection. Antiretroviral therapy (ART) has a favorable overall risk-to-benefit ratio and can decrease liver-related mortality. However, it can cause short and long-term liver injury, and chronic hepatitis C is a risk factor for ART-related hepatotoxicity. The current treatment for HCV was approved in 2001 for mono-infected patients. Subsequently, in 2004, the US FDA approved pegylated interferon (PEG-IFN)-α-2a for HCV treatment of HIV-infected patients in the USA. PegIFN-α-2b was approved for co-infection in Europe in 2007. Since then, the importance of hepatitis C in co-infected patients has been increasingly emphasized, and healthcare providers are confronted with the challenge of HCV treatment in HIV-positive patients.

Basis of Treatment of HCV in HIV-co-infected Individuals

The standard of care for the treatment of co-infected patients is PEG-IFN and ribavirin (RBV; weight-based: 1000 mg/day for persons < 75 kg and 1200 mg/day for persons ≥75 kg in genotype-1 patients, and 800 mg fixed-dose in genotypes 2 or 3) for 48 weeks, independent of genotype. This is based on five major studies, of which four are randomized, controlled trials. AIDS Pegasys Ribavirin International Co-infection Trial (APRICOT) enrolled 868 patients. Treatment-naive, co-infected patients of all genotypes were randomized in one of the three treatment arms to receive either pegIFN-α-2a 180 μg plus RBV 800 mg, pegIFN-α-2a 180 μg plus placebo or standard interferon (3 million units [MU] three times a week) plus RBV 800 mg for 48 weeks. The PEG-IFN and RBV combination demonstrated superiority, with an overall sustained virological response (SVR) of 40% compared with 20% for PEG-IFN monotherapy and 12% in the standard interferon arm. Higher baseline HCV RNA levels (> 800,000 IU/ml) were associated with lower rates of SVR. They also found that the high negative-predictive value of the absence of an SVR to predict virological failure applied to co-infected patients. During HCV treatment, CD4 cell count decreased but the mean percentage of CD4$^+$ lymphocytes increased. Safety issues were mostly hematologic and comparable to HCV mono-infected patients. RIBAVIC compared standard interferon (3 MU three times a week) to pegIFN-α-2b (1.5 μg/kg/week). A total of 412 patients were enrolled. The overall SVR rate was 27% in the PEG-IFN group. A total of 40% of the patients had bridging fibrosis or cirrhosis, a negative prognostic factor for SVR. However, SVR rate in the control arm was 20%, higher than the 12% seen in the APRICOT trial. Many patients' experienced adverse events not related to treatment and discontinued therapy. Serious adverse events, although similar in both groups, were higher than reported in the mono-infected population. A total of 11 cases of pancreatitis hyperlactatemia were diagnosed in patients taking didanosine (ddI) as part of their ART regimen. AIDS Clinical Trials Group (ACTG)-5071 randomized 133 subjects to receive either pegIFN-α-2a (180 μg/week) or standard IFN-α-2a plus RBV in a dose–escalation schedule: 600 mg for 4 weeks, then 800 mg for 4 weeks, then 1000 mg for a total of 48 weeks. PEG-IFN demonstrated superiority over standard interferon with a reported SVR rate of 27% compared with 12%. A liver biopsy was performed at week 24 of treatment in patients with no virological response and 35% of them had histologic improvement, suggesting that benefits from the therapy extend beyond virological control. In this study, the inadequate schedule of RBV may have explained the low SVR (high relapse) rates observed. Laguno and coworkers randomized patients to receive either standard interferon (3 MU three times a week) or pegIFN-α-2b (100–150 μg/week), with weight-based RBV 800–1200 mg for 48 weeks. Genotype-2 and -3 infections with HCV RNA levels of less than 800,000 IU/ml were treated for 24 weeks only. A total of 95 patients, of whom 30% had bridging fibrosis or cirrhosis, were randomized. Overall, 44% achieved SVR. This was the first randomized trial to use weight-based RBV and it yielded an increase in SVR. Patients with genotype-2 and -3 infection experienced a high relapse rate after 24 weeks of treatment. Side effects were frequently reported, including depressive symptoms in 43% of patients. They diagnosed nine cases of mitochondrial toxicity in patients receiving ddI and/or stavudine (d4T). The most recent trial is Peginterferon Ribavirin España Co-infection (PRESCO). It sought to determine the role of weight-based RBV

and extended duration of therapy in co-infected patients. A total of 389 patients received pegIFN-α-2a (180 μg/week) plus RBV (1000 mg or 1200 mg, according to weight). Genotype-1 participants were treated for 48 versus 72 weeks and genotype-2 and -3 patients were treated for 24 versus 48 weeks. They achieved an overall SVR rate of 49.6%. In genotype-1 subjects, SVR rates were significantly greater in the 72-week arm, but with a withdrawal rate of 36 out of 45 (80%). Higher dosage of RBV was well-tolerated.

Every patient for whom the benefits of treatment of liver disease outweigh the risks of toxicity should be offered treatment. Response should be assessed at week 4. Undetectable HCV RNA at week 4 is called rapid virological response (RVR). Week 12 response is either partial–early virological response (pEVR; decrease of at least 2 logs from baseline viral load with still-detectable HCV RNA) or complete–early virological response (cEVR; undetectable HCV RNA). These measures correlate with SVR in mono- as well as co-infected patients. In the absence of EVR, treatment should be discontinued, since SVR can be achieved in only 2% of those patients. HCV RNA undetectability at 24 weeks is necessary to achieve SVR.

Sustained Virological Response Rates are Lower in Co-infected Patients

The goal of HCV treatment is to achieve and maintain undetectability of plasma HCV RNA 24 weeks after the end of treatment – a SVR. The expected SVR rates in co-infected patients are 14–38% with genotype-1 infection and 44–73% with genotypes -2 or -3. These numbers probably overestimate the actual proportion of patients who achieve SVR in reality. The two major predictors of SVR to treatment of HCV are genotype (-2 and -3) and a low baseline serum viral load (HCV RNA). In co-infected individuals, baseline serum and hepatic HCV RNA levels are generally higher. Other factors that may affect SVR rates negatively, but to a lesser extent, are frequently found in HIV-co-infected patients and may be modifiable. It is also known that there is a lower rate of HCV treatment initiation in co-infected patients. Possible explanations are the providers' perception that SVR will not be achieved, the lack of trust in patient adherence, the multiple comorbidities and the reluctance to treat owing to potential adverse events with ART and drug interactions. Better knowledge of the distinguishing features of co-infection and its treatment, together with belief in patients motivation, are the first steps toward treatment success. The most likely to respond to treatment are those with genotypes -2 or -3 and those with low baseline viral load (< 800,000 IU/ml in APRICOT and Laguno and coworkers, and < 500,000 IU/ml in PRESCO).

The Effect of HAART in Liver Disease

Beneficial Effects

Multiple studies demonstrated an accelerated progression of hepatic fibrosis in co-infected individuals. There is now evidence that ART, and particularly HAART, slows the rate of liver fibrosis. In one study, co-infected patients on HAART with undetectable HIV RNA (< 400 copies/ml) had a slower rate of fibrosis than those with viremia but

similar to HCV mono-infected individuals. There is also data demonstrating that co-infected patients on HAART have a lower probability of developing cirrhosis. With the hindsight of more than 20 years of ART, data demonstrate an improved overall survival and a decreased mortality from HCV-associated liver disease in co-infected patients treated with HAART. In this cohort of 285 co-infected subjects, predictors of increased liver-related mortality were the absence of HAART, a low CD4 cell count and older age. In the Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) study, the elevated risk of liver-related deaths was strongly associated with advanced immunodeficiency. Those studies emphasize the role of ART in improving liver outcome by controlling HIV. Data also suggest that this population would benefit from earlier treatment of HIV (at a higher CD4 cell count) based on the beneficial effects on the liver disease.

Hepatotoxicity of Antiretroviral Therapy/drug-induced Liver Injury

The dark side of HIV treatment is hepatotoxicity, which is more frequent among patients with HCV. It ranges from mild, asymptomatic transaminases elevations to rare fulminant hepatic failure. Liver enzyme elevations are more frequent in co-infected patients receiving HAART compared with HIV mono-infected patients. One study reported that the occurrence of fulminant hepatic failure was increased by a factor of five in co-infected patients on HAART. In the D:A:D study, ART was directly responsible for 2.7% of all liver-related deaths. All classes of antiretrovirals have caused hepatotoxicity, but the non-nucleoside reverse transcriptase inhibitors class, and mostly nevirapine, has been associated with liver damage more frequently. In HIV-seropositive patients taking nevirapine, co-infection with HCV, female gender and increased CD4 cell count have been found to increase the risk of hepatotoxicity by a hypersensitivity reaction. This has led to the recommendation of avoiding initiation of nevirapine in women with a CD4 cell count over 250 cells/mm^3 and in men with a CD4 cell count over 400 cells/mm^3. There are four main mechanisms by which ART can induce hepatotoxicity: mitochondrial toxicity, hypersensitivity reactions, direct liver injury and immune reconstitution in the presence of chronic hepatitis C. Steatosis can also be considered a form of hepatotoxicity. Sulkowski and coworkers found steatosis in 40% of co-infected individuals with extensive ART exposure. The strongest association was with d4T. The most recently recognized manifestation of ART-induced hepatotoxicity is noncirrhotic portal hypertension. This condition is thought to be due to cumulative exposure to the nucleoside reverse transcriptase inhibitor (NRTI) ddI. One possible explanation is a direct endothelial cell injury in the portal system, with resultant portal hypertension without association with cirrhosis.

HAART has truly revolutionized the treatment of HIV. Every HIV-positive individual should be offered treatment when indicated and the presence of chronic hepatitis C should not prohibit treatment. Control of HIV is recommended before starting HCV treatment. Many antiretroviral agents with favorable liver safety profiles are currently available. However, owing to the risk of increased toxicity in HCV infection, when HIV treatment can be safely deferred, it may be strategic to treat HCV first to limit liver injury and toxicity. One study did report that successful treatment of hepatitis C reduces the risk of ART toxicity. When HIV therapy is indicated, the challenges are to judiciously choose

the HAART regimen and provide a close and regular follow-up of clinical and biochemical parameters.

HCV Treatment: Toxicity & Drug Interactions With Antiretroviral Therapy

Mitochondrial Toxicity

The signature toxicity of NRTIs is mitochodrial toxicity. These compounds interfere with mitochondrial DNA synthesis by binding to the mitochondrial γ-DNA polymerase. Depending on the binding affinity of a drug, this results in a loss and dysfunction of mitochondrial DNA. Depletion of cellular mitochondrial DNA presents with malaise, nausea and vomiting, peripheral neuropathy or myopathy, and has caused fatal cases of hepatic steatosis, pancreatitis, lactic acidosis and SVR. *In vitro*, zalcitabine has the highest toxicity followed by ddI, d4T, lamivudine, zidovudine, abacavir (ABC) and tenofovir. Simultaneous use of RBV, a guanosine nucleoside analog, and some HIV nucleoside analogs, mostly ddI and d4T, have been linked to an increased incidence of mitochondrial toxicity and lactic acidosis. In co-infected patients with cirrhosis, the use of ddI is also a risk factor for hepatic decompensation during treatment with PEG-IFN and RBV. RBV interacts with ddI and leads to the accumulation of intracellular dideoxyadenosine triphosphate (ddA-TP). This consequence has led to the current recommendation to avoid use of RBV with ddI and/or d4T.

Anemia & Other Cytopenias

HIV can affect the three hematologic cell lines resulting in anemia, leukopenia and thrombocytopenia. It is expected that HIV–HCV co-infected patients present with a lower baseline level of blood cells. Zidovudine is a well-known bone marrow suppressor and a frequent cause of anemia and neutropenia in HIV-mono-infected individuals. As for HCV treatment regimen, interferon also suppresses bone marrow, whereas RBV causes dose-dependent hemolytic anemia. Peripheral destruction of red blood cells in combination with a poor bone marrow reserve has led to cases of severe anemia and neutropenia in co-infected patients on a zidovudine-containing regimen treated with PEG-IFN and RBV. The use of zidovudine during HCV treatment should therefore be avoided. In three major treatment studies of HIV–HCV co-infection, hematologic events were the most frequent cause of treatment discontinuation in 17, 12 and 8.5% of patients. In a recent retrospective study of 237 co-infected patients treated with PEG-IFN and RBV, 34% presented with severe hematologic toxicity. Among multiple risk factors of hematologic toxicity, the use of zidovudine had an adjusted odds ratio of 3.3. A low baseline level of hemoglobin, white blood cells and platelets also predicted significant decrease during treatment. Multiple studies have emphasized the role of optimal exposure to RBV to maximize SVR and reduce relapse rate in HCV mono-infection. Efforts should be made to use growth factors instead of reducing RBV dosage. A study published in 2005 evaluated the effectiveness of epoietin-α (EPO) once weekly to correct anemia and avoid RBV dose reductions in HIV–HCV co-infected patients treated with PEG-IFN and RBV. A total of 66 anemic patients were enrolled, 34 of whom received EPO. Anemia was corrected in all patients receiving EPO, and it resulted in significantly more patients

allowed to continue on optimal dosage of RBV. Neutropenia is a frequent event during combination therapy in co-infected individuals, and severe neutropenia (absolute neutrophil count < 500 cells/mm^3) has usually not been associated with infection.[58] Filgrastim can be used to increase neutrophil count but is not FDA-approved for treatment of neutropenia owing to hepatitis C treatment. There is currently no approved growth factor to increase platelet count, although the thrombopoietin receptor agonist eltrombopag has completed Phase II clinical trial and demonstrates promise.

Drug–drug Interactions

In vitro studies have suggested that RBV could reduce the activity of the HIV–nucleoside analogs by interfering at the level of intracellular phosphorylation. This has not been confirmed in a pharmacokinetic study. Recently, studies of co-infected patients have reported a better response to PEG-IFN plus RBV in those with Tenofovir-based regimens compared with ABC. SVR rates of 45 versus 29% have been reported in one study. Ribavirin and ABC are both guanosine analogs and an intracellular inhibitory competition between the two might be the explanation. In the study by Vispo and coworkers, ABC was only an independent predictor of lack of SVR in patients with less RBV exposure (plasma through < 2.3 µg/ml). One study did not support these findings.

Optimal Duration & Dosage of Anti-HCV Therapy

Duration of Therapy

Duration of therapy in co-infected patients is still an area of uncertainty. The current recommendation is to treat all genotypes for 48 weeks. This is based on clinical trials in this population that have been designed to evaluate the SVR after 48 weeks of treatment, independent of the genotype, for the majority. The study by Laguno and coworkers offered a 24 week-treatment with weight-based RBV for genotype-2 and -3 co-infected patients. They obtained end-of-treatment response (EOTR; HCV undetectable at end of treatment) in 68% but the relapse rate was high, with only 53% achieving SVR compared with 41% EOTR and 38% SVR in genotype-1-infected patients treated for 48 weeks. The kinetics of the response to HCV treatment in HIV-infected individuals is slower. In mono-infected patients, slow responders are usually defined as those who achieve at least a 2-log decrease at 12 weeks of therapy, still with detectable viremia and who have undetectable viremia at 24 weeks. One study evaluated extension of therapy to 72 weeks for those mono-infected patients with genotype-1 infection. After a 48-week treatment, slow responders were randomized either to stop therapy or continue for a total of 72 weeks. SVR rates were of 38% in the 72-week group versus 18% in the 48-week treatment arm (p = 0.026). One group investigated prolonged therapy in co-infected patients of all genotypes with no EVR and found no benefit. However 68% of patients randomized to the extension arm dropped out. In the PRESCO trial, treatment extension also seemed to offer benefit in a subset of patients but the high rate of treatment discontinuation in the genotype-1 and -4 groups precluded the authors from making this conclusion. The current trend in HCV treatment is to individualize treatment based on early viral kinetics. This approach certainly offers each individual the best chance of

treatment success. A substudy from the RIBAVIC trial reported no chance of SVR when the viral load could not be suppressed below 460,000 IU/ml at week 4 (negative predictive value of 100%). A substudy from APRICOT demonstrated that RVR is the best predictor for SVR, while the absence of an EVR is the best predictor of the lack of SVR. This is with a 48-week treatment. More recently, a retrospective review of two prospective single center studies in HIV–HCV co-infected patients was published. It suggested that when RVR is achieved in those with genotype-2 or -3, HIV–HCV co-infected individuals could be successfully treated with a 24-week treatment with optimal dose of RBV and peg-IFN (positive predictive value: 100%; 39 patients). Further research will help determine the best treatment duration and whether shorter treatment in rapid responders or extended treatment in slow responders can be beneficial in this population.

Ribavirin Dosage

Another area of uncertainty in co-infected patients is the optimal RBV dosage. In HCV mono-infection, one randomized trial compared a fixed 800-mg dose of RBV to weight-based dosing (1000 mg in those < 75 kg and 1200 mg in those ≥75 kg) in all genotypes and concluded that weight-based RBV leads to higher SVR rates in those with genotype-1. The same observation was not found in genotype-2 and -3-infected patients. This study formed the basis of the current recommendation to treat HCV mono-infection with weight-based RBV in genotype-1 and use the fixed 800-mg dose for treatment of genotypes-2 and -3. A comparable study has been performed in the co-infection setting and is presently being analyzed (The Pegasys and Ribavirin for AIDS: Dose Study in Genotype 1 Management [PARADIGM] Study). The PRESCO trial was a nonrandomized, prospective, open, comparative trial of weight-based RBV (1000 mg for those weighing < 75 kg and 1200 mg for those ≥75 kg, regardless of genotype) and extended duration of treatment. The use of higher RBV dosage led to an overall SVR rate of 49.6%, the highest reported in this population. For comparison, SVR rates were 40% in APRICOT and 27% in RIBAVIC; two studies that used the fixed 800-mg dose of RBV. Stratified by genotype, it gives a SVR of 35% for genotype-1 treatment (29% in APRICOT and 17% in RIBAVIC) and 72% for genotypes-2 and -3 (62% in APRICOT and 44% in RIBAVIC). Weight-based RBV-use did not increase the incidence of adverse events and led to better SVR rates for all genotypes. We use weight-based RBV, independent of genotype, for the treatment of HCV in HIV-infected individuals.

Treatment of Nonresponders & Relapsers

Nonresponders are defined as those who were adherent to an optimal treatment with adequate dose of PEG-IFN and weight-based RBV for the appropriate duration and either did not achieve the 2-log reduction in viremia at week 12 or undetectability at week 24. Relapsers are those who completed therapy with EOTR and subsequently experienced a rebound in viremia without re-exposure to HCV. The first step in evaluating a previous nonresponder or relapser is to ask weather the patient was treated according to the current standard treatment. Was the patient adherent to treatment? Were the PEG-IFN and RBV dosage optimal when ordered? Did the patient need dose reduction owing to side effects?

For how long was he treated and was that appropriate according to the viral kinetics? If the previous failure to respond to treatment can be explained by suboptimal treatment schedule, it is advisable to try retreatment with the adequate dosage of both PEG-IFN and RBV and for the appropriate duration. A second reason to explain previous failure can be that treatment was limited due to poor adherence or side effects. In the case of hematologic abnormalities, the use of growth factors such as EPO and granulocyte colony-stimulating factor can allow the completion of treatment. Regarding psychiatric issues, prophylactic use of antidepressants can be very useful. In fact, the knowledge of previous obstacles to treatment success in a particular patient can be seen as an advantage for the second attempt, since anticipation can allow for prevention of side effects and more rapid intervention when needed. For those patients with prior virological failure with optimal treatment, it can be useful to look at other factors that could have been responsible for prior treatment failure. Was the HIV infection optimally controlled? Insulin resistance is a negative prognostic factor for RVR and EVR in co-infected patients. It is presently unknown if improvement in insulin sensitivity will lead to better SVR rates. One study evaluated the retreatment of 101 nonresponders and 53 relapsers to standard interferon and RBV. They reported SVR rates of 58.5% for relapsers and 13% for nonresponders (p < 0.001). Favorable predictors of SVR by multivariate analysis were prior relapse compared with nonresponse, mild-to-moderate fibrosis, genotypes other than one and baseline viral load less than 2 million copies/ml. In those patients, the lack of RVR and EVR had high negative-predictive values of SVR – 94 and 97%, respectively. In prior nonresponders with advanced liver fibrosis, another option is to treat with the goal of improving histology, despite the absence of virologic response. The last option is to wait for new agents to be approved, such as the protease inhibitors (PIs) that are currently in Phase III clinical trials for the treatment of HCV.

Acute HCV

A growing number of HCV infections are observed in HIV-positive men who have sex with men (MSM). In several outbreak reports, acquisition of acute HCV in MSM was associated with high-risk sexual behavior, ulcerative sexually transmitted infections and use of recreational, nonintravenous drugs such as methamphetamines. HIV-positive patients have a lower likelihood of spontaneous clearance of HCV than seronegative patients. Treatment of acute HCV infection in HIV-positive patients leads to higher SVR rates compared with the chronic stage. This argues in favor of early treatment initiation in co-infected individuals. A 12-week period after exposure is traditionally offered to give the opportunity for spontaneous clearance to occur. Treatment should then be instituted if viremia is persistent, since the chance of response may decrease if treatment is further delayed. Otherwise, we recommend to monitor HCV RNA every 2 weeks and to institute treatment if viremia establishes a plateau or steadily increases. Treatment recommendation is PEG-IFN plus weight-based RBV for 24 weeks, independent of genotype. This is based on experience with acute mono-infection, case reports and expert opinion. Factors associated with better rates of SVR in the acute infection are genotypes-2 and -3, RVR and elevated alanine aminotransferase. CD4 cell count, HCV and HIV viremia, and age are not associated with SVR. SVR rates of 60–90% have been reported.

End-stage Liver Disease

HIV infection accelerates the progression of liver fibrosis in co-infected individuals. The co-infected experience a more rapid evolution toward cirrhosis and ESLD. Once HIV-co-infected patients present with decompensated liver diseases, the median survival was 16 months compared with 48 months in mono-infected subjects. Several studies found a positive impact of HAART on liver-related mortality in co-infected patients with ESLD. PEG-IFN and weight-based RBV can be used in cirrhotic patients with Child-Pugh stage-A. Cirrhosis is a negative predictive factor of SVR, but treatment can significantly benefit the patient at that stage and can serve as a bridge whilst awaiting liver transplantation (LT). In Child-Pugh stages-B and -C cirrhotics, PEG-IFN treatment can be deleterious and life-threatening and LT remains the only option.

A recent retrospective cohort study was performed to ascertain the impact of HIV on survival after LT in the HAART era. Data were extracted from the United Network for Organ Sharing database. A total of 138 HIV-positive individuals were included and compared with 30,520 HIV-negative controls. In HIV-positive patients, the estimated 2-year survival probability was 70% compared with 81% for controls. The excess risk was seen in co-infected patients (hepatitis B and C). The HCV–HIV co-infected group had a significantly poorer outcome and lower survival rate than HIV-negative controls. In a second study, survival after LT in co-infected patients was compared with HCV mono-infected patients, and led to similar conclusions. Of the 79 HIV-positive patients, 35 were receiving HAART. They differed from the control arm by being younger and having a higher Model for ESLD (MELD) score. The survival rates at 2 and 5 years in co-infected subjects were 73 and 51% respectively, compared with 91 and 81% patients, respectively, in HCV-monoinfected subjects. The MELD score was the only predictor of poor survival, meaning that they had more severe disease at the time of LT. After LT, co-infected patients experience an almost universal recurrence of HCV in the graft, with an increased incidence of rapid fibrosis progression, fibrosing cholestatic hepatitis and early cirrhosis. Management becomes more complex considering interactions between HCV and HIV drugs and immunosuppressive agents in the setting of a vulnerable liver and frequently altered renal function.

Conclusion

HCV–HIV co-infection is a major public health issue. Studies have made it clear that the two simultaneous infections result in a rapid progression of liver disease. Every HIV–HCV co-infected patient should be evaluated to determine if treatment is indicated. With PEG-IFN and weight-based RBV, nearly one half of patients can be expected to clear the infection. Anticipation of possible side effects can prompt prophylactic or rapid interventions, which will increase treatment adherence and maintain adequate doses of medication. Delaying intervention allows for the progression of fibrosis, increases the chance of toxicity owing to ART and reduces the likelihood of future treatment response. New HCV drugs with different targets are under development. This could have a real impact on liver-related mortality in the future of HIV infection.

Future Perspective

The burden of HCV co-infection in HIV-positive patients is likely to increase with the constant advances in the field of ART and the resulting aging of the HIV population. We will see an increasing number of older patients with long-standing HIV infection and past exposure to ART with less favorable toxicity profiles (zalcitabine, ddI and d4T). The era of true antiviral HCV treatment is just beginning. Presently, over 50 compounds are under development. Two PIs, telaprevir and boceprevir, are in Phase III trials as we go to press and FDA approval is expected in the next few years. These drugs are used with PEG-IFN and RBV, and greatly improve HCV SVR rates. Drug development in HCV will have many parallels to HIV drug development. After telaprevir and boceprevir, other PIs and polymerase inhibitors with different resistance patterns are in the pipeline. This should allow the addition of PIs and polymerase inhibitors to PEG-IFN and RBV sometime in the next few years. PEG-IFN and RBV will be the cornerstone of HCV treatment for the next decade. At least one of the new PIs will be studied in HIV patients before FDA approval. Drug–drug interaction studies with ART are obviously a prerequisite. The FDA has suggested that all the new HCV compounds be studied in underserved populations in Phase III and not in Phase IV studies. That gives us hope that HCV antivirals will be studied more frequently and earlier in HIV patients in the future.

Summary

Therapy of HCV in HIV-infected individuals

- The standard of care is PEG-IFN and weight-based ribavirin (RBV) for 48 weeks in all genotypes.

- Treatment should be offered when benefits of treatment outweigh risks of toxicity.

- Sustained virological response (SVR) rates are lower than in the mono-infected patient.

The effect of HAART on liver disease

- HAART slows the rate of liver fibrosis and decreases mortality from HCV-related liver disease in co-infected patients.

- Co-infection is a risk factor for hepatotoxicity to HAART.

- Overall, HAART has a favorable profile.

Drug toxicities

- The use of didanosine is contraindicated during HCV treatment owing to increased mitochondrial toxicity with RBV.

- Anemia is the more frequent hematological side effect during HCV treatment and simultaneous use of zidovudine is discouraged. Epoietin-α can correct anemia.

Optimal duration & dosage of anti-HCV therapy

- The current recommendation is to treat for 48 weeks; however, individualized treatment extension of treatment could be beneficial in slow responders.

- RBV should be weight-based and optimal dose maintained throughout treatment.

Treatment of nonresponders & relapsers

- SVR rates of 13 and 58.5% have been reported in a retreatment trial in nonresponders and relapsers, respectively.

Acute HCV

- Early treatment with PEG-IFN plus RBV for 24 weeks leads to high SVR rates.

End-stage liver disease

- Co-infected patients have a worst outcome compared with HCV mono-infected after liver transplantation.