Mitchell L. Shiffman
Editor

Chronic Hepatitis C Virus

Advances in Treatment, Promise for the Future

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Editor
Mitchell L. Shiffman, MD
Liver Institute of Virginia
Bon Secours Health System
Richmond and Newport News, VA, USA
mitchell_shiffman@bshsi.org

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This book is dedicated to the numerous patients with chronic HCV whom I have cared for over the past 20 years; for all they have taught me about this disease and its management; and for contributing to numerous clinical trials which have improved the treatment of chronic HCV.

Preface

I first began caring for patients with chronic hepatitis C virus (HCV) and participating in clinical trials to treat this virus in 1990. I distinctly remember enrolling my first patients with non-A, non-B hepatitis into a clinical trial in which they received either standard interferon alfa-2α 1, 3, or 5 million IU or a placebo injection three times weekly. Standard interferon had not yet been approved by the Food and Drug Administration for treatment of non-A, non-B hepatitis (HCV). The HCV had just been identified when this trial was initiated and no tools were yet available to measure HCV RNA. The entry criteria were based on having a serum level of alanine aminotransferase (ALT) at least 1.5 times the upper limit of normal, and the end point was a biochemical response defined as normalization of serum ALT. We were ecstatic when we realized that 12% of patients who received active drug had achieved a sustained virologic response.

Over the next 2 decades, my staff and I participated in numerous clinical trials designed to either slow fibrosis progression and/or improve sustained virologic response (SVR) and "cure HCV."

During this time, naming studies became fashionable and having a good name became almost as important as having a good study design. Some of the more memorable studies my team and I participated in, either because of their clinical significance or simply because the study had a good name (listed in alphabetical order), included the following: 007, 107, ACCELERATE, ACHIEVE, ELEVATE, ENHANCE, HALT-C, IDEAL, NCORE, PROVE, SPRINT, STEALTH-C3, REALIZE, and RESPOND. Although many of the studies we participated in were unsuccessful, each taught us a bit more about this virus, its natural history, and how to treat it more successfully. Over the years, a slow stepwise increase in SVR rates did occur. However, these steps paled in comparison to the magnificent success we have finally realized with the development of HCV direct-acting antiviral agents. As this book goes to press, the first two HCV protease inhibitors, boceprevir and telaprevir, have been approved by the Food and Drug Administration. When either of these protease inhibitors is utilized along with peginterferon and ribavirin to treat chronic HCV, an SVR can be achieved in up to 80% of patients. Our ability to "cure" HCV has increased sevenfold in just 20 years.

I am truly grateful to the thousands of patients who have entrusted me with their care and for many, their willingness to enroll into one or more of the hundreds of clinical trials I have directed during this time. Our mutual goal to

develop treatments which will "cure" HCV in all persons infected with this virus is on the brink of becoming reality.

This book chronicles the advances we have made in our understanding of chronic HCV and the various methods we have utilized to try and eradicate this virus. The title was selected because these "Advances in Treatment" will soon enable us to make good on our "Promise for the Future" and "cure" HCV in the vast majority of our patients.

I am extremely grateful to my friends and colleagues who agreed to contribute to this project. The hard work and thought that these outstanding clinicians and scientists have put into their contributions is clearly evident. The book is composed of 25 chapters divided into four parts.

Part 1 The Natural History of Chronic HCV

This part starts off with an outstanding summary of the epidemiology of chronic HCV, how the epidemic started, where we are now, and what may happen in the future. The chapter on acute HCV looks at risk factors for acquiring HCV, why and how spontaneous resolution occurs, and the impact of treating HCV soon after the onset of infection. Other chapters in this part deal with tools to assess fibrosis progression to cirrhosis, the impact of hepatic steatosis, the role of HCV and its treatment on the development of hepatocellular carcinoma, how HCV and its treatment affects extrahepatic organs, HCV in persons coinfected with HIV, and how HCV impacts patients with chronic renal failure and the limitations of our current treatment in this population. This part concludes by discussing the impact of treatment on the natural history of chronic HCV.

Part 2 Treatment of Chronic HCV with Interferon-Based Therapy

This part starts by reviewing the development of interferon and ribavirin for treatment of chronic HCV and whether these agents will remain the backbone for all future therapies. The next chapter evaluates how immune modulators have been explored as a treatment for HCV and whether there will be a role for such agents in the future. The next several chapters deal with assays to measure HCV RNA and how to assess viral response, the concepts of response-guided therapy and how this concept could be utilized to maximize SVR rates, tips on how to manage the side effects of peginterferon and ribavirin, how host genetics affect response to peginterferon and ribavirin, and how genetic testing could be utilized to guide treatment decisions in the future. The final chapter in this part deals with maintenance peginterferon therapy as a treatment for chronic HCV; why this treatment was conceived and why this approach failed to meet our expectations.

Part 3 Antiviral Therapy for Chronic HCV

This part starts by reviewing the various cellular and viral targets which could be utilized to attack HCV. The second chapter discusses viral resistance, how this could emerge during HCV treatment, and the possible long-term impact of mutations which develop within the hepatitis C viral genome. The data which led to the approval of boceprevir and telaprevir for treatment of chronic HCV and preliminary data on future protease and polymerase inhibitors are then reviewed. The final two chapters in this part deal with whether we will ever be able to "cure" HCV without peginterferon and ribavirin and if not whether oral antiviral agents will be utilized as a maintenance cocktail to control HCV and prevent fibrosis progression in the future.

Part 4 Liver Transplantation for Chronic HCV

The final part of the book deals with issues related to liver transplantation. Cirrhosis and hepatocellular carcinoma secondary to chronic HCV are collectively the leading indication for liver transplantation in most countries. The first two chapters in this part review the natural history of chronic HCV following liver transplantation, and discuss whether HCV positive organs could be utilized for transplantation now or in the future when antiviral agents might be able to suppress or eradicate HCV. The final two chapters deal with treating HCV in patients prior to and or following liver transplantation and the impact that oral antiviral agents will have in these settings.

It is my hope that you will find *Chronic Hepatitis C Virus: Advances in Treatment: Promise for the Future* a useful addition to your reference collection. The historical perspective provides a very nice summary of the obstacles we have overcome to improve our treatments of this virus. I am hopeful that the projections made by many of the contributors will become reality and our promise for the future will be realized.

Richmond and Newport News, VA, USA Mitchell L. Shiffman, MD

Contents

Contributors

Nezam H. Afdhal, MD Department of Medicine, Liver Center, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

Andrew Aronsohn, MD Center for Liver Disease, Section of Gastroenterology, Hepatology and Nutrition, University of Chicago Medical Center, Chicago, IL, USA

Tarik Asselah, MD, PhD Service d'Hépatologie, Hôpital Beaujon, Clichy, France

Bruce R. Bacon, MD Department of Internal Medicine, Division of Gastroenterology and Hepatology, Saint Louis University School of Medicine, St. Louis, MO, USA

Yves Benhamou, MD, PhD Department of Hepatology, Pitié-Salpêtrière Hospital, Paris, France

Nathalie Boyer, MD Service d'Hépatologie, Hôpital Beaujon, Clichy, France

Robert S. Brown, Jr., MD, MPH Center for Liver Disease and Transplantation, Columbia University College of Physicians and Surgeons, New York, NY, USA

Vincenza Calvaruso, MD Department of Dibimis, Sezione Di Gastroenterologia & Epatologia, DIBIMIS, Policlinico Paolo Giaccone, Università Di Palermo, Palermo, Italy

Michael Chang, MD Department of Medicine, University of California at Los Angeles, Los Angeles, CA, USA

Edgar D. Charles, MD Laboratory of Virology and Infectious Disease, The Rockefeller University, New York, NY, USA

Michael R. Charlton, MD, MBBS Mayo Clinic, Division of Gastroenterology and Hepatology, Rochester, MN, USA

Curtis Cooper, MD, FRCPC Department of Medicine, Division of Infectious Diseases, University of Ottawa, The Ottawa Hospital, Ottawa, ON, Canada

Antonio Craxì, MD Department of Dibimis, Sezione Di Gastroenterologia & Epatologia, DIBIMIS, Policlinico Paolo Giaccone, Università Di Palermo, Palermo, Italy

Gonzolo Crespo Liver Unit, IDIBAPS and CIBERehd, Hospital Clinic de Barcelona, Barcelona, Spain

Gary L. Davis, MD Director, General and Transplant Hepatology, Baylor University Medical Center, Simmonds Transplant Institute, Dallas, TX, USA

Katja Deterding, MD Department of Gastroenterology, Hepatology, and Endocrinology, Hannover Medical School, Hannover, Germany

Geoff Dusheiko, FCP(SA), FRCP, FRCP (Edin) Centre for Hepatology, Royal Free Hospital, London, UK

Gregory T. Everson, MD Department of Internal Medicine, University of Colorado Denver, Aurora, CO, USA

Fabrizio Fabrizi, MD Division of Nephrology, Maggiore Hospital, IRCCS Foundation, Milan, Italy

Xavier Forns, MD Liver Unit, IDIBAPS and CIBERehd, Hospital Clinic de Barcelona, Barcelona, Spain

Brett E. Fortune, MD Department of Gastroenterology and Hepatology, University of Colorado Denver, Aurora, CO, USA

Edward J. Gane, MBChb, MD, FRACP Liver Unit, Auckland City Hospital, Auckland, New Zealand

Anna Maria Geretti, MD, PhD Institute of Infection & Global Health, University of Liverpool, Liverpool, and University College London, London, UK

Stephen A. Harrison, MD Department of Medicine, Division of Gastroenterology and Hepatology, Brooke Army Medical Center, San Antonio, TX, USA

Patrick Ingiliz, MD Department of Infectious Diseases, Medical Center for Infectious Diseases, Berlin, Germany

Ira M. Jacobson, MD Department of Medicine, Weill Cornell Medical College, New York Presbyterian Hospital, New York, NY, USA

Alison B. Jazwinski, MD Departments of Gastroenterology and Hepatology, Duke University School of Medicine and Duke Clinical Research Institute, Durham, NC, USA

Donald Jensen, MD Center for Liver Disease, Section of Gastroenterology, Hepatology and Nutrition, University of Chicago Medical Center, Chicago, IL, USA

Fasiha Kanwal, MD, MSHS Department of Internal Medicine, Division of Gastroenterology and Hepatology, John Cochran VA Medical Center, St. Louis, MO, USA

William Kemp, MBBS, FRACP Department of Gastroenterology, The Alfred Hospital, Melbourne, VIC, Australia

Bernd Kronenberger, MD Department of Internal Medicine 1, Hospital of the Johann Wolfgang Goethe-University, Frankfurt, Germany

Maud Lemoine, MD, PhD Hepatology Department, St. Antoine Hospital, Paris, France

Raza Malik, MD, PhD Department of Medicine, Liver Center, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

Michael P. Manns, MD Department of Gastroenterology, Hepatology, and Endocrinology, Hannover Medical School, Hannover, Germany

Patrick Marcellin, MD, PhD Department of Hepatology, Beaujon Hospital, Clichy, France

Paul Martin, MD Division of Hepatology, University of Miami, Miami, FL, USA

Stella M. Martinez Liver Unit, IDIBAPS and CIBERehd, Hospital Clinic de Barcelona, Barcelona, Spain

Alvaro Martinez-Camacho, MD Department of Gastroenterology and Hepatology, University of Colorado Denver, Aurora, CO, USA

Andrew J. Muir, MD, MHS Department of Medicine, Duke University Medical Center, Durham, NC, USA

Jean-Michel Pawlotsky, MD, PhD Department of Virology, National Reference Center for Viral Hepatitis B, C and Delta, Hôpital Henri Mondor, Université Paris-Est, Créteil, France

INSERM U955, Créteil, France

Stuart K. Roberts, MBBS(Hons), MD, FRACP Department of Gastroenterology, The Alfred Hospital, Melbourne, VIC, Australia

Sammy Saab, MD, MPH, AGAF Department of Medicine, University of California at Los Angeles, Los Angeles, CA, USA

Department of Surgery, Pfleger Liver Institute, University of California at Los Angeles, Los Angeles, CA, USA

Morris Sherman, MB, BCh, PhD, MRCP(C) Department of Medicine, University Health Network, Toronto General Hospital, University of Toronto, Toronto, ON, Canada

Mitchell L. Shiffman, MD Liver Institute of Virginia, Bon Secours Health System, Richmond and Newport News, VA, USA

Hubert Sung, MD Department of Surgery , Pfleger Liver Institute, University of California at Los Angeles, Los Angeles, CA, USA

Dawn McDowell Torres, MD Department of Medicine, Walter Reed Army Medical Center, Washington, DC, USA

Bart J. Veldt, MD Department of Gastroenterology and Hepatology, Erasmus MC University Medical Center, Rotterdam, The Netherlands

Elizabeth C. Verna, MD, MSc Center for Liver Disease and Transplantation, Columbia University College of Physicians and Surgeons, New York, NY, USA

Heiner Wedemeyer, MD Department of Gastroenterology, Hannover Medical School, Hannover, Germany

Stefan Zeuzem, MD Department of Internal Medicine 1, Hospital of the Johann Wolfgang Goethe-University, Frankfurt, Germany

Part I

Natural History of Chronic HCV

Epidemiology of Chronic HCV

1

Gary L. Davis

Keywords
Hepatitis C • Epidemiology • Natural history • Cirrhosis • Hepatocellular carcinoma

Introduction

Epidemiology is the study of factors affecting the health and illness of populations, and serves as the foundation and rationale for interventions made in the interest of the public health. The epidemiology of hepatitis C virus (HCV) infection includes the incidence and risk factors for infection, development of disease (chronic hepatitis), outcomes (cirrhosis, hepatocellular carcinoma, liver-related death), and how these influence our strategies for treatment.

History of Hepatitis C

The transmission of icteric illness by a percutaneous route was first recognized in the late 1880s with the introduction of the smallpox vaccine, and such events became especially apparent in the early twentieth century among patients receiving vaccines or injections for diabetes or syphilis [1–3]. The first association of blood transfusion with hepatitis was in 1943 [4]. The landmark Willowbrook studies by Krugman clarified the transmissibility of a hepatitis agent from human plasma [5], but the source of the infection remained a mystery until hepatitis B surface antigen was reported in 1967 to be associated with many, but not all, of these cases [6]. It soon became apparent that most parenterally transmitted infections were not caused by hepatitis A (discovered in 1973) or B, and they were attributed to an elusive agent that became known as non-A, non-B virus [7]. Scientists in the United States and Japan finally identified this major cause of parenterally transmitted hepatitis in 1989 and designated it as the HCV [8–10].

Acute Infection

Estimates of the number of acute HCV infection in the United States are shown in Fig. 1.1. These estimates have been extrapolated from age-specific prevalence data and the Centers for Disease Control Sentinel Counties studies and show a low infection rate through about 1965, followed by a tenfold increase over the next 25 years that peaked

G.L. Davis, MD (✉)
Director, General and Transplant Hepatology, Baylor University Medical Center, Simmonds Transplant Institute, 3410 Worth Street, Suite 960, Dallas, TX 75246, USA
e-mail: GaryDav@BaylorHealth.edu

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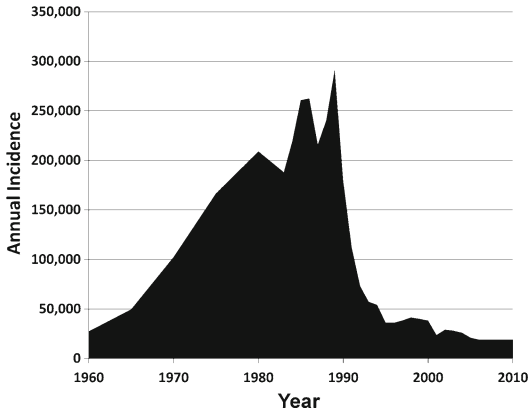

Fig. 1.1 Incidence of acute hepatitis C virus infection in the United States (adapted from refs. [11, 12])

in 1989 at approximately 300,000 cases per year before rapidly declining back to earlier levels [11, 12]. Availability of serologic markers for the virus with resulting screening of blood donors in 1990 essentially eliminated transfusion as a cause. Further reduction occurred as drug users adapted safer injection practices over concern for HIV. Currently, there are an about 19,000 new cases of HCV infection per year in the United States [12].

Acute HCV infection has been most common among young adults (aged 20–39) with a slight predominance of males. Since most cases are asymptomatic and anicteric, they typically are unrecognized. The major liability of HCV infection is the high likelihood of chronicity, although the paucity of symptoms keeps most cases of chronic hepatitis from being diagnosed until many years after the acute episode. There is considerable variability in the risk of progression to chronic infection with a range from 50 to 90% [12–15]. Resolution of acute infection appears to be more common in young people and women, but may be also related to genetic factors such as IL28B haplotype [16]. Spontaneous eradication of chronic infection can occur months or years after acute infection and after chronicity has been well established, but this is unusual [17].

Identifiable risk factors are present in most cases of acute cases of hepatitis C. Intravenous drug use (IVDU) has been the major mode of acquisition in the United States since at least 1960 and currently accounts for between 60 and 90% of new cases [18]. The risk among active drug users is 17% to nearly 80% per year and is most related to sharing of drug preparation equipment rather than needles [19, 20]. In addition, a history of remote high-risk behavior is common in the 10–40% who do not have a recent or readily identifiable risk factor for infection. Thus, most of these so-called sporadic cases are probably related to remote drug exposure.

Blood transfusion was a major risk factor for acquisition of HCV before 1970 and accounted for up to half of all cases [18]. Multiply transfused patients were at particularly high risk. Implementation of a volunteer blood donor pool and HBsAg screening at that time resulted in a precipitous drop in risk by about two-thirds, though a 4–10% risk in transfusion recipients continued until anti-HCV screening became available in 1990 [21]. The risk of transfusion-acquired HCV infection is now exceedingly rare, estimated at 0.01–0.001% per unit transfused [22].

The route of acquisition in the small number of remaining cases has been difficult to prove with certainty, but probably results from sexual and nosocomial transmission. While epidemiological studies have suggested sexually acquisition may be a common route of infection, the risk of sexual transmission is so low that it is more likely that other factors explain many of these cases [23, 24]. Interestingly, most of these cases have a remote history of other high-risk behaviors that might better explain infection [25]. There is, however, a low risk of transmission between monogamous couples without other risk factors. Furthermore, there is circumstantial evidence suggesting sexual transmission among those who practice certain high-risk sexual behavior, particularly men who have sex with men [26].

Although uncommon in most parts of the world today, nosocomial transmission (other than via transfusion) has historically been a frequent route of infection over the last century. This has resulted from reuse of needles and injection equipment, incomplete cleaning of surgical equipment, inadvertent exposure by an infected

healthcare worker, and using multidose vials for different patients [27, 28]. Fortunately, these practices have largely disappeared in most areas and transmission of HCV from medical procedures or personnel is exceedingly rare when proper precautions are followed. Nonetheless, some cases of nosocomial HCV infection have been reported, even in modern medical facilities, though they are usually associated with breaks in technique or surreptitious sharing of drug vials by an infected healthcare worker [29]. In the absence of such gross violations of protocol, the risk of transmission from an infected healthcare worker or surgeon to a patient is 0–0.014% or less than 1:10,000 [30].

Perhaps a greater risk in the healthcare setting is transmission from HCV infected patients to workers. Serologic surveys of emergency department patients found that 18% were infected with HCV [31]. The proportion with HCV infection was even higher in patients with a history of IV drug use (83%), past blood transfusion (21%), and amongst men who have sex with men (21%). Despite this theoretical risk, it remains debatable whether healthcare workers, even those with regular exposure to blood, have a higher prevalence of HCV infection [32, 33]. Although all potential routes of HCV transmission to hospital workers are not obvious, needle-stick injuries probably account for the few documented cases. Serological follow-up of health care workers who sustained after accidental needle sticks/sharps exposures to an anti-HCV–positive patient documented anti-HCV seroconversion in just 1.8% (range, 0–6.6%). The risk is greatest with hollow needles used to draw blood, as compared to hollow infusion needles or suture needles [34].

Finally, perinatal transmission is known to occur. Although this route probably contributes few cases in the United States and Europe, it may represent a major route of transmission in countries with a high prevalence of chronic HCV infection. Antibody to HCV is usually passively transferred from the infected mother to the infant and may remain detectable in the baby for up to a year. The risk of transmission of HCV from viremic (HCV RNA positive) mothers to their infants is 3.2% [35]. The risk of transmission in viremic women coinfected with HCV and HIV (HCV RNA positive) is 7.9% [35]. This higher rate of transmission may, in part, relate to higher levels of HCV RNA in coinfected women; however, some studies have not shown a relationship between risk and viral levels. The rate of mother-to-infant transmission is similar for vaginal and cesarean delivery [36]. However, prolonged duration of membrane rupture appeared to increase the risk of infant infection in one study [37]. HCV is not transmitted by breast feeding [37].

Chronic Hepatitis C

Prevalence

The prevalence of HCV infection throughout the world is low, averaging 2–3% or 170 million persons [38]. However, these estimates are often based on volunteer blood donor prevalence rates and may therefore underestimate the true population prevalence. Nonetheless, these numbers provide some idea of the global distribution of HCV. The antibody prevalence is low (0.01–0.1%) in the United Kingdom and Scandinavia; slightly higher in the United States, Western Europe, Australia, and parts of South America and Africa; and intermediate (1–5%) in Eastern Europe, the Mediterranean, Middle East, Indian subcontinent, Brazil, and parts of Africa and Asia. The highest prevalence is in Northern Africa, the Middle East, and Far East. Wasley suggests that this country-to-country variation is due to the predominant risk factors [39]. For example, in the United States and Europe, the prevalence is low and concentrated in young males who predominantly acquire infection in early adulthood from injection drug use [11, 12]. In Japan and Italy, infection is quite common in older persons indicating a risk in the distant past, perhaps through community inoculation programs. Similarly, the high prevalence across all adult age groups in Egypt indicates a common risk factor, namely medical injections of treatment for schistosomiasis (bilharziosis) [39].

Several studies have come to a relative consensus that there are 3–4 million infected persons

in the United States which represents roughly 1.8% of the population [11, 12]. However, these prevalence studies were largely based on the National Health and Nutrition Examination Survey (NHANES) which did not include groups such as institutionalized persons in whom the prevalence of HCV is very high [40]. Prevalence is highest in middle-aged persons consistent with acquisition of infection at a young age during the peak years of infection between 1970 and 1990 [11, 12]. Chronic infection is higher among males, particularly African-Americans and Hispanics [41]. IVDU accounts for most cases; transfusion is a common identifiable risk factor only in patients older than 50 years [42].

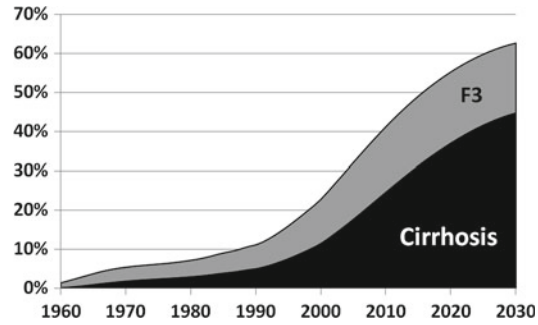

Fig. 1.2 Projection of proportion of HCV-infected persons with bridging fibrosis (F3) or cirrhosis (F4) over time. The percent with either F3 or F4 fibrosis may exceed 60% by the year 2030. Adapted from Davis et al. [12]. © 2010, with permission from Elsevier

Natural History

The medical and quality-of-life liabilities associated with HCV infection are related to the complications of advanced fibrosis including cirrhosis, portal hypertension, and hepatocellular carcinoma. Thus, an understanding of the natural history and progression of HCV infection is important. While approximately 80% of acutely infected individuals develop chronic hepatitis C, the disease progresses slowly in most patients [12]. However, the actual rate of histological progression is variable and has been the subject of debate [13–15]. Some have suggested that progression to severe end-stage liver disease is inevitable provided the infected person does not succumb first to another lethal illness [43, 44]. Indeed, mathematical models of the aging cohort of HCV-infected persons project that an increasing proportion of these individuals will develop bringing fibrosis (F3) or cirrhosis in coming decades [12]. The proportion with cirrhosis could reach more than 40% of all infected persons by the year 2030 (Fig. 1.2). However, others have concluded that disease progression is extremely unusual and restricted to a limited few. These opposing views can be accounted for by the slow pace of progression, the usual lack of symptoms in chronically infected patients, the limitations of available natural history data, and the variation in progression rate related to host factors such as age, gender, alcohol use, and insulin resistance [13]. The influence of these factors, particularly age and gender, on disease progression and outcomes has been best demonstrated in mathematical models of disease progression [12, 14, 15].

Several long-term follow-up studies of recipients of contaminated blood products clearly demonstrate these differences in progression rates according to gender and age of acquisition. They also show that overall few patients with acute hepatitis C progress to liver failure and liver-related death [45, 46]. For example, long-term retrospective observation of healthy young women who received HCV-contaminated lots of immunoglobulin products more than 20 years ago shows that just slightly more than half developed chronic hepatitis and a large proportion appeared to recover [45]. Nonetheless, fibrosis was beginning to become apparent after 17 years, though only 2% had cirrhosis. In contrast, the classic studies by Seeff of mostly older patients followed prospectively after blood transfusion between 1968 and 1980 found that half of surviving individuals developed clinically apparent chronic hepatitis with elevated aminotransferases and, of these, slightly more than 30% had developed cirrhosis [13]. Evidence of hepatic decompensation eventuates in more than 40% of cirrhotic patients and liver-related mortality after 18 years was higher among hepatitis patients (3.2%) than in controls (1.5%). A follow-up report 7 years later found liver-related mortality

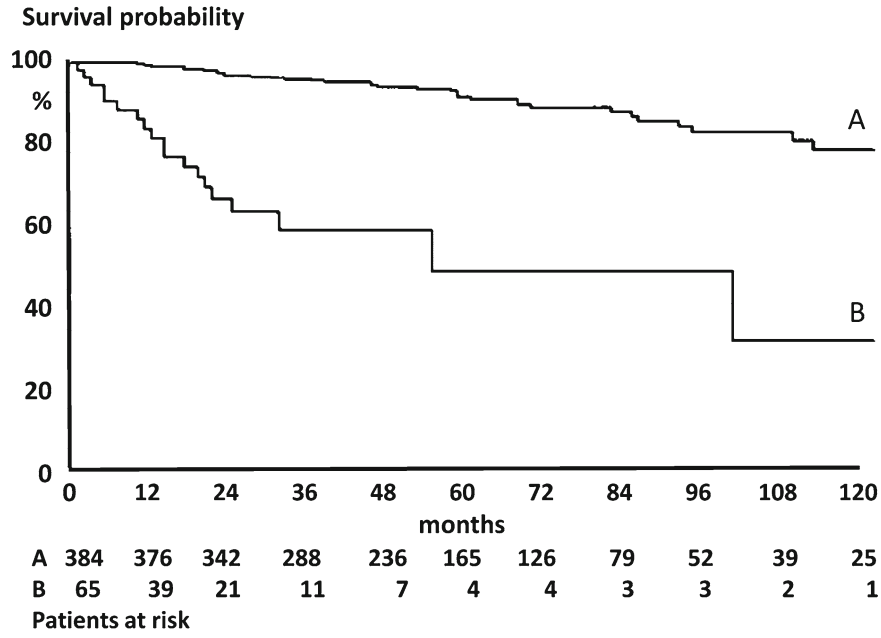

Fig. 1.3 (**a**) Survival probability in HCV-infected persons with compensated cirrhosis (**b**) and decompensated cirrhosis (reprinted from Fattovich et al. [51]. © 2007, with permission from Elsevier)

of 4.1 vs. 1.3% [47]. A similar study among HIV-negative, transfusion-dependent patients with hereditary bleeding disorders found liver-related mortality of just 3.4% after 25 years [48]. Progression of fibrosis appears to continue relentlessly as the duration of infection increases. The prevalence of cirrhosis may increase to more than 40% after 40 years [43, 44, 49]. Furthermore, the subset of patients who present with established chronic hepatitis C appears to have an even higher rate of progression to cirrhosis, liver failure, and liver-related death, suggesting that the rate of progression may accelerate once fibrosis begins [49, 50].

Complications of chronic hepatitis C occur in patients with advanced hepatic fibrosis (bridging fibrosis or cirrhosis) and include hepatic synthetic dysfunction, complications of portal hypertension, and hepatocellular carcinoma. Fortunately, most patients with cirrhosis retain normal hepatic function as assessed by routine laboratory testing and do not have such complications; survival in such compensated cirrhotics is greater than 90% at 5 years, approximately the same as normal age-related survival (Fig. 1.3) [51]. Unfortunately,

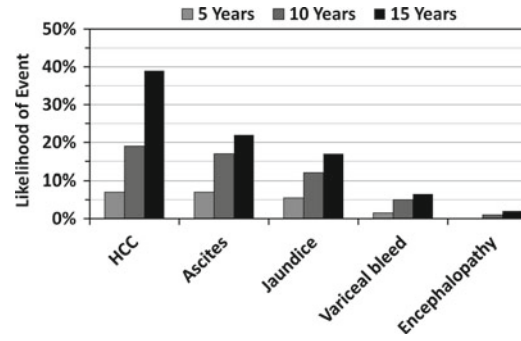

Fig. 1.4 The cumulative probability of decompensation events and hepatocellular carcinoma in patients with previously compensated cirrhosis (modified from Sangiovanni et al. [52], with permission from John Wiley & Sons, Inc.)

however, the risk of complications is reasonably high and cannot be accurately predicted in individuals. Hepatic decompensation (jaundice, ascites, variceal bleeding, encephalopathy) occurs in approximately 3–4% per year (Fig. 1.4) [51, 52]. The risk of developing hepatocellular carcinoma is 3–7% per year [52]. Once one of these complications has occurred, 5-year survival is just 50% [51, 52].

Several environmental and host factors may accelerate the progression of fibrosis and disease progression. The most important of these cofactors is alcohol intake which increases the risk of cirrhosis from 1.5- to 3-fold [53]. Patients who die from the liver disease are more likely than others to be alcohol users. The effect appears to be dose dependent with a 15-fold higher risk in the heaviest consumer compared to teetotalers [54]. Regular and heavy alcohol represents a greater risk for progression than duration of infection, age, gender, or coinfection with either HBV or HIV. Age of acquisition of HCV infection plays a role in both the risk of chronicity as well as the rate of fibrosis progression. The rate of progression appears to be more rapid if HCV infection is acquired after the age of 50 years [12, 14, 49]. Age may also play a role in those already chronically infected and might explain the more rapid progression that sometimes occurs as the disease advances beyond the second decade [49]. The rates of chronicity and disease progression are also less in young women than young males [45]. African-Americans appear to have histologically somewhat less severe liver disease than do Hispanics or Caucasians [55]. Hepatic steatosis has also been linked to more rapid progression of fibrosis [56]. Finally, progression is more rapid in immune suppressed persons such as the HIV-coinfected patient or liver transplant recipients. Progression to cirrhosis is greater in HIV-infected persons and this is partly related to low CD4 counts though other cofactors such as alcohol use confound interpretation of these observations [57]. Progression of HCV after liver transplantation is dramatically increased. The risk of cirrhosis is 3.7, 8.5, and 28% after just 1, 2, and 5 years following transplant [58]. More than 40% of these cirrhotic patients will decompensate within 1 year and survival is only 41% within a year of the decompensation event [59]. Surprisingly, however, rapid progression and decreased early survival does not appear to occur in recipients of other solid organs or bone marrow.

There is currently little convincing data to suggest that viral factors directly impact the rate of disease progression, although both virus level and genotype influence the chance of viral eradication with antiviral therapy and so are important in our ability to modify the course of the disease.

Future Burden of Disease

As stated in the introduction to this chapter, epidemiology serves as the foundation and rationale for interventions made in the interest of the public health. So it is helpful to review and summarize the trends that make intervention imperative.

In the United States, the high chronicity rate after acute HCV infection has kept the prevalence of chronic hepatitis C relatively stable at 3–4 million persons over the last decade despite the marked fall in incident cases since 1990 [11, 12, 18]. Importantly, however, the proportion of individuals with chronic hepatitis who have now had their disease for longer than 20 years is increasing and will continue to do so for another 2 decades [12]. Notably, while the overall prevalence of HCV is 1.8%, the prevalence after the age of 40 is up to threefold higher and the majority of individuals with HCV infection are thus now in their fourth to fifth decade of life [11, 12, 18]. Baby boomers comprise about 66% of HCV-infected patients in the United States [18, 60]. Outpatient and hospital visits for hepatitis C have increased steadily since the early 1990s [61].

The number of cases of chronic hepatitis with advanced fibrosis or cirrhosis is increasing as patients with chronic infection age [12]. It is estimated that 25% of patients with chronic hepatitis C have cirrhosis and an additional 15% have bridging fibrosis. The proportion of cases with cirrhosis is anticipated to be 37 and 45% by 2020 and 2030, respectively [12]. Since the annual risks of hepatic decompensation and hepatocellular carcinoma among cirrhotics are more than 3% and 3%–7%, respectively [51, 52], we can anticipate that these complications will be seen much more commonly. Cirrhosis due to chronic hepatitis C accounts for about 40% of deaths due to liver disease and is the leading indication for liver transplantation in the United States; in 2006, HCV infection was responsible for nearly 40% of all liver transplants performed [61]. HCV infection

is also the most common cause of hepatocellular carcinoma, the leading cause of death among those with HCV-related cirrhosis [62]. Age-adjusted HCC incidence rates tripled in the United States between 1975 and 2005 [62, 63]. HCV explains the rapid increase in HCC in the United States in recent years, while rates for HBV- and alcohol-related HCC have decreased or remained stable [62, 63]. The risk of HCC appears to increase with age and the duration of infection [64]. Thus, a chronic hepatitis C represents a major liability in healthcare's future. Mathematical models estimate that both hepatic decompensation and HCC are expected to roughly double by 2020, while liver-related deaths will almost triple [11, 12]. These morbid and often mortal events will occur primarily in persons in their sixth, seventh, and eighth decades of life.

Antiviral treatment of hepatitis has been evolving over the last 20 years and great strides in effectiveness should be seen with the introduction of direct acting antiviral (DAA) agents beginning in 2011. Treatment of chronic hepatitis C with pegylated interferon and ribavirin eradicates the virus in about half of patients [65]. DAA promise to improve this further, perhaps to 70% or higher [66]. Sustained viral clearance reduces or even reverses liver fibrosis [67]. In successfully treated patients with cirrhosis, the risk of liver failure is essentially eliminated and the risk of hepatocellular carcinoma is reduced by about two-thirds [68]. Newer antiviral treatment therapies might be able to significantly reduce the societal impact of HCV infection in coming decades. If viral clearance can be achieved in 80% of treated patients, treatment of just half of infected persons would reduce cirrhosis by 15.2%, liver failure by 39%, HCC by 30%, and liver-related deaths by 34% over the next decade [12]. However, major obstacles remain. Most infected patients remain undiagnosed in this country, both patients and physicians have been slow to accept therapy for a number of reasons, a number of cases have contraindications to interferon-based treatment, and many lack adequate insurance coverage (Zobair Younossi, personal communication, 22 Sept. 2010). Thus, the current and future challenge for hepatologists is to identify HCV-infected patients; develop safe, effective, and affordable treatment; and reduce the burden of this disease in coming years.

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Acute HCV

2

Katja Deterding, Heiner Wedemeyer,
and Michael P. Manns

Keywords
Acute HCV • Antiviral therapy • PEG-IFNa-2b

Introduction

The rate of chronicity in patients with acute hepatitis C is 50–90%. Early control of hepatitis C virus (HCV) infection by interferon alpha-based therapies has been shown to be possible in the majority of patients. Treatment of acute hepatitis C should be considered not only because chronic HCV infection can lead to further serious clinical sequelae like liver cirrhosis or hepatocellular carcinoma but also because HCV viremia may be associated with a risk for transmission of HCV to other persons and because hepatitis C can have significant social, legal, and economic consequences, especially for infected members of the health care system. While treatment of acute hepatitis C with type I interferons is well established, there has been considerable debate as to which therapy and which time point of therapy is optimal. To determine this we must consider efficacy, side effects, cost, and whether the addition of ribavirin is necessary as it is when treating chronic hepatitis C infection [1].

K. Deterding, MD • M.P. Manns, MD (✉)
Department of Gastroenterology, Hepatology,
and Endocrinology, Hannover Medical School,
Carl-Neuberg-Strasse 1, Hannover 30625, Germany
e-mail: manns.michael@mh-hannover.de

H. Wedemeyer, MD
Department of Gastroenterology,
Hannover Medical School, Hannover, Germany

Epidemiology and Natural Course of Acute Hepatitis C

The incidence of acute hepatitis C differs significantly between countries. HCV is highly endemic in some African countries. New HCV infections still occur in countries with a low human development index since only half of the blood products are screened for anti-HCV in these countries and about 40% of all injections are still given via re-used equipment. However, acute hepatitis C is also still present in Western countries. In Italy, the incidence ranges from 1 to 14 infections per 100,000 according to the national surveillance agency [2], the Italian blood donor program [3], or evaluation in the general population [4].

The cause of transmission of HCV is often difficult to define. Since screening of blood products for the HCV by PCR was introduced, the risk for transfusion-associated acute hepatitis C has been dramatically reduced. Thus, the main reason for HCV infection nowadays is intravenous drug use. The incidence in the high-risk group of drug abusers is up to 39/100 person

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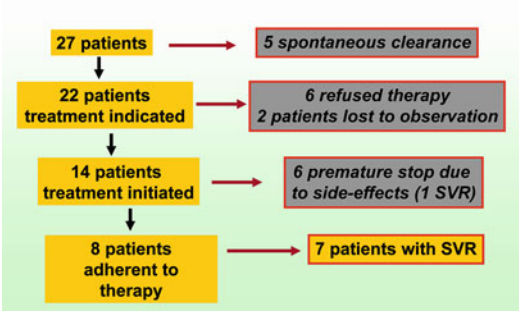

Fig. 2.1 Treatment of acute hepatitis C in IV drug addicts: The Swiss Association for the Study of the Liver Study (SASL 18). I.V. drug user from the Swiss HCV cohort showed rather low overall sustained response rates (based on data from ref. [6])

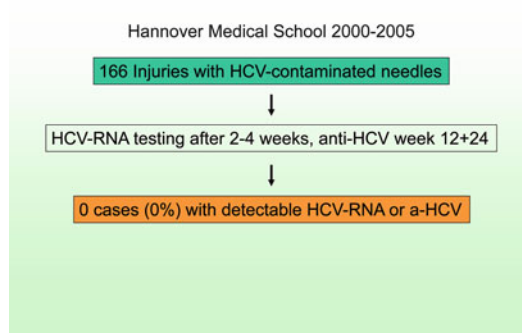

Fig. 2.2 Low rate of HCV seroconversion after occupational exposure to HCV (based on data from ref. [12])

years [5]. Long-term IV drug users show HCV infection rates of 50–80%. However, IV drug users may be difficult to treat for acute hepatitis C as data from the Swiss HCV cohort showed rather low overall sustained response rates (Fig. 2.1). This was due to noncompliance, loss of follow up, and to prematurely stopping therapy in a significant number of patients [6]. On the other hand, Italian investigators reported much better experiences in treating acute hepatitis C [7] justifying treatment attempts in well-established settings of experienced physicians treating not only infectious diseases but also addiction and psychiatric disorders.

Other possible modes of acquisition are medical procedures, sexual intercourse, or needlestick injuries in health care professionals [8–11]. In particular the last group of patients may ask for immediate treatment of acute hepatitis C. Furthermore, the risk to acquire HCV after occupational exposure might be lower than previously reported (Fig. 2.2) [12].

HCV-RNA can be detectable in serum within 3–7 days after exposure. HCV-RNA levels rise rapidly during the first weeks followed by a rise of serum aminotransferases 2–8 weeks after exposure [13]. The elevation of serum alanine aminotransferase (ALT) indicating hepatic injury, inflammation, and necrosis commonly may reach levels greater than 10 times the upper limit of normal. Unfortunately, the serological development of acute HCV infection is accompanied by clinical symptoms only in a minority of cases. Jaundice occurs in only 20–30% of patients, mostly between 2 and 12 weeks after infection [14, 15]. More commonly, nonspecific symptoms, such as fatigue, low-grade fever, myalgia, nausea, vomiting, or itching, are clinical correlates of the infection leading to high rates of unrecognized cases in the acute phase of the disease. It is quite well established that patients are more likely to recover spontaneously if they are symptomatic. We were able to show that young male patients with HCV genotype 3 infection recovered more than individuals infected with genotype 1 [16]. However, in other cohorts of patients with more severe symptoms, the HCV genotype failed to be significantly associated with recovery or chronicity. There is only limited data regarding how different factors, such as ALT levels, bilirubin levels, age, sex, or HCV genotype, are associated with the outcome of interferon treatment in acute hepatitis C. In the Hep-Net Acute HCV-II study, only baseline ALT levels of greater than 500 U/L but none of the other factors were associated with SVR to 6 months of PEG-IFNa-2b treatment (Fig. 2.3) [17]. Thus, patients with more severe hepatitis may require less stringent therapies and the natural course of the infection can be monitored for some time before treatment is initiated. Importantly, in none of the studies conducted to

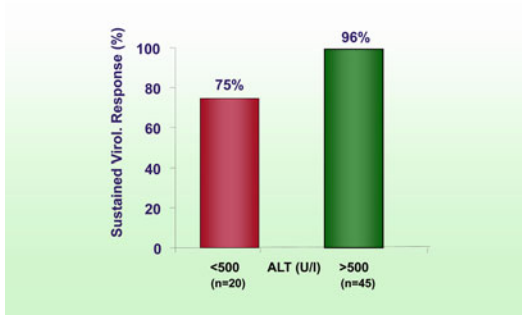

Fig. 2.3 Baseline ALT levels and treatment response in the Hep-Net Acute HCV-II study. In the Hep-Net Acute HCV-II Study, only baseline ALT levels of greater than 500 U/L but none of the other factors were associated with SVR to 6 months of PEG-IFN-α-2b treatment (based on data from ref. [17])

date was HCV genotype clearly associated with treatment outcome. This is significantly different than what is observed in patients with chronic HCV infection.

Conventional Recombinant Interferon Alpha for the Treatment of Acute Hepatitis C

Several small trials performed in the 90s indicated that HCV infection can be treated very effectively in its acute phase. Some of these studies were controlled [18–23], while others did not include a control group [24]. Most of these studies had substantial limitations. Some included only a limited number of patients [22, 24] or only individuals with transfusion-associated HCV infection [18–23]. The treatment schedules differed between the administered type of interferon, interferon dosage, and treatment duration. Therapeutic efficacy was not determined on the basis of HCV-RNA measurement in all studies [18, 23]. All but one study [21] indicated a beneficial effect of therapy. Higher doses of interferon seemed to be associated with better treatment response. In a trial by Vogel et al., 10 MU interferon alfa-2b daily achieved a virological response in 90% of cases after a follow-up of 7–42 months [24]. Patients cleared HCV-RNA within 4–12 days. Aminotransferases normalized after 18–43 days of therapy. Thus, the results of the pilot studies indicated that virological response rates were dose dependent and increased with longer treatment duration. A daily administration of interferon seemed to be more effective than an intermittent dosage.

The treatment of 44 consecutive patients with acute hepatitis C published by Jäeckel et al. in 2001 received much attention [25]. Patients were treated for 24 weeks with an induction dosing of 5 MU interferon alfa-2b daily for 4 weeks followed by 3 MU interferon alfa-2b thrice weekly for additional 20 weeks. After 24 week follow-up, 98% of cases had undetectable HCV-RNA and normal ALT levels.

Thus, the study showed that progression to chronicity can be prevented by early treatment with interferon-based monotherapy. Importantly, no combination with ribavirin was necessary. A further follow-up showed that virological response rates were sustained for up to 224 weeks after the end of therapy [26].

Subsequent studies from Belgium [27] and Japan [28] suggested that even shorter therapies of only 1–2 months with daily injections of interferon alpha might be possible leading to sustained response rates between 84 and 100% if treatment is initiated soon after the acute exposure.

Pegylated Interferon Alpha for the Treatment of Acute Hepatitis C

Pegylated interferons have been investigated by several investigators to treat acute hepatitis C. In 2005, Santantonio published on a cohort of 16 Italian patients with acute HCV treated with PEG-IFNa-2b for 24 weeks [29]. Sustained clearance of HCV-RNA was observed in 15 patients (94%). Treatment was initiated 12 weeks after the clinical onset of hepatitis. The proportion of individuals infected with HCV genotypes 2 or 3 was high (63%).

The fairly good tolerability and the high sustained response rate to PEG-IFNa-2b treatment

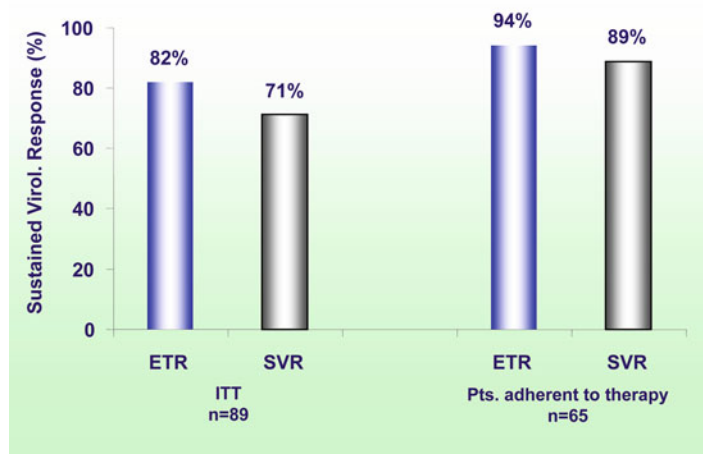

Fig. 2.4 Response rates in the Hep-Net Acute Study HCV-II. Virological response rates defined as undetectable HCV-RNA via polymerase chain reaction (<600 IU/mL). *ETR* end-of-treatment response; *SVR* sustained virological response (based on data from ref. [17])

of acute hepatitis C was confirmed by the German Hep-Net Acute HCV-II study [17]. Eighty-nine patients were recruited receiving at least one dose of PEG-IFNa-2b. Treatment was scheduled for 24 weeks and no ribavirin was given. This study reflects to a large extent the "real-life" setting in Germany since patients were recruited via the German network of excellence on viral hepatitis "Hep-Net" [30]. Subsequently, patients were included not only by 18 university hospitals but also by 26 municipal hospitals and even by nine gastroenterologists in private practice. Possible sources of infections were medical procedures, IV drug abuse, and sexual exposure accounting for about three-fifths of cases. Sixty-six percent of patients were infected with HCV genotype 1 and maximum ALT levels before treatment ranged between 24 and 3,399 U/L (median 599). The median time from the most likely date of infection to start of therapy was 76 days and the time from the onset of symptoms to the start of therapy ranged between 5 and 131 days with a median of 27 days. Thus, therapy was started 1–2 months earlier than in most of the other recent studies on acute hepatitis C where treatment was usually delayed until 3 months after the patient first presented.

The Hep-Net-Acute HCV-II study showed end-of-treatment and sustained virological response rates of 82 and 71% in the intent-to-treat analysis, respectively (Fig. 2.4). Thus, response rates were lower than in several previous studies including the German Acute-HCV-I study using conventional interferon alpha [25]. However, only 70 patients fulfilled the so-called 80/80-criteria of adherence to therapy receiving at least 80% of the PEG-IFN dose and completing at least 80% of treatment duration. A rather high number of patients (15%) were lost to follow-up and protocol violations were performed in another four patients – possibly reflecting the high number of participating centers, including some rather inexperienced sites. Additionally, eight individuals had to stop treatment due to side effects and only four of those achieved an SVR. The sustained response in the group of patients who was adherent to therapy and completed follow-up ($n=65$) was 89%. These results are similar with that reported in other studies. As already mentioned earlier, only baseline ALT levels but not HCV genotype, HCV-RNA levels, age, or sex were associated with sustained response rate in this study.

Another study from Italy suggested that a PEG-IFNa-2b dose of at least 1.3 μg/kg should be administered in acute hepatitis C since lower doses may reduce the chance to achieve a sustained response [31]. However, de Rosa and colleagues treated their patients for only 12 weeks.

None of the other studies treating acute hepatitis C for 24 weeks with PEG-IFNa-2b including the Swiss study on IV drug addicts [6] reported a similar dose effect.

Timing of Therapy; Early Treatment of Acute Hepatitis C or Delayed Treatment?

Data on the optimal timing of treatment for acute hepatitis C is limited since the various studies are difficult to compare and the general approach to delay treatment [32] has been defined as either 12 weeks after the acquisition of HCV or after the clinical onset of hepatitis. In a Japanese population, Nomura compared early "immediate" treatment to treatment starting 1 year following infection [28]. A superior result was achieved when treatment was initiated sooner after infection than waiting a full year after presumed exposure.

The Hep-Net-Acute-HCV-III study was designed in 2004 as a prospective, randomized study in patients with symptomatic acute hepatitis C comparing the efficacy and safety of immediate PEG-IFNa-2b treatment for 6 months vs. delayed treatment with PEG-IFNa-2b plus ribavirin for 6 months starting 12 weeks after randomization in patients who were still HCV-RNA positive. All asymptomatic patients were assigned to early treatment with PEG-IFNa-2b. A planned analysis of 108 patients randomized until December 31, 2007 confirmed that early immediate treatment with PEG-IFNa-2b was highly effective in both symptomatic and asymptomatic patients. Delayed IFNa+ribavirin treatment resulted in a lower overall response rate. However, patients who were adherent to the prescribed regimen had similar efficacy rates in symptomatic patients [33].

If frequent monitoring of HCV-RNA levels is possible, HCV-RNA kinetics may also be considered for timing therapy as repeated measurement of HCV-RNA may predict spontaneous clearance of acute hepatitis C [34].

Duration of Therapy

As mentioned earlier, most trials using pegylated interferon alpha-2b have treated patients for 24 weeks. However, shorter therapies are very likely to be possible [31], in particular, in individuals with baseline parameters being associated with a high likelihood to achieve a sustained response.

Ribavirin

The need for ribavirin is well established in the treatment of chronic hepatitis C. However, there appears to be no need to use ribavirin in patients with acute hepatitis C since approximately 90% of patients appear to achieve a sustained viral response with interferon alpha alone. Ribavirin can be associated with significant side effects and costs and thus, in our opinion combination therapy for acute hepatitis C is not justified. However, the addition of ribavirin can be considered in patients with delayed HCV-RNA kinetics after the onset of treatment, and in those patients with HCV genotype 1 and a low or normal baseline value for serum ALT value.

Influence of Interferon Alpha on Cellular Immune Responses in Acute Hepatitis C

Cellular immunity has been studied extensively in acute hepatitis C showing that HCV specific T-cell responses play an important role in the natural course of the infection. The adaptive T-cell response is mediated both by CD4+ helper T-cells and CD8+ killer T-cells. Involvement of CD 4+ lymphocytes in successful recovery of acute HCV infection was first proposed by Diepolder et al., who observed a strong proliferative immune response mainly against the NS3 protein and a significant production of interferon-gamma by HCV-specific CD4+ T-cells in patients with self-limited disease [35]. Thereafter, several groups consistently found an association between

a strong, multispecific and maintained HCV specific CD4+ und CD8+ T-cell response and the resolution of acute HCV infection [36].

CD4+ T-cells seem to be present for several years after recovery [37], there are conflicting data whether HCV-specific CD8+ T-cell responses persist [37] or decline [38] over time. However, several studies observed durable HCV-specific T-cells in HCV seronegative individuals, who were exposed to HCV by occupational exposure or as household members of HCV-positive partners, but who never became HCV-RNA positive [39]. These observations suggest that HCV-specific T-cells might be induced upon subclinical exposure and might contribute to protection against clinically apparent HCV infection.

Studies of interferon therapy on CD4+ und CD8+ T-cells in patients with acute HCV could not detect a clear relationship between treatment outcome and T-cell immunity [40–42]. Overall, HCV-specific cellular immunity in the peripheral blood cells seems to decline during and after interferon alpha-induced recovery. Possible explanations are that (1) interferon alpha has antiproliferative properties, which could prevent homeostatic and TCR-ligation-driven proliferation of T-cells and thus explain in part reduced frequency of HCV-specific T-cells and weaker proliferative responses; (2) interferon alpha may also have caused apoptosis of activated T-cells since interferon alpha sensitizes cells to antigen-induced cell death occurring at the end of an immune response; (3) HCV-specific T-cells may have disappeared from the circulation and homed to the primary site of inflammation, the liver. We have shown that the decline of T-cells during interferon alpha therapy may be a consequence of both, apoptosis and homing [43]. Thus, the balance between cell death vs. regulation of chemokine receptors potentially can lead to different long-term outcomes.

Conclusions

Interferon alpha therapy of acute hepatitis C is well established. Response rates are high and pegylated interferons can be recommended while ribavirin administration is usually not required. Early immediate treatment with PEG-IFNa-2b is highly effective in both symptomatic and asymptomatic patients. Delayed IFNa + ribavirin treatment resulted in lower overall response rates. However, if patients who were adherent to treatment this strategy seems to be of similar efficacy in symptomatic patients. Asymptomatic patients with genotype 1 infection should be treated as early as possible while treatment might be delayed in individuals presenting with significant symptoms, at least 10 times elevated ALT levels and in patients with genotype 2 or 3 infections. Currently, we still would recommend a 24-week course of treatment although shorter treatment regimens are likely to be effective in a significant proportion of patients.

The optimal management of patients with acute hepatitis C infection should include a careful workup of clinical and virological data as well as the consideration of the individual patient's history.

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Assessing the Progression of Chronic HCV to Cirrhosis

3

Gonzolo Crespo, Stella M. Martinez, and Xavier Forns

Keywords
Noninvasive • Fibrosis • Serum markers • Elastography

Noninvasive Assessment of Liver Fibrosis: Historical Perspective

Liver biopsy is considered the gold standard to assess the degree of liver damage (inflammation, fibrosis) in chronic liver diseases. The assessment of necroinflammatory changes and fibrosis is important for therapeutic decisions, particularly in individuals with chronic hepatitis B and C. Moreover, the fibrosis stage provides prognostic information.

Despite its wide use, liver biopsy has several limitations. First, liver biopsies only represent an extremely small portion of the liver and therefore, sampling errors can occur, especially when smaller sized biopsies are analyzed [1, 2]. In addition, histological examination is prone to intra- and interobserver variation, which may occur even when widely validated systems are used to score liver damage. An additional limitation is the fact that a liver biopsy provides only a static (transversal) measure of liver damage: standard histological methods do not allow for any determination as to whether fibrosis is under the process of deposition-degradation or if it is an established process (e.g., as a consequence of a previous liver injury). Finally, liver biopsy is an invasive procedure with associated morbidity: pain occurs in 20% of patients and major complications (such as bleeding or hemobilia) in 0.5% [2]. For this reason, liver biopsy has poor tolerance, particularly if it needs to be repeated over time in an individual patient.

In patients with chronic hepatitis C, liver biopsy has long been part of the initial evaluation of potential candidates for antiviral therapy. During the era of interferon monotherapy only ~20% of treated patients achieved a sustained virological response. Thus, careful selection of individuals who could benefit from this treatment was crucial. Patients with low response probability and mild disease (no fibrosis or fibrosis restricted to the portal tract) were not good candidates to undergo a long, expensive, and poorly tolerated treatment. On the contrary, treatment was indicated in those individuals with more advanced disease. The increase in treatment efficacy after the combination of interferon with ribavirin, as well as the implementation of stopping rules (avoiding long treatment courses in individuals with very low response probability), has led to a decrease in the necessity of liver

G. Crespo • S.M. Martinez • X. Forns, MD (✉)
Liver Unit, IDIBAPS and CIBERehd, Hospital Clinic de Barcelona, Villarroel 170, Barcelona 08036, Spain
e-mail: xforns@clinic.ub.es

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biopsy for patients with chronic hepatitis C. Moreover, the development in recent years of noninvasive methods to assess liver fibrosis has challenged the commonplace use of liver biopsy. Not only are physicians more comfortable using fast and noninvasive methods, but also patients today are aware of such procedures and are thus increasingly reluctant to undergo a liver biopsy.

The development of noninvasive methods for assessing liver fibrosis, along with heightened awareness of the limitations of liver biopsy, is slowly changing the routine clinical management of patients with chronic hepatitis C. However, there has been a clear resistance to accept noninvasive diagnosis of liver fibrosis as a viable and preferable alternative to liver biopsy. The reasons for this are various. First, the lack of sufficient external validation for some of the proposed methods. Second, the fact that liver biopsy itself is not an ideal gold standard. Finally, and not least important, there is still significant opposition to change what has long stood as dogma.

Current Status of Noninvasive Assessment of Liver Fibrosis

The area of chronic hepatitis C has proven to be a pioneer in the evaluation of noninvasive methods for replacing liver biopsy. The fact that most of the published studies addressing the use of serum markers for the assessment of liver fibrosis have been performed in patients with chronic hepatitis (particularly in those with chronic hepatitis C) explains their current widespread application in this setting. Indeed, some of the proposed methods have been validated in large cohorts of patients and liver disease specialists start to feel more comfortable using them in routine clinical practice [3–12] (Table 3.1).

Serological Markers

The most common approach used to assess the degree of liver fibrosis by noninvasive means consisted of routine biochemical and/or hematological tests [13]. These biomarkers are identified from retrospective studies in which combinations of routine laboratory tests associated with liver function are evaluated for their predictive value to identify significant fibrosis or cirrhosis. Some of the markers included in these scores are platelet counts, ALT or ALT, GGT, bilirubin, and cholesterol. As stated earlier, some of these scores have been widely validated in large cohorts of patients [3–10, 12, 14] (Table 3.1). Although identification of individual fibrosis stages is not possible, some of the published scores do allow the classification of 50–70% of individuals (significant fibrosis/nonsignificant fibrosis) with high positive and negative predictive values (Fig. 3.1). Their major drawback is that they are not sensitive enough to identify patients with mild degrees of fibrosis but who are at risk of progression.

More recently, a better understanding of the pathophysiology of liver fibrosis has prompted investigators to use more refined markers to identify different fibrosis stages. The latter are intended to detect extracellular matrix turnover and/or fibrogenic cell changes [15, 16]. Although liver fibrosis is a local reaction of the liver to chronic injury, serum levels of fibrogenic cytokines, extracellular matrix proteins, and degradation products are markedly increased in cases of advanced fibrosis (bridging fibrosis or cirrhosis) [17]. The most common markers used in current assays involve measuring products of extracellular matrix synthesis or degradation and the enzymes that regulate their production or modification: hyaluronic acid, serum collagenases and their inhibitors (TIMP), and profibrogenic cytokines (such as TGFβ1) [11, 18, 19] (Table 3.1). An example is the ELF score, a combination of the amino terminal propeptide of type III collagen, hyaluronic acid, and the tissue inhibitor of metalloproteinase 1 (TIMP-1); this composite sum has proven useful in identifying patients with stage 3 and 4 liver fibrosis [11]. Some limitations inherent to fibrosis biomarkers are their lack of sensitivity during the initial stages of liver fibrosis and their lack of specificity: they can detect fibrogenesis in organs other than the liver and can be affected by renal or liver failure. The fact that some of the markers are not routinely available in most clinical laboratories will prove less of a

Table 3.1 Performance of noninvasive methods to assess liver fibrosis in patients with viral chronic hepatitis C

Score	Serum markers	Etiology	*n*	≥F2 (%)	AUC≥F2	F4 (%)	AUC F4
Fibrotest [3]	GGT, hapatoglobin, bilirubin, apolipoprotein A, alpha-2-macroglobulin	HCV	339	40	0.83	12	0.92
Castera et al. [27]		HCV	183	74	0.85	25	0.87
Cales et al. [10]		HCV/HBV	383+	56	0.81	16	–
			120	**47**	**0.87**[a]	**12**	
Bourliere et al. [32]		HCV	235	42	0.81	7	0.82
Wilson et al. [73]		HCV(HIV)	119	38	0.74	3	
Sebastiani et al. [33]		HCV	125[a]	60	0.81	15	0.71
Sene et al. [74]		HCV	138	47	0.83	14	–
Halfon et al. [75]		HCV	356	41	0.79	4	0.86
Leroy et al. [34]		HCV	180	50	0.84	14	
Coco et al. [76]		HCV	164	33	0.89	23	0.88
Forns [5]	Age, GGT, cholesterol, platelets	HCV	351+	24	0.86	3	–
			125	**25**	**0.81**	6	
Cales et al. [10]		HCV/HCV	383+	56	0.82	16	–
			120	**47**	**0.86**	**12**	
Bourliere et al. [32]		HCV	235	42	0.76	7	–
Sebastiani et al. [33]		HCV	125[b]	59	0.79	15	–
Sene et al. [74]		HCV	138	47	0.77	14	–
Leroy et al. [34]		HCV	180	50	0.78	14	
Coco et al. [76]		HCV	228	32	0.91	20	–
Sanchez-Conde et al. [39]		HCV/HIV	97	43	0.75	8	–
APRI [6]	AST, platelets	HCV	192+	47	0.80	15	0.89
			78	**50**	**0.88**	**17**	**0.94**
Castera et al. [27]		HCV	183	74	0.78	25	0.83
Cales et al. [10]		HCV/HCV	383+	56	0.79		–
			120	**47**	**0.82**	**12**	
Lackner et al. [77]		HCV	194	50	0.80	16	0.90
Kelleher et al. [78]		HCV/HIV	95	27	0.71	–	–
Borroni et al. [79]		HCV	228	35	–	13	0.86
Parise et al. [80]		HCV	206	42	0.82	21	0.84
Wilson et al. [73]		HCV (HIV)	119	38	0.70	3	–
Bourliere et al. [32]		HCV	235	42	0.71	7	0.81
Sebastiani et al. [33]		HCV	125[b]	59	0.69	15	0.61
Sene et al. [74]		HCV	138	47	0.73	14	–
Halfon et al. [75]		HCV	356	41	0.76	4	0.92
Leroy et al. [34]		HCV	180	50	0.81	14	
Coco et al. [76]		HCV	228	32	0.80	20	0.84
Sanchez-Conde et al. [39]		HCV/HIV	100	43	0.77	8	–
FIB-4 [8]	Age, ALT, AST, platelets	HCV/HIV	555+	21[b]	0.74[b]	–	–
			277	**22**[b]	**0.76**[b]		
Vallet-Pichard et al. [81]		HCV	847	36	0.85[b]	7	0.91

(continued)

Table 3.1 (continued)

Score	Serum markers	Etiology	*n*	≥F2 (%)	AUC ≥ F2	F4 (%)	AUC F4
Sanchez-Conde et al. [39]		HCV/HIV	99	43	0.69	8	–
Hepascore [9]	Age, sex, alpha-2-macroglobulin, hyaluronate, bilirubin, GGT	HCV	117+	44	0.85	6	0.94
			104	**57**	**0.82**	**16**	**0.89**
Halfon et al. [75]		HCV	356	41	0.76	4	0.89
Leroy et al. [34]		HCV	180	50	0.79	14	
Guechot et al. [82]		HCV	512	48	0.81	15	0.88
Becker et al. [83]		HCV	203+	39	0.83	20	0.88[c]
			188	**52**	**0.81**	**19**	
Fibrometer [10]	Platelets, prothrombin time, macroglobulin, AST, hyaluronate, age, urea	HCV	383+	56	0.88	16	–
			120	**47**	**0.89**	**12**	
Halfon et al. [75]		HCV	356	41	0.78	4	0.94
Leroy et al. [34]		HCV	180	50	0.86	14	
ELF [11]	N-terminal propeptide of collagen type III, hyaluronic acid, TIMP-1, age	**HCV**	**496**	**27**[b]	**0.77**[b]	**12**	–
Cales et al. [10]		HCV/HBV	383+	56	**0.83**	16	–
			120	**47**		**12**	
Parkes et al. [84]	Modified ELF (age not included)	HCV	347	56–64[d]	0.74–0.87[d]	**–**	0.87–0.90[d]
HALT-C [12]	Hyaluronic acid, TIMP-1, platelets	HCV	512	93	–	38	0.81
Sud [4]	AST, cholesterol, HOMA, age, alcoholic intake	HCV	170+	48	0.84	6	–
			126	**59**	**0.77**	**13**	–

Reports and studies including more than 100 patients validating the original data are included. In studies including an estimation group and a validation group, data from the validation cohort are depicted in bold

ALT alanine aminotransferase; *AST* aspartate aminotransferase; *GGT* gamma glutamyl transpeptidase; *TIMP-1* tissue inhibitor of metalloproteinase; *HOMA* homeostatic model assessment

[a]Patients with elevated ALT

[b]Severe fibrosis (F3–F4)

[c]Entire cohort

[d]Range within different cohorts (US and English)

problem in the near future, since some of the assays based on fibrogenesis markers have been patented and will soon be commercialized.

Imaging Techniques

The imaging methods used in routine clinical practice (ultrasonography, CT scan, or magnetic resonance imagining [MRI]) are able to detect changes in the liver parenchyma when there is significant fibrosis (bridging fibrosis and mainly cirrhosis) and signs of portal hypertension (enlarged spleen, collateral venous circulation, enlarged portal vein). However, these methods are not useful for identifying patients with less advanced stages of fibrosis. Optical analysis of computed tomography images of the liver (Fibro-CT) has been used to assess fibrosis in patients with chronic hepatitis C [20]. Fibro-CT showed good accuracy in diagnosing advanced fibrosis (AUC > 0.85) and revealed that heterogeneous distribution of liver fibrosis was associated with generally less accurate assessments.

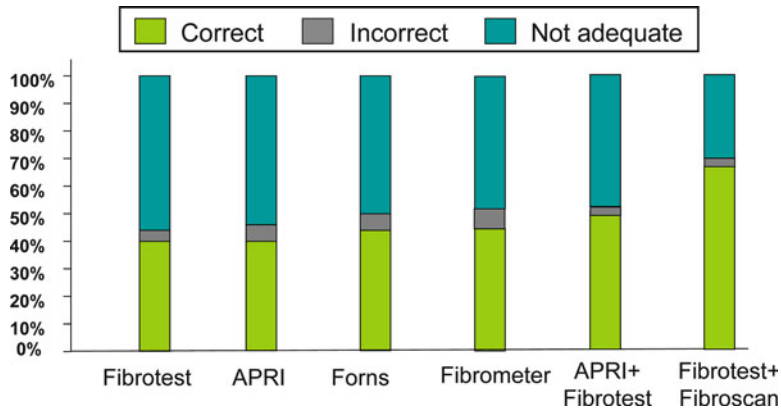

Fig. 3.1 Accuracy of different serological noninvasive methods and transient elastography to identify significant liver fibrosis (≥F2) in patients with chronic hepatitis C. Liver biopsy was used as a gold standard. Combination of two different methods seems to increase the diagnostic accuracy and avoid additional liver biopsies (adapted from refs. [3, 5, 6, 10, 47, 49])

The latter finding is relevant, since it emphasizes the limitations of histological analysis of a small liver fragment and brings into question the use of liver biopsy as a gold standard for fibrosis quantification. The use of fibro-CT, however, is time consuming and more expensive than the noninvasive serum markers currently in use.

Transient Elastography

Transient elastography (TE) is probably the most widely used noninvasive method in Europe for assessing the degree of liver fibrosis (Fibroscan®) [21]. The concept is simple: a vibration of mild amplitude and low frequency is transmitted to the tissue, which induces an elastic shear wave that propagates within the liver. Pulse-echo ultrasonic acquisitions follow the shear wave and measure its speed (Fig. 3.2). The velocity of wave propagation relates directly to tissue stiffness (the harder the tissue, the faster the shear propagates), which is measured in kilo Pascals (kPa). The method is rapid, noninvasive, and reproducible. Importantly, TE acquires information from a much larger portion of the tissue compared with liver biopsy, and therefore, the risk of sampling error is significantly lower. The experience of the operator (more than 500 tests is optimal) appears to be crucial [22]. Any interpretation of the results should take into account variabilities among different measurements (the higher the variability, the less reliable the results) [23]. The limitations of the method are its high failure rate in individuals with narrow intercostal spaces and morbid obesity and the fact that an increased liver stiffness is not always a surrogate of fibrosis. In fact, the presence of necroinflammation (acute hepatitis) or extrahepatic cholestasis may significantly increase liver stiffness values in the absence of fibrosis [24]. Despite the above-mentioned limitations, several studies have already evaluated the accuracy of TE in identifying patients with significant fibrosis or cirrhosis [25–29] (Table 3.2). The diagnostic accuracy is sufficiently good in identifying significant fibrosis (particularly if the underlying disease is taken into account) and is excellent in identifying liver cirrhosis [30, 31].

Current Assessment of Liver Fibrosis in Routine Clinical Practice

In patients with chronic hepatitis C, liver fibrosis is assessed in order to help select the appropriate antiviral therapy algorithm. Although the identification of significant fibrosis (≥F2) has been regarded as an important target, its real value as a

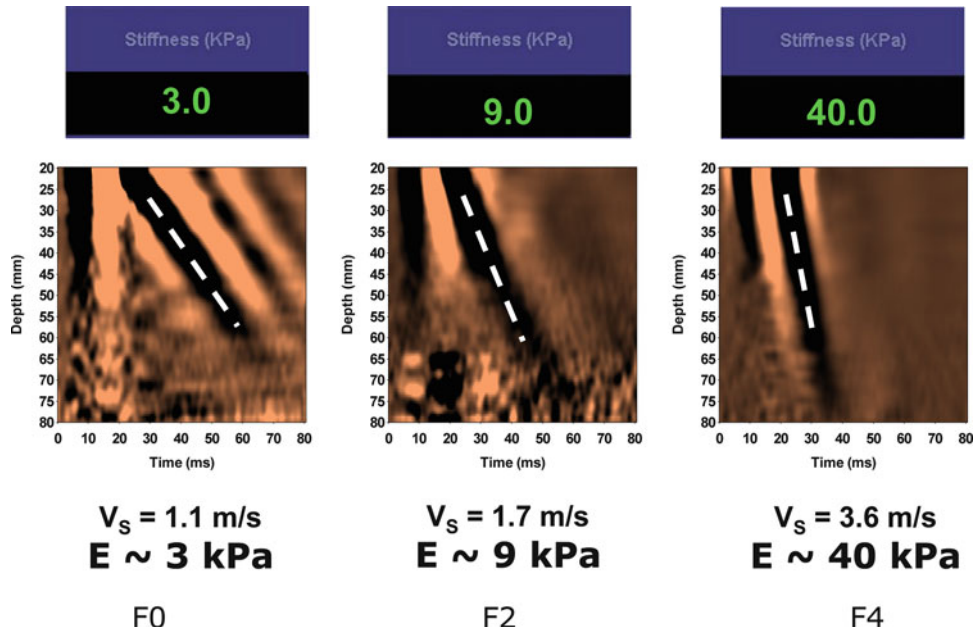

Fig. 3.2 Transient elastography performed in three patients with different degrees of liver fibrosis. The velocity of wave propagation relates directly to tissue stiffness (the harder the tissue, the faster the shear propagates), which is measured in kilo Pascals (kPa)

Table 3.2 Studies assessing the accuracy of transient elastography to identify patients with significant fibrosis or cirrhosis in different liver diseases

References	Etiology	Patients (*n*)	$F \geq 2$ (%)	AUROC for $F \geq 2$	F4 (%)	AUROC for F4
Ziol et al. [25]	HCV	251		0.79		0.97
Kettaneh et al. [26]	HCV	935		0.78		0.90
Castera et al. [27]	HCV	183		0.83		0.95
Carrion et al. [28]	HCV-LT	124		0.90		0.98
De Ledinghen et al. [29]	HCV-HIV	72		–		0.97
Ganne-Carrie et al. [31]	CLD	1,007				0.95
Kamphues et al. [85]	HCV-LT	94	68	0.81	10	0.87
Beckebaum et al. [43]	HCV-LT/other	157	59	0.87	15	0.97
Sánchez-Conde et al. [39]	HCV-HIV	100	43	0.80	8	0.99
Kirk et al. [86]	HCV-HIV	192	37	0.87	25	0.87
Vergara et al. [87]	HCV-HIV	169	62	0.83	39	0.95
Arena et al. [88]	HCV	150	56	0.91	19	0.98
Rigamonti et al. [42]	HCV-LT	95	36	0.85	17	0.90
Fraquelli et al. [89]	CLD	200	50	0.86	12	0.90
Coco et al. [76]	HCV or HBV	228	62	0.93	20	0.96
Chang et al. [90]	CLD	150	44	0.86	10	0.92

static measure of disease severity is arguable. As stated earlier, the fact that treatment efficacy continues to increase over time makes it less relevant to identify the individual stages of fibrosis. In any case, most well-validated noninvasive methods have shown good diagnostic accuracy in identifying patients with a significant degree of fibrosis (i.e., fibrosis expanding beyond the portal tract). Sensitivities and specificities above 85% can be considered sufficient due to the lack of relevant clinical consequences for false positive and false negative cases. In addition, noninvasive tests can be repeated over time, and in cases of indeterminate results two or more methods can be combined.

The identification of individuals with bridging fibrosis (F3) or liver cirrhosis (F4) is more critical: in such cases, sensitivity must be very high, not only due to the potential indication of a specific treatment, but also because screening for hepatocellular carcinoma (HCC) and gastroesophageal varices is mandatory in patients with liver cirrhosis. Fortunately, the performance of noninvasive methods to assess the presence of cirrhosis is excellent. This probably explains why in several European countries the use of noninvasive methods (such as TE) has largely replaced liver biopsy for identifying individuals with liver cirrhosis.

The use of two or more noninvasive methods has been shown to increase the diagnostic accuracy of an individual assay [32–35], which has prompted some investigators to use a sequential algorithm in order to better assess liver fibrosis. Castera et al. [27] showed that the combination of TE and Fibrotest was very useful for diagnosing significant fibrosis (≥F2) and cirrhosis (F4), with areas under the ROC curve measuring 0.88 and 0.95, respectively. Sebastiani et al. [36] investigated the viability of combining APRI with Fibrotest-Fibrosure (Sequential Algorithm for Fibrosis Evaluation, SAFE) to identify significant fibrosis and cirrhosis in more than 2,000 patients with chronic hepatitis C. The algorithm used to identify significant fibrosis avoided 50% of the liver biopsies, whereas an algorithm used to identify cirrhosis avoided more than 80% of the biopsies. The rates of discordance between the histological findings and SAFE were low (around 10%) and in most cases, TE confirmed the results of the noninvasive test.

Assessment of Liver Disease Progression in Special Situations: Patients with HIV HCV Coinfection and Liver Transplant Recipients

Hepatitis C infection is the most common cause of liver disease in HIV-infected patients. As progression of chronic hepatitis C is accelerated in this patient population, an accurate assessment of liver fibrosis is clearly relevant. However, some of the available serum markers (e.g., cholesterol or apolipoprotein levels) currently being used for immunocompetent patients may not be adequate for this population. Nevertheless, most of the methods that have been tried in HCV monoinfected populations have proven similarly useful in HCV-HIV co-infected patients [37, 38]. TE has also been shown to be very effective [39] and is widely used in this setting.

Recurrent hepatitis C infection following liver transplantation is the main cause of graft loss in most liver transplant patients. The presence of significant fibrosis 1 year after transplantation has been clearly linked to heightened probabilities of developing cirrhosis and clinical decompensation [40]. Thus, such patients typically undergo frequent liver biopsies following the procedure. It is therefore quite obvious that replacing liver biopsy with noninvasive methods is imperative. The use of liver fibrosis serum markers (particularly when based on indirect fibrosis markers) is problematic in patients undergoing liver transplantation. Some of the variables included in the scores (ALT, platelet counts, cholesterol) may vary due to causes unrelated to the deposition of collagen in the liver graft. For this reason, indirect serum markers of fibrosis have not proven to be sufficiently accurate in the context of recurrent hepatitis C. On the contrary, those markers intended to detect extracellular matrix turnover and/or fibrogenic cell changes appear to be more appropriate in this particular setting. A recent study has shown how the ELF

score can discriminate between patients with mild and progressive hepatitis C recurrence after liver transplantation early after the procedure [41].

TE also appears to be a promising tool in the setting of hepatitis C recurrence after LT. There are several studies that have shown TE to be very accurate in identifying patients with advanced fibrosis and portal hypertension in the context of hepatitis C recurrence after LT [28, 42–44]. In the study carried out by Carrión [28], 124 liver transplant recipients with HCV infection underwent 169 liver biopsies and 129 liver hemodynamic analyses, paired with liver stiffness measurements (LSMs). As expected, the presence of portal hypertension (hepatic venous pressure gradient, HVPG ≥ 6 mmHg) was indicative of advanced liver fibrosis (≥F2). Importantly, there was a good correlation between portal pressure and liver stiffness; the area under the ROC curve for diagnosing portal hypertension was 0.93. This is particularly relevant since the presence of significant fibrosis or increased portal pressure can identify those patients at risk of clinical decompensation [40].

Noninvasive Assessment of Liver Fibrosis: Promise for the Future

New Methods

In addition to the use of serum biomarkers and TE, strides are being made in the field of liver imaging for clinical assessments of liver fibrosis or liver damage. One example is the technological advances surrounding the clinical application of liver MRI: contrasted-enhanced MRI, diffusion-weighted MRI, and magnetic resonance elastography [45]. The latter uses a modified phase-contrast method to image the propagation characteristics of the shear waves within the liver [46, 47]. Some of the advantages of MRI are the assessment of the entire liver parenchyma, the lack of an acoustical window requirement, and operator independence. In addition, this method may aid in quantifying hepatic fat content [48]. The drawbacks of MR elastography are its cost and the fact that it is time consuming. Recently, the incorporation of proton magnetic resonance spectroscopy (which allows for the analysis of molecular tissue composition) appears to be a safe and reproducible tool for assessing hepatic fat content. In contrast to liver tissue examination (in which the percent of hepatocytes with fat droplets are assessed), this method determines the volume fractions of lipids; livers affected by fat infiltration exhibit an increase in the intensity of the lipid resonance peak [48, 49].

Recently, acoustic radiation force impulse (ARFI) imaging technology has been implemented as a valid method to assess liver fibrosis. ARFI imaging permits evaluation of the elastic properties of a region of interest (ROI) while performing a real-time B-mode conventional hepatic ultrasonography. Recent studies have shown excellent diagnostic accuracy in identifying significant fibrosis and cirrhosis in patients with various liver diseases [50–57]. Its inclusion in a conventional ultrasound machine may offer an advantage in some centers. However, studies with higher numbers of patients under different settings are needed in order to successfully incorporate this promising new tool into clinical practice.

The Use of Noninvasive Markers of Liver Fibrosis to Assess Disease Outcomes and Follow-Up Disease Progression

Most of the studies reported so far provide only a transversal assessment of liver damage. However, it is much more relevant to give dynamic information on a disease. The latter is very relevant since the progression of liver fibrosis over time is commonly not linear and can be influenced by many variables [58, 59]. Thus, transversal evaluation of liver fibrosis (by means of liver biopsy or via a noninvasive test) may not provide an accurate view of the long-term outcome of a disease. The advantage of noninvasive assessments of liver fibrosis is the fact that over time these tests can be repeated during patient follow-up (Fig. 3.3).

Recent data suggest that noninvasive tests can play a role in identifying those patients at risk of disease progression (e.g., clinical decompensation, HCC, and liver-related death). Indeed, two

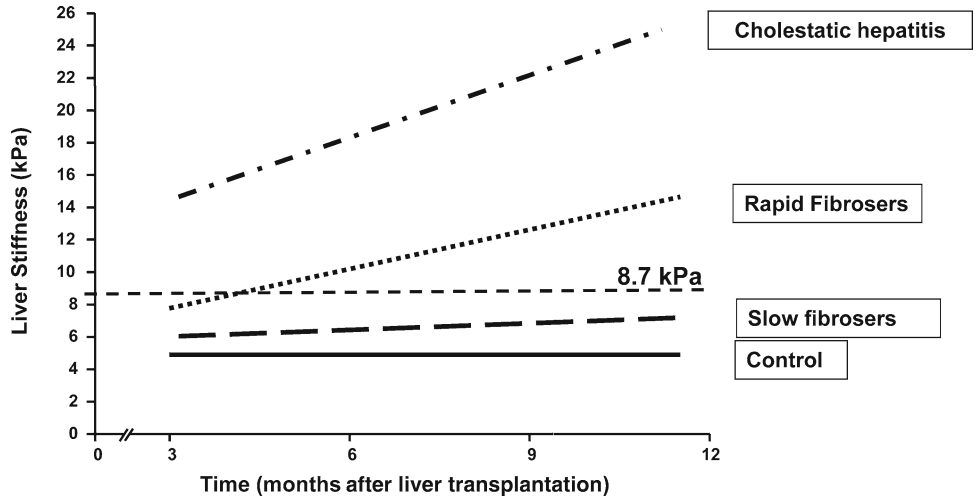

Fig. 3.3 Measurement of liver stiffness over time in patients with hepatitis C recurrence after liver transplantation. Longitudinal assessment of liver stiffness identifies different patterns of fibrosis progression (slow fibrosers, rapid fibrosers). Slow fibrosers are defined as individuals with F0–F1 1 year after transplantation; rapid fibrosers are patients with significant fibrosis (≥F2) 1 year after transplantation (adapted from Carrion et al. [44], with permission from John Wiley & Sons, Inc.)

studies derived from the HALT-C trial explored the association of serum fibrosis makers levels with the risk of clinical and histological disease progression in a large cohort of patients with advanced chronic hepatitis C. Baseline bilirubin, albumin, INR, and YKL-40 levels were independently related to the development of clinical events, whereas baseline platelet count and hyaluronic acid levels accurately predicted histological progression [60, 61]. The ability of serum fibrosis markers to predict liver-related mortality was recently assessed in a cohort of 303 HCV-infected patients, 68% of whom were also HIV infected [62]. Patients were followed for a mean period of 3.1 years, and an expert committee identified 35 deaths directly or probably attributable to liver disease, with HIV status not affecting the predictability of liver-related mortality. The performance of hyaluronic acid (HA), APRI, and FIB-4 in predicting liver-related mortality was equivalent to that obtained in MELD or Child-Turcotte-Pugh (CTP) scoring. In predicting 1-year liver-related mortality, the AUROCs for HA, APRI, FIB-4, MELD, and CTP were 0.92, 0.9, 0.9, 0.84, and 0.93, respectively. In multivariate analyses, these markers independently predicted liver mortality in models including MELD or CTP, suggesting that their addition may improve our ability to identify patients at risk of death.

The potential role of TE to assess clinical outcomes is supported by its good correlation with portal pressure, which accurately predicts clinical events. A study that included 165 patients with cirrhosis demonstrated that liver stiffness values below 19 kPa were highly predictive of the absence of large varices, with a negative predictive value of 93% [63]. It is important to remember, however, that the correlation between portal pressure and LSM decreases when HVPG values exceed 12 mmHg [28, 64, 65]. LSM may also be useful in identifying patients at risk of developing HCC. In a recent Japanese study [66], 866 HCV-positive patients underwent LSM at baseline and were followed for a mean period of 3 years. HCC developed in 77 patients. The 3-year cumulative probability of developing HCC correlated to a significant degree with baseline LSM, with rates as low as 0.4% in patients with LSM ≤ 10 kPa and as high as 38% in those with LSM > 25 kPa. Although these two studies may have clinical implications in terms of screening for varices and HCC, both need validation in a larger cohort of patients.

One area of interest is the possible value of these markers for assessing the effects of therapy on liver damage, particularly on liver fibrosis. Most studies have focused on patients with hepatitis C and B, since there is a specific therapy and a clear definition of response (clearance of viral genome). In this setting, the use of direct fibrogenesis markers and TE appears to detect improvements in patients achieving a response [67, 68]. The lack of a liver biopsy following treatment interruption represents a clear limitation when assessing changes in serum biomarkers. Nevertheless, it has been well documented that a sustained virological response is associated with improvements in both necroinflammatory scores and fibrosis [69, 70]. Recently, Halfon et al. [71] studied 114 co-infected patients treated with pegylated interferon and ribavirin who had undergone a liver biopsy both before and 6 months after end of treatment. In virological responders (25%), Forns' score, Fibrotest, FIB-4, Fibrometer, and APRI decreased significantly after viral clearance, correlating with a decrease in the fibrosis stage and in necroinflammatory activity, an outcome not observed in nonresponders.

In the future, fibrosis biomarkers might potentially prove helpful in monitoring the effects of antifibrotic therapies.

What we need in the near future is to establish which of the current methods currently being used to assess and follow-up disease progression is most effective and most suitable for use in routine clinical practice. Tests with the highest potential to be implemented are those that (1) have been extensively validated in independent cohorts of patients; (2) incorporate analytical validation; (3) contain precise information on the diagnostic accuracy and potential causes of unreliable results; and (4) are useful for establishing disease outcomes, which ultimately is the most relevant endpoint [72].

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Chronic HCV and Steatosis

4

Dawn McDowell Torres and Stephen A. Harrison

Keywords
Steatosis • Insulin resistance • Obesity • HOMA-IR • Diabetes • Metabolic syndrome

Historical Perspective

Simply put, when non-A non-B (NANB) hepatitis was first identified in 1975, the world was a thinner and more active place. According to the National Health and Nutritional Examination Survey (NHANES) data, the prevalence of obesity increased from 14.5 to 22.5% between 1976–1980 and 1988–1994 [1]. This timelines coincides with progress in the identification of the hepatitis C virus (HCV) and development of tests to screen donor blood for the virus [2]. The role of hepatic steatosis in HCV infection would not be addressed until much later.

Before the identification of NANB hepatitis, nonalcoholic fatty liver disease (NAFLD) had already been described in the literature as hepatic steatosis in the absence of significant alcohol consumption [3, 4]. The most clinically relevant subset of these patients with the additional findings of hepatocyte injury as evidenced by ballooning and inflammation with variable fibrosis were classified as nonalcoholic steatohepatitis (NASH) in 1980 by Ludwig and colleagues [5].

The association of NAFLD and NASH with obesity, IR, and metabolic syndrome became clear over a period of focused research in the 1990s. Initially described as the two-hit hypothesis, the development of NASH from NAFLD was based upon first a background of steatosis and IR (hit #1), and second, the presence of oxidative stress (hit #2) [6]. This concept has evolved to include aberrant cellular repair mechanisms and dysregulation of the immune response in addition to IR and oxidative stress [7]. Study is ongoing to better characterize the pathways that lead to steatohepatitis and fibrosis.

As the twentieth century ended, both diseases were relatively common with early data suggesting a 20–30% prevalence for NAFLD and 1.8–3% prevalence for chronic hepatitis C (CHC), thus the coexistence of CHC and NAFLD was not unexpected [8, 9]. However, hepatic steatosis was identified in 40–86% of patients with HCV infection, an observed incidence 2.5-fold higher than expected if these disease processes occurred

D.M. Torres, MD
Department of Medicine, Walter Reed Army Medical Center, Washington, DC, USA

S.A. Harrison, MD (✉)
Department of Medicine, Division of Gastroenterology and Hepatology, Brooke Army Medical Center, 1506 Palmer View, San Antonio, TX 78260, USA
e-mail: stephen.harrison@amedd.army.mil

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independent of one another [10]. This is where the stories begin to come together.

Current Treatment

As improved understanding of the two clinical entities of chronic HCV infection and NAFLD was obtained, treatment regimens were developed, particularly for HCV infection. Over the last 20 years, treatment has evolved from alpha (α) interferon monotherapy to pegylated interferon to our current standard of care (SOC), pegylated interferon plus ribavirin [11–13]. The stepwise improvements in sustained virologic response (SVR) have coincided with the advent of combination interferon/ribavirin therapy and subsequent use of pegylated interferon.

At present, overall SVR rates typically are reported around 50% with numerous factors associated with better or worse outcomes. More advanced liver disease (i.e., more fibrosis) is often associated with poorer response to therapy, as is genotype 1 infection, African American or Hispanic ethnicity, high baseline viral load, significant alcohol use, and the focus of this discussion, the presence of hepatic steatosis and IR.

The role of IR and hepatic steatosis with or without steatohepatitis in CHC is quite complex with both host and viral factors contributing to the presence of these findings. This complex interplay may be broken down into three broad categories of discussion: first, what is the mechanism for hepatic steatosis in CHC infection; second, what is the significance of hepatic steatosis and insulin resistance (IR) in CHC infection (does it affect disease progression and response to therapy), and finally, do treatments to reduce IR and hepatic steatosis improve response rates to current SOC therapy.

Mechanisms of Hepatic Steatosis in CHC Infection

Hepatic steatosis occurs based on a combination of viral and host factors. Host factors are the same as those that predispose to NAFLD along with significant alcohol consumption, use of chronic medications such as steroids, ethnic predisposition, and other less common causes detailed in Table 4.1 [14]. Viral factors are often genotype specific and either directly cause steatosis or indirectly lead to hepatic steatosis via increased IR.

Table 4.1 Host and viral factors related to hepatic steatosis

Host factors	Viral factors
Obesity/metabolic syndrome/ insulin resistance	Genotype 1 and4 • Increased insulin resistance (indirect) ◦ IRS-1 disruption ◦ SOC-3 expression increased • Increased tumor necrosis factor-alpha
African American or Hispanic ethnicity	Genotype 3 infection • Activation of FAS • Down regulation PPARα→↓FA oxidation
Alcohol use	All genotypes • Increased oxidative stress
Medications • Tamoxifen • Amidarone • Methotrexate • Calcium channel blockers • Irinotecan/Oxaliplatin • Valproic acid Ibuprofen • Aspirin • Tetracycline • Zidovudine/Didanosine/ Stavudine	
Other factors • Jejunal-ileal bypass/ Bilopancreatic diversion/ Extensive small bowel resection • Total parenteral nutrition	

Host Factors

The major mechanism by which host factors lead to hepatic steatosis is thought to be mediated by

IR. The subsequent pathways from hepatic steatosis to steatohepatitis are also well studied but less completely understood. Obesity is a state of excess energy availability, often stored in increased adipose depots throughout the body. Excess energy leads to increased fatty acid uptake in the liver as well as increased fatty acid production by hepatocytes [15]. Obesity in the form of visceral adipose tissue also leads to increased IR via the secretion of metabolically active cytokines including tumor necrosis factor-alpha (α), leptin, angiotensinogen, and adipokines. The net result is hepatic steatosis.

IR and proinflammatory cytokines also play a role in the development of steatohepatitis and fibrosis although other mechanisms have also been shown to be important. Hepatic iron, lipid peroxidation, mitochondrial dysfunction, endoplasmic reticulum stress, bacterial endotoxins, and derangements in cellular repair mechanisms all have been implicated in steatohepatitis [16]. Genetics also plays a role with single nucleotide polymorphisms in a gene known to be associated with hyperlipidemia (apoliprotein C3) resulting in substantially higher rates of IR and NAFLD compared to wild-type homozygotes [17]. Other genes such as patatin-like phospholipase domain-containing protein 3 (PNPLA3) have also been linked to NAFLD independent of IR and specifically correlate with disease severity [18, 19]. It would appear that no one pathway reigns supreme in the development of NASH from a background of NAFLD. Similarly, the absence or presence of fibrosis in NASH patients seems to be determined by an intricate web of factors that lead to stellate cell activation and subsequent fibrosis. Ongoing study will provide further understanding of this complicated series of events that can lead to end stage liver disease and its complications independent of CHC.

Viral Factors

As previously mentioned, the prevalence of hepatic steatosis in the setting of CHC significantly exceeds expected prevalence rates should this process be based solely on background NAFLD in a CHC population. This would suggest that CHC either indirectly or directly causes hepatic steatosis. In fact, it would appear the virus does both, with variable mechanisms depending on viral genotype.

Indirectly, HCV infection leads to increased steatosis by increasing IR. Evidence suggests this occurs independently of body weight, diabetes, and the presence of cirrhosis with impaired glucose tolerance seen in early HCV infection before significant fibrosis occurs [20, 21]. Animal studies in genotype 1 constructs have supported the role of HCV infection in impairment of the ability of insulin to lower plasma glucose levels, again independent of weight gain or significant fibrosis [22].

More recently, this was confirmed in a prospective study comparing humans with genotype 1 and 4 HCV infection to chronic hepatitis B (CHB) controls [23]. This large population of French patients was found to have similar rates of metabolic syndrome to the country as a whole, but 32% more IR as evidenced by a Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) >3. These authors also correlated high serum HCV RNA with IR, and in subgroup analysis of 145 patients with normal BMI, showed average HOMA-IR to be 2.8 vs. 1.7 in CHB controls. These findings substantiate the notion that IR in HCV infection is a feature of viral infection and not just a cohabitating disorder in an obese host.

It would appear that the mechanisms leading to viral induced-IR include impairment of the host's insulin signaling pathway. The genotype constructs used by Shintani et al. pointed to a disruption of insulin receptor substrate-1 (IRS-1) tyrosine phosphorylation. Other studies have implicated upstream HCV core protein-induced expression of suppressor-of-cytokine-signaling-3 (SOCS-3) and subsequent proteosomal degradation of IRS-1 and IRS-2 as a direct mechanism of IR [24].

The findings of Aytug et al. supported the role of HCV infection in IR and illustrated the downstream implications of impaired IRS-1 signaling. Human liver specimens infected with HCV

showed decreased IRS-1 tyrosine phosphorylation, and subsequent decreased IRS-1/pI5 phosphatidylinositol 3-kinase (PI3-kinase) and IRS-1-associated PI3-kinase enzymatic activity [25]. As HCV infection resulted in an increase in insulin receptor and IRS-1 substrates, these authors suggested a HCV-inducted postreceptor defect.

Increased tumor necrosis factor-alpha in the setting of HCV infection is a parallel pathway that also leads to impairments of tyrosine phosphorylation of IRS-1 substrate [26]. Subsequent study by the same group showed the downstream effect of this impairment is increased IR and administration of anti-TNF alpha to the transgenic mice partially restored insulin sensitivity [20].

These studies have provided compelling evidence that HCV infection increases IR, independent of host metabolic abnormalities (obesity, metabolic syndrome). Increased levels of SOC-3 may also directly contribute to hepatic steatosis independent of increasing IR via activation of sterol regulatory element-binding protein-1c (SREBP-1c).

Viral driven steatosis may also contribute to IR and it remains under study if IR is predominantly a cause for or result of hepatic steatosis in genotype 1 CHC [27].

Genotype 3 CHC and Steatosis

Genotype 3 virus warrants separate discussion given its unique association with hepatic steatosis. These patients have a higher prevalence and quantity of steatosis than their genotype 1 counterparts and this appears to be the result of specific viral mechanisms on lipid metabolism [28].

All genotypes of CHC use host lipid machinery as a means to replicate and circulate throughout the body [29]. The lipid cell droplet membrane has been shown to be essential for HCV replication, and mutations of viral proteins that result in decreased association with this membrane result in decreased viral replication [30]. The composition of the host cell membrane is important and the attachment of certain proteins to this membrane occurs in a process called protein prenylation. This process relies on lipids called isoprenoids, specifically geranylgeranyl and farnesyl lipids [31]. Inhibition of the production of geranylgeranyl lipids occurs with the use of 3-hydroxy-3-methyglutaryl-CoA (HMG-CoA) reductase inhibitors such as lovastatin. Administration of lovastatin in HCV cell culture has been shown to disrupt viral replication with restoration of replication with the administration of the downstream product geranylgeraniol [32]. Subsequent studies demonstrated that specific inhibition of geranylgeranylation of a host protein FBL2 inhibited HCV replication [33].

While all genotypes of HCV infection utilize host lipid membranes for replication, genotype 3 virus is exceptional in its use of host lipid machinery. Hourioux et al. showed greater lipid accumulation in cells producing genotype 3 HCV core protein vs. genotype 1a core protein [34]. These authors suggested this was due to a phenylalanine residue specific to genotype 3 which had a higher affinity for lipids than the genotype 1 residue tyrosine. Piodi et al. confirmed increased lipid accumulation in genotype 3 CHC compared to genotype 1 [35]. This lipid accumulation was characterized by more large and neutral lipid droplets and occurred without a change in core protein processing by signal peptide peptidases. They also compared genotype 3 infections with and without steatosis and did not find a genetic or functional difference between the genotype 3a core proteins. The authors concluded that HCV core protein–lipid droplet interaction was important in hepatic steatosis although it seemed that viral and host mechanisms were both involved.

Other viral processes specific to genotype 3 infection have been implicated in hepatic steatosis. Both fatty acid synthesis and breakdown are altered by HCV infection. Significant up-regulation of fatty acid synthase (FAS) in genotype 3 infection compared to genotype 1 via the activation of a promoter mediated by SREBP-1 has been described [36]. Decreased fatty acid oxidation secondary to down-regulation of peroxisome proliferators-activated receptor alpha (PPARα) also occurs in HCV infection and appears to be more affected by genotype 3 infections [37, 38].

Several studies also suggest that genotype 3 infections lead to greater inhibition of microsomal triglyceride transfer protein (MTP) and very low-density lipoprotein (V-LDL) secretion compared to genotype 1 constructs [39]. The net result of less MTP and V-LDL secretion is increased hepatic steatosis [40]. Genetic analysis via microarrays using genotype 3 virus strains has also shown a nearly fivefold elevation of stearoyl coenzyme A desaturase 4 (SCD4) [41]. SCD4 is the rate-limiting enzyme in the synthesis of monounsaturated fatty acids and subsequent study has shown reduced SCD4 in the livers of ob/ob mice reduced hepatic steatosis [42].

Genotype 3 infected patients also tend to have lower baseline cholesterol levels than genotype 1 infected patients, likely via these and other genotype-specific modifications of host lipid metabolism [43]. In fact, genotype 3 patients that obtain a SVR showed improved hepatic steatosis unlike their genotype 1 counterparts as well as a significant increase in serum cholesterol compared to genotype 3 patients who did not achieve SVR. These findings cumulatively support a role for genotype 3 infection in host lipid metabolism that is distinct from genotype 1 infections.

Oxidative Stress

The generation of oxidative stress is a nongenotype specific effect of CHC infection that appears to relate to the development of steatosis and advanced hepatic histology. The generation of reactive oxygen species (ROS) is important in the pathogenesis of CHC infection and results in mitochondrial malfunctioning, endoplasmic reticulum stress, and immune cell-mediated damage [44]. Studies have demonstrated 3.4-fold increased production of lipid peroxidation products in cells expressing HCV core protein and this has been isolated to a mitochondrial source in cell culture study [45, 46]. Evidence suggests that the net result of increased oxidative stress leading to DNA damage may be steatosis, necroinflammation, fibrosis, and even hepatocellular carcinoma [47].

Clinical Significance of IR and Steatosis

While the presence of IR and steatosis in the setting of HCV infection is the sum result of the complex interactions between host and viral factors, the clinical significance of these findings is more straightforward, albeit genotype specific. The severity and progression of disease, as well as response to therapy, are all affected by IR and steatosis.

Severity of CHC infection is usually defined by hepatic histology and degree of necroinflammation and fibrosis. In genotype 1 CHC, the presence of IR, steatosis, and steatohepatitis has generally been associated with the presence of more advanced liver disease. In a study of 201 treatment-naïve genotype 1 CHC patients seen in an Italian liver center, the presence of IR and overt diabetes were strongly predictive of severe fibrosis as defined by stage 3 or 4 disease [48]. In fact, as IR progressed to frank diabetes, there was a corresponding increase in fibrosis. This study confirmed the findings of a previous study associating IR with the degree of steatosis in nondiabetic patients with genotype 1 CHC [49].

Another large study in an English patient population of treatment-naïve genotype 1 and 3 CHC patients, further defined the relationship between IR, steatosis, and fibrosis [50]. IR was a major independent determinant of fibrosis, regardless of genotype or the confounding effect of increased BMI. As expected, steatosis was associated with BMI and HOMA-IR for genotype 1 infected patients, but not genotype 3 patients, where high viral load was linked to steatosis. This substantiates the notion that metabolic-associated steatosis is more consistent with genotype 1 (and 4) infection, whereas steatosis in genotype 3 infection is more directly related to viral processes.

Disease Progression and the Role of Steatosis and IR

Steatosis and IR also have been implicated in disease progression in CHC patients, both in the pre- and posttransplant populations. Disease

progression is typically defined as increasing levels of fibrosis until cirrhosis is established. The role of steatosis in disease progression requires careful examination because as a general rule, increasing fibrosis usually is associated with decreasing steatosis. This phenomenon was well described in analysis of the 892 patients from the Hepatitis C Antiviral Long-Term Treatment Against Cirrhosis (HALT-C) trial [51]. In this analysis of predominantly genotype 1 patients (94%), the mean steatosis score decreased equivalently in the follow-up biopsies of both the interferon-treated patients and controls and this correlated to worsening of their liver disease.

On first glance the results from the HALT-C trial seem to contrast the findings of four other studies where baseline steatosis was an independent predictor of fibrosis progression [52–55]. In contrast to the HALT-C data, the patients in these four studies mostly had early or no fibrosis, with one study composed entirely of genotype 3 patients. The complicated role of steatosis in fibrosis progression was further investigated by Castera et al. who found in a multivariate analysis of 96 noncirrhotic patients followed for an average of 48 months, steatosis was the only factor independently associated with fibrosis progression [56]. Yet another study found steatosis associated with fibrosis only in univariate analysis of 494 mixed genotype CHC patients [57].

From this large body of evidence, it would appear that steatosis does predict an accelerated progression to cirrhosis, at least at early stages of disease. As fibrosis becomes advanced, steatosis diminishes and does not predict progression to cirrhosis. The exact mechanism for this terminal decline in steatosis is unclear, but mirrors the sequence of events seen in NASH [58].

IR has also been associated with accelerated rates of progression to cirrhosis [20, 56]. This is true even in the posttransplant population where CHC patients with higher rates of IR have been noted to have higher rates of fibrosis progression in multivariate analysis [60]. Steatosis was not associated with increased rates of fibrosis in this population. Several mechanisms for this have been proposed with the end result of stellate cell activation and hepatic fibrosis. Again, speculated mechanisms mirror the sequence of events seen in fibrosis progression in NASH patients and include increased levels of the hormone leptin which in turn increases TNF-α and transforming growth factor-β, cytokines that are proinflammatory and involved in fibrinogenesis [61, 62].

Standard of Care Treatment in the Setting of Steatosis and IR

Steatosis and IR appear predictive of and associated with more advanced liver disease in CHC patients, making treatment of these patients all the more important. Current SOC therapy with pegylated interferon and ribavirin results in SVR in only around 50% of all genotype 1/4 and 80% of genotypes 2/3 CHC patients [63, 64]. Factors associated with increased and decreased rates of SVR are important to identify in order to select patients most likely to benefit from therapy and appropriately counsel patients as to their chances of response should they decide to undergo an often difficult treatment regimen.

The individual roles of hepatic steatosis and IR in response to therapy have been studied. One prospective study of 399 treatment-naïve patients with genotype 1 CHC showed that IR was independently associated with a lower rate of SVR (relative risk 0.87, $p=0.028$) when adjusted for known factors that predict response [65]. Interestingly, increasing rates of IR as measured by quartile increases in HOMA-IR scores correlated in a stepwise fashion with decreased SVR rates. On the other hand, patients with steatosis were only slightly less likely to have a sustained response to therapy (30 vs. 46%, $p=0.09$). This study was unique in that 50% of the patients were African American, a patient population with a high prevalence of risk factors for NAFLD, but comparatively lower prevalence of hepatic steatosis, and in this study, African Americans were half as likely to have hepatic steatosis as Caucasians for any given degree of obesity or IR [66]. The correlation with increased IR and decreased SVR was seen in both Caucasians and African American patient populations, although African Americans did have lower overall SVR.

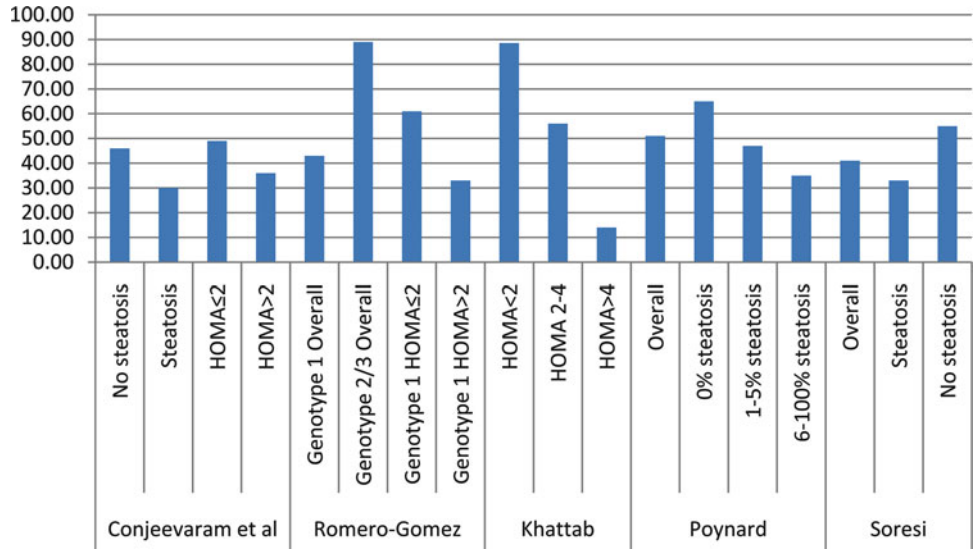

Fig. 4.1 Relationship of steatosis and insulin resistance to SVR in SOC therapy

These findings are consistent with previous studies showing African American or Hispanic ethnicity predicts nonresponse to therapy.

The association of IR and decreased rates of SVR was also seen in a prospective study by Romero-Gomez et al. of 159 patients with genotypes 1, 2, and 3 CHC [67]. Overall SVR was 43.4% of genotype 1 and 89% of genotype 2/3 patients. Genotype 1 patients with IR (HOMA-IR >2) had 32.8% patients with SVR vs. patients with no IR (HOMA-IR <2) who had nearly double the rate of SVR at 60.5%. Steatosis was not associated with SVR.

Similarly, a recent study of genotype 4 patients showed IR as measured by HOMA-IR was independently associated with both RVR and SVR [68]. Overall SVR was 60.3% with HOMA-IR <2 patients having 88.6% SVR compared to 56% SVR in patients with HOMA-IR between 2–4 and 14.2% SVR in patients with HOMA-IR >4. Degree of steatosis was also associated with treatment response in univariate although not multivariate analysis. Poynard et al. and Soresi et al. also found patients with steatosis at baseline were less likely to achieve SVR [43, 69]. In total five studies outlined in Fig. 4.1 suggest that IR and steatosis predict nonresponse to treatment for CHC patients, particularly genotype 1.

Treatment of Steatosis and IR as Part of Combined Regimen for CHC

With previous studies establishing steatosis and IR as predictors of nonresponse to therapy, researchers have begun to assess if treatments to improve IR and hepatic steatosis in conjunction with SOC therapy may increase SVR rates. To date, there is only limited data available to address this question. One early pilot study by Hickman et al. demonstrated that a mean weight loss of 5.9 kg over 3–6 months improved steatosis and fibrosis in treatment-naïve CHC patients [70]. This study did not address treatment response and was limited by small patient numbers with postweight loss liver biopsy only occurring in ten patients.

Other efforts to determine if targeting IR is valuable in the treatment of CHC have produced mixed results. Thiazolinediediones (TZDs) are a class of medications used in the treatment of diabetes that act as selective agonists for

the peroxisome-proliferator-activated receptor-γ (PPAR-γ), reducing IR in muscle, liver, and adipose tissue.

One multicenter prospective study in the nonresponder patient population was terminated after the initial five patients receiving the TZD pioglitazone, in addition to SOC therapy with pegylated interferon and ribavirin, failed to have a satisfactory virologic response after 12 weeks [71]. Criticisms of this early effort were that triple therapy was begun simultaneously rather than with a lead-in phase of the insulin sensitizer, and it was conducted in a small nonresponder patient population without a SOC control group.

A second study in 97 treatment-naïve genotype four patients also used pioglitazone [72]. Patients were randomized to receive pegylated interferon-alpha(α)-2b and ribavirin alone or in combination with pioglitazone 30 mg once daily for 48 weeks. SVR in the three-drug group was 60.4% compared to 38.7% in the SOC group ($p=0.04$). These promising results must be tempered by the single center nature of this study with only genotype four patients, but certainly suggest insulin sensitizers may be useful adjuvants to current SOC therapy.

Metformin is another medication traditionally used in the treatment of diabetes mellitus whose mechanism of action is to lower blood glucose and insulin secretion via suppression of hepatic glucose output as well as increased glucose uptake in skeletal muscle. Romero-Gomez et al. conducted a randomized, double-blinded, placebo-controlled trial using SOC therapy with pegylated interferon and ribavirin with and without metformin in insulin-resistant genotype 1 treatment-naïve patients [73]. In intention-to-treat analysis, there was nonsignificant trend in SVR between metformin containing treatment arm vs. placebo, 53 vs. 42% ($p=$NS). SVR was statistically higher in female patients receiving metformin compared to placebo, 58 vs. 29% ($p=0.03$) although any conclusions drawn from subgroup analysis should be done cautiously.

Larger multicenter trials are underway and are required to definitely determine if targeting IR will significantly improve SVR. These studies have been designed with a lead-in phase where IR is targeted prior to beginning pegylated interferon and ribavirin with preliminary results expected in 2011.

Future Treatment

Unquestionably, the future of treatment lies in DAAs, particularly in combination with current SOC therapy with pegylated interferon and ribavirin. NS3A protease inhibitors such as telaprevir and boceprevir can improve SVR rates from 50% to 60–75% in treatment-naïve genotype 1 patients [74–76]. Polymerase inhibitors in conjunction with SOC therapy show similar efficacy in early clinical trials [77]. Treatment regimens typically carry the same side effect profile as SOC therapy with pegylated interferon and ribavirin with the addition of more frequent skin rashes, anemia, and dysgeusia.

While considerable hope is being pinned on these new small molecules, realism dictates critical analysis of the future of HCV treatment. Certainly, more patients will achieve SVR, but a critical minority (25–40%) of patients will not obtain and or sustain virus negativity using the three-drug combination treatment. This number may be further decreased by combination therapy with four drug regimens using protease/polymerase/pegylated interferon/ribavirin, but the tolerability of such intensive treatment is unknown. In addition, there is the possibility of the propagation of resistant strains of HCV infection, particularly given the knowledge that 8.6% of treatment-naïve genotype 1a patients carry at least one dominant resistance mutation at baseline [78].

This is where a critical analysis of nonresponder and relapser populations is crucial. What is it about these patients that results in treatment failure? Recent research developments have discovered that certain genes predict responsiveness to interferon therapy, including a two-gene signature (IF127 and CXCL9) that is 80% accurate in predicting response [79]. Genetic tests for key resistance mutations for the protease inhibitors also exist although are not commercially available as of yet. Fortunately, these mutations do not

affect sensitivity to interferon and ribavirin, but it seems reasonable that having diminished sensitivity to interferon/ribavirin based on two-gene signature analysis above in combination with clonal expansion of a resistant protease species would predispose towards nonresponse.

The place where hepatic steatosis and IR fits into this big picture is evolving. Patients with hepatic steatosis and IR are less likely to response to SOC therapy, but it is uncertain if this also lowers their chances of SVR using three- and four-drug regimens. The importance of evaluating IR and steatosis prior to treatment is that these are *modifiable* risk factors. Diet, exercise, and medications can all improve IR and hepatic steatosis. This is a completely different and novel pathway that may offer new hope to those unfortunate individuals who for whatever reason fail to obtain SVR using state-of-the-art treatment. A treatment algorithm of the future may include initial assessment for IR and modification of host risk factors for IR prior to consideration of therapy with three- or four-drug regimens. Genetic analysis of the host for interferon sensitivity as well as tests for common resistance mutations pertaining to protease and polymerase inhibitors may also be useful in determining who is at risk of failing treatment, and thus who could benefit most from modification of IR/hepatic steatosis. The future of HCV therapy is brighter than years past but still with room for improvement.

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Chronic HCV and Hepatocellular Carcinoma

5

Morris Sherman

Keywords
Chronic hepatitis C • Hepatocellular carcinoma • Cancer screening • Ultrasonography • BCLC staging

Epidemiology

Past

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world and the third most common cause of cancer death [1]. Worldwide, chronic infection with the hepatitis C virus is the second most common cause of HCC, accounting for 20% of all HCC [1]. The proportion of cases attributable to hepatitis C varies in different geographic areas. In Japan, Pakistan, and Southern Europe, chronic hepatitis C is the most common cause of HCC [1], whereas elsewhere in Asia and in Africa hepatitis B is the dominant cause.

The incidence of HCC has risen in many countries, including Japan, Israel, Canada, Australia, Italy, and Spain, as well as in the United States and France [2–6]. In most of these countries, the major contributor to this increased incidence is chronic hepatitis C. This is because epidemics of hepatitis swept various parts of the world at different times in the past. In Japan, the epidemic started after World War I associated with parenteral medical procedures. In the USA, the epidemic started in the 1960s associated with injection drug use. In Europe, the epidemic occurred in the 1950s, also associated with medical interventions.

The risk of HCC in patients with chronic hepatitis C is highest and has been best studied in patients who have established cirrhosis [7–9]. A prospective population-based study of the risk of HCC in patients with hepatitis C showed that being anti-HCV-positive conferred a 20-fold increased risk of HCC compared to anti-HCV-negative subjects [10]. The presence or absence of cirrhosis was not evaluated. The incidence of HCC in cirrhotic populations ranges from 1.3%/year to about 5%/year [7–9]. HCC can occur in noncirrhotic hepatitis C as well, although the incidence is much lower [11].

Present

More recent data suggests that HCC rates may have stabilized in some countries in Europe or may even be falling in, e.g., Japan [12, 13].

M. Sherman, MB, BCh, PhD, MRCP(C) (✉)
Department of Medicine, University Health Network, Toronto General Hospital, University of Toronto, Rm # NCSB 11C 1252, 585 University Avenue, Toronto, ON, Canada M5G 2N2
e-mail: morris.sherman@uhn.on.ca

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Molecular clock studies, in which the rate of change in viral genetic sequences is plotted as a function of time, suggest that in Japan the hepatitis C epidemic started after World War I [14]. This together with other epidemiological data suggests that the incidence of HCC is or soon will be peaking in Japan, and can be expected to decline in the future.

Future

In contrast, molecular clock analysis of the hepatitis C epidemic in the USA has suggested that the peak incidence of HCC is yet to come, perhaps 10 years or more into the future [14]. The incidence of HCC in the USA has already started to rise increasing from 1.4/100,000/year to 2.4/100,000/year between 1976 and 1995 [6]. This is mostly attributable to chronic hepatitis C.

Other aspects of HCC epidemiology remain to be clarified. Treated patients with sustained virological response are at lower risk of HCC than those still infected [15]. Few studies, however, have stratified patients into those with cirrhosis and those without, so that the posttherapy decline in incidence in the highest risk subjects after treatment has not been quantitated. Currently, the presumption is that successful treatment of hepatitis C in patients who do not have cirrhosis carries with it a very low or negligible risk of HCC. However, data are lacking. Once the new direct-acting antiviral agents are introduced and a greater number of patients are successfully treated this question will become more urgent.

Surveillance

Past

HCC develops silently. There is no opportunity for early detection by self-examination as with breast or skin cancer, nor does it call attention to itself by bleeding into a hollow organ, as bladder or bowel cancer might. Therefore, in the absence of surveillance and early detection programs, HCC presents late in the course of the disease with the onset of symptoms due to liver failure or constitutional symptoms. At this late stage of disease, curative therapy can seldom be applied safely, and when feasible is seldom effective. Noncurative but life-extending therapy may also not be possible because of advanced hepatic failure. Furthermore, progression of disease at this stage is usually rapid with a prognosis of only a few weeks to 3 months.

There are no randomized controlled trials of surveillance in chronic hepatitis C. The optimal surveillance interval and the optimal method of surveillance had not been established. It used to be common practice to perform surveillance using alphafetoprotein (AFP) and or ultrasound at intervals of between 3 and 12 months. A single cost-efficacy analysis suggested that surveillance with ultrasonography was cost-effective [16].

Present

The current recommendation for HCC surveillance is that patients with cirrhosis due to hepatitis C should undergo 6 monthly surveillance using ultrasonography [17]. Again, there are no randomized controlled trials supporting this recommendation. However, these recommendations are supported by cost-efficacy analyses [18–23], and by cohort studies that demonstrate that serological surveillance is neither sensitive nor specific [24], and that patients who undergo 6 monthly surveillance have a better survival than patients who undergo 12 monthly surveillance [25].

Defining the At-Risk Population

Currently, the at-risk population is defined as patients with hepatitis C and cirrhosis. However, additional analyses are required to determine whether surveillance might also be effective for patients with precirrhosis under certain circumstances. Refining the at-risk population will likely broaden the precirrhotic population who might warrant surveillance, as well as eliminate some patients from the cirrhotic population either because they are at low risk, or because

the development of HCC is far in the future. It is also clear that patients with cirrhosis who successfully clear virus with therapy have a reduced risk of developing HCC, but the risk does not disappear completely. Several studies and a meta-analysis have confirmed this finding. Whether the risk continues to decrease over time or remains more or less constant after a period is not clear.

Serological Surveillance Is Ineffective

Among serological surveillance tests the performance characteristics of AFP have been best studied [26]. AFP levels are frequently elevated in patients with established HCC. However, it is clear that AFP is not a good test for small HCCs. The receiver operating curve analysis of AFP used as a diagnostic test suggests that a value of about 20 ng/mL provides the optimal balance between sensitivity and specificity [24]. However, at this level the sensitivity is only 60%, i.e., AFP surveillance would miss 40% of HCC if a value of 20 ng/mL is used as the trigger for further investigation. This is inadequately sensitive for general use. If the AFP cut-off is raised to, e.g., 200 ng/mL the sensitivity drops to 22%. Conversely, reducing the cut-off means that more HCCs would be identified, but at the cost of a progressive increase in the false-positive rate. This analysis was performed in a case–control study where the prevalence of HCC was artificially set at 50%. At this prevalence the positive predictive value (PPV) of an AFP of 20 ng/mL was 84.6%. However, if the HCC prevalence rates were more like those seen in most liver clinics, i.e., about 5%, the PPV of an AFP of 20 ng/mL is only 41.5%, and even at a cut-off of 400 ng/mL the PPV is only 60% [24]. In cohorts undergoing surveillance, the incidence of HCC may be even lower than 5%, depending on the criteria for entry into surveillance. AFP is not specific for HCC. Recent data from the HALT-C study confirm that AFP is frequently elevated in chronic viral hepatitis C, even in the absence of HCC [27]. In the era of sensitive radiological tests, when ultrasound can identify lesions smaller than 2 cm, the role of AFP is questionable.

Another serological test used is des-gamma carboxyprothrombin (DCP), also known as Prothrombin Induced by Vitamin K Absence II (PIVKA II) [28–32]. However, like AFP, DCP is insufficiently accurate for routine use. There are also reports that DCP is a marker for portal vein invasion by tumor [33]. This would also suggest that DCP is not a good screening test. A screening test should be able to identify early stage disease and not late stage disease. Other tests that have been reported as screening tests include the ratio of glycosylated AFP (L3 fraction) to total AFP [29, 34–40], alpha fucosidase [41, 42], and glypican 3 [43, 44]. None of these have been adequately investigated and they cannot be recommended as screening tests.

Recently, the HALT-C team has addressed the issue of using AFP and DCP for surveillance for HCC [27]. They analyzed the serum concentrations of these markers at the time of diagnosis of HCC and 12 months prior to the diagnosis. The performance characteristics of both tests were not optimal at diagnosis, and at 12 months prior to diagnosis were downright poor.

In another study, the value of DCP as a surveillance test was evaluated in a population with known HCC [28]. However, evaluating a test for HCC in the presence of known HCC, even small HCC, is not the same as using the test in a surveillance population. Furthermore, in a population with known HCC, further bias may be introduced by the use of DCP to make the initial diagnosis. Thus, DCP remains to be proven as a useful marker for early detection of HCC.

The radiological test most widely used for surveillance is ultrasonography. Ultrasound has been reported to have a sensitivity of between 65 and 80% and a specificity greater than 90% when used as a surveillance test [45]. However, the surveillance performance characteristics have not been as well defined in nodular cirrhotic livers [46, 47]. These performance characteristics, although not ideal, are considerably superior to any of the serological tests. The major drawback to using ultrasound for HCC surveillance is that it is very operator dependent. In addition, scanning

is difficult in obese subjects. This does not negate the use of ultrasound as a surveillance test. It means that some patients are not good candidates for surveillance. Ideally, ultrasonographers performing HCC surveillance should receive special training, much as is done for mammographic surveillance in some jurisdictions. In good hands ultrasound can detect lesions smaller than 1 cm. The challenge is proving that the lesion is HCC or not.

Surveillance, in order to be successful in reducing HCC mortality should detect HCC at a stage when cure is possible. This means detecting the earliest possible stage of HCC. There is data that suggests that once HCC is larger than 2 cm the frequency of cure decreases, compared to smaller lesions, whatever intervention is applied [48, 49]. Lesions larger than 3 cm are even less likely to be cured. This means that any analysis of the efficacy of surveillance has to target lesions that are smaller than 3 cm, and preferably smaller than 2 cm. However, identifying lesions larger than 3 cm represents a failure of surveillance.

The most difficult ultrasounds are in the obese with fatty liver disease and cirrhosis. However, no alternative strategy for surveillance in obese patients has been adequately tested. Some reports suggest the use of CT scanning as a screening test for HCC [50–52]. The performance characteristics of CT scanning have been developed in diagnostic/staging studies in which some other test has raised the suspicion of HCC. Thus, these are biased populations. The performance characteristics of CT scanning in HCC surveillance are unknown. In addition, for CT scanning to have maximum sensitivity will require four-phase scans, with the attendant high levels of radiation.

Cost-Efficacy Analyses

There are now several cost-efficacy analyses that confirm that ultrasonographic surveillance is effective and cost-effective and that 6 monthly surveillance is more effective than 12 monthly surveillance [18–23]. Furthermore, these analyses indicate that surveillance with CT scan or MRI is cost-ineffective, with an incremental cost-efficacy ratio compared to ultrasonography of more than $100,000/life year saved. The studies differ considerably in several aspects, including the disease model, the treatment interventions that were followed, and the size of lesion at detection.

None of the currently available cost-efficacy analyses include modern thinking about HCC. The ideal model should identify most HCCs at a size smaller than about 2–2.5 cm that can be treated with radiofrequency ablation (RFA). As disease progresses, or if disease presents later, modern concepts of staging and prognosis according to stage must be applied. None of the existing cost-efficacy analysis includes such factors in their models. Thus, there is a pressing need for these analyses to be repeated using modern concepts.

Future

There are two major aspects to the future of surveillance. The first is to refine the tools to determine who is at risk. The second is to improve surveillance tools. The first steps in risk stratification have already been taken. Once again, the HALT-C study has provided data [11]. Patients who developed HCC were compared to those who did not develop HCC, and using multiple logistic regression several risk factors were identified. These were combined in a formula that allowed a calculation of a high intermediate and low risk of HCC. The calculation was rather complex and not easily applicable. Furthermore, this data does not tell us what degree of risk is sufficient to warrant surveillance. Nonetheless, this represents a good start. Additional studies will no doubt refine the assessment of risk. The best evidence is likely to come from molecular studies on liver tissue. In this regard, it is worth noting a study in which liver tissue adjacent to a resected HCC was examined by micro-array technology and identified a molecular signature that predicted late recurrence in the patient [53]. Late recurrences are thought to be de novo tumors, rather than metastases from the earlier lesion. Thus, apparently normal tissue removed at resection predicted tumor developing in the remaining liver. This can only be explained by postulating that there are genetic changes in normal

looking liver that are part of the carcinogenic process. These changes must have led to cellular proliferation so that the liver, or at least large parts of it, were a clonal population. Thus it might, at least theoretically be possible to obtain liver tissue prior to the development of HCC and identify HCC risk, but perhaps more important, be able to identify patients who are not at risk and who do not need surveillance.

Improvement in surveillance tools may come from the use of proteomics to identify serological markers of HCC risk or of the presence of small HCCs. Since we are looking for lesions smaller than about 2 cm it is unlikely that conventional techniques will be able to identify tumor-related proteins in the blood. Immunoassay or enzymatic assays are just not sufficiently sensitive. However, proteomics, in which trace amounts of protein are detectable using mass spectrometry may help identify a unique protein, or more probably a set of proteins that either defines HCC risk or indicates the presence of HCC. This can then be sought with radiological techniques.

Diagnosis

Past

Before the advent of ultrasonography and CT scanning, the diagnosis of HCC was only made when a patient presented with a mass in the abdomen and liver failure or constitutional symptoms. If the AFP was massively elevated the diagnosis was confirmed. However, if the AFP was not elevated hepatic artery angiography or a blind biopsy was required to make the diagnosis. Ultrasound and CT made it easier to find a liver mass and to characterize it as likely malignant. However, confirmation once again required AFP testing or a biopsy.

Present

Today the role of liver biopsy in the diagnosis of HCC is much reduced. The use of four-phase CT scanning with multidetector array machines, dynamic contrast-enhanced MRI using liver-specific agents, and contrast ultrasound has allowed a firm diagnosis to be made in the majority of HCC larger than about 1 cm in diameter [17].

The radiological features of HCC are highly specific [54, 55]. However, the smaller the lesion, the less likely that that typical features will be found. HCC exhibits hypervascularity on the arterial phase of a dynamic study (CT, MRI, or contrast ultrasound) and "washout" during the venous phase. During the arterial phase, the portal venous blood in the liver dilutes the contrast agent in the arterial flow. The tumor is fed by only arterial blood so that the contrast remains undiluted. Therefore, the tumor contains a higher concentration of contrast agent and appears "brighter" than the surrounding liver. During the venous phase the portal blood contains contrast, whereas the arterial blood feeding the tumor no longer contains contrast and the HCC lacks a portal venous supply. Thus, the liver will be "brighter" than the lesion, or, in the terminology used, the lesion exhibits "washout" of contrast. Other vascular tumors tend to have a dual arterial and venous blood supply, so they may enhance more than the liver in the arterial phase, but they do not "washout."

When these features are present a biopsy is not needed to confirm the diagnosis. However, the very earliest stage of HCC, such as might be detected on surveillance might not display these typical appearances. For these lesions a biopsy is required.

An algorithm for the investigation of masses in the liver that might be HCC is given in Fig. 5.1 [17]. This algorithm applies to patients with cirrhosis or noncirrhotics in whom the pretest probability of HCC is high, such as chronic hepatitis B. The algorithm should not be applied to patients with a mass in the liver but who are not at risk for HCC.

Pathological Diagnosis of Dysplasia and Early HCC

One of the consequences of surveillance programs is the identification of smaller and smaller HCCs, and of dysplastic nodules. The smaller the HCC the more difficult it is to distinguish malignant from benign nodules. This is true both radiologically and histologically.

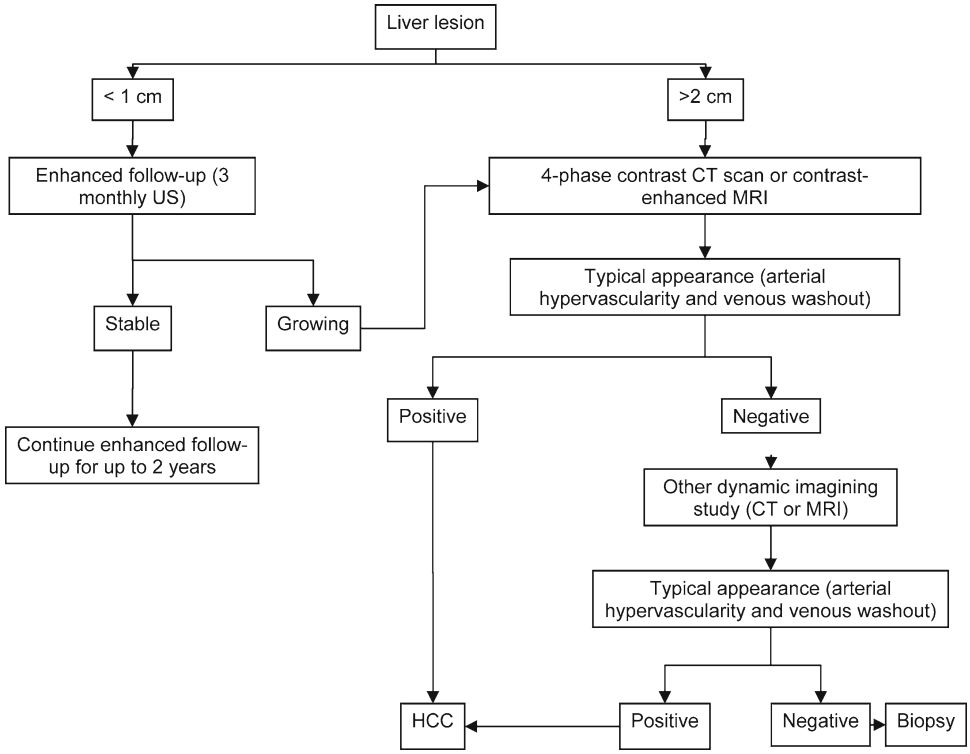

Fig. 5.1 Algorithm for the investigation of small screen-detected lesions on ultrasound

Recently, a distinction has been made between "very early HCC" [56, 57] and "small HCC" [58]. Very early HCC, as defined by Japanese pathologists, is generally hypovascular and has ill-defined margins. Thus, it has a somewhat vague outline on ultrasound and may be hypovascular on CT scanning. Histologically, there are few unpaired arteries, but the cells show varying grades of dysplasia. The pathology of these "very early HCC" lesions has been defined in resected specimens, and therefore, the natural history of these lesions is unknown. The characteristic that stamps these as malignant is known as stromal invasion, i.e., invasion of the portal space by hepatocytes. Vascular invasion is absent. In contrast, "small HCC" have well-defined margins on ultrasound and exhibit the typical features of moderate- or well-differentiated HCC on CT and on histology [57]. These lesions often show microvascular invasion, despite their small size. The presence of microvascular invasion suggests that the prognosis of these lesions after treatment is less good than for "early HCC" where vascular invasion is rare. However, this has not been proven in clinical studies.

In addition to morphological features that help distinguish dysplasia from HCC, staining characteristics may be helpful. Markers of HCC vs. benign tissue include glypican 3 [59, 60], heat shock protein 70 (HSP-70) [61], and glutamine synthetase [61]. HSP-70 is positive in about 70% of HCC and negative in normal liver. GS when positive stains diffusely, in contrast to normal liver where GS stains only a few cells around the terminal vein. Staining for vascular endothelium with CD 34 is more usually positive and strongly positive in HCC [62], identifying the unpaired arteries more clearly, whereas in benign tissue the sinusoidal epithelium stains only weakly with this antibody. Cytokeratin stains for biliary epithelium (CK 7 and CK 19) should be negative, and a positive biliary cytokeratin stain makes

HCC less likely [63]. Given the difficulty of making a positive diagnosis in tissue from small lesions, pathologists should use the full panel of stains listed earlier to help distinguish high-grade dysplastic nodules from HCC.

Patients with liver nodules with a nonspecific vascular profile who have a negative biopsy should continue to undergo enhanced follow-up. A negative test result cannot, on its own, adequately rule out the presence of HCC. There is always the possibility of biopsy sampling error or incorrect interpretation (both pathology and radiology). Only lack of growth over a prolonged period of time indicates that a lesion is not malignant. Since small HCC may be slow growing, follow-up should be for a minimum of 18–24 months. There are no data to establish the best follow-up policy at this point, but repeated biopsy or follow-up CT/MRI to detect further growth should be considered.

Future

The new AASLD guidelines for the management of HCC include a diagnostic algorithm that has been validated to have high specificity and when combined with biopsy should also have a high sensitivity for the detection of HCC at an early stage, optimally smaller than 3 cm [17, 64–66]. New diagnostic tools are also or will soon be available. These include MRI with supraparamagnetic iron oxide (SPIO) which is taken up by Kupffer cells and other macrophages but not HCC cells [67]. There are vascular MRI contrast agents that are taken up by hepatocytes and which undergo biliary excretion. These would also be excluded from HCC providing additional information about the nature of the lesion. PET scanning has not been fully evaluated in HCC.

Treatment

Treatment of HCC in years past, whether in patients with hepatitis C or other conditions was largely surgical, provided liver function allowed it. However, most patients presented late in the course of their disease, when surgery was rarely feasible, and even when possible was seldom curative. Transarterial chemoembolization (TACE) came into use in the early 1990s, but was not initially widely used. In 1996, a randomized controlled study failed to show improved survival in those undergoing TACE compared to those who were treated with best supportive care [68]. Despite this TACE continued to be used. In 2001, two randomized controlled trials of TACE vs. best supportive care and a subsequent meta-analysis all showed that survival could be enhanced [69–71]. The major difference between the first and the second two trials was that the second two studies restricted TACE to patients with Child's A cirrhosis.

Smaller lesions could be treated by ethanol injection, which in turn was supplanted by RFA.

Present

Today, many patients are diagnosed at an early stage when liver function is preserved and there are no cancer-related symptoms. There are also now several active treatments available that can potentially improve survival. However, to achieve the best outcomes requires the careful selection of candidates for each treatment option and the expert application of these treatments. The therapies that are known to offer a high rate of complete responses and thus, a potential for cure, are surgical resection, transplantation, and percutaneous ablation. For patients with solitary HCC in the setting of decompensated cirrhosis and for those with early multifocal disease (up to three lesions, none larger than 3 cm) the best option is liver transplantation [72], but for patients with solitary tumors in well-compensated cirrhosis the optimal treatment strategy remains, with the options being local ablation, resection, or transplantation.

Among noncurative therapies, TACE and sorafenib have been shown to positively impact survival [73, 74]. Other options such as arterial embolization without chemotherapy or radioembolization do show some antitumor activity but there is as yet no evidence of improved survival.

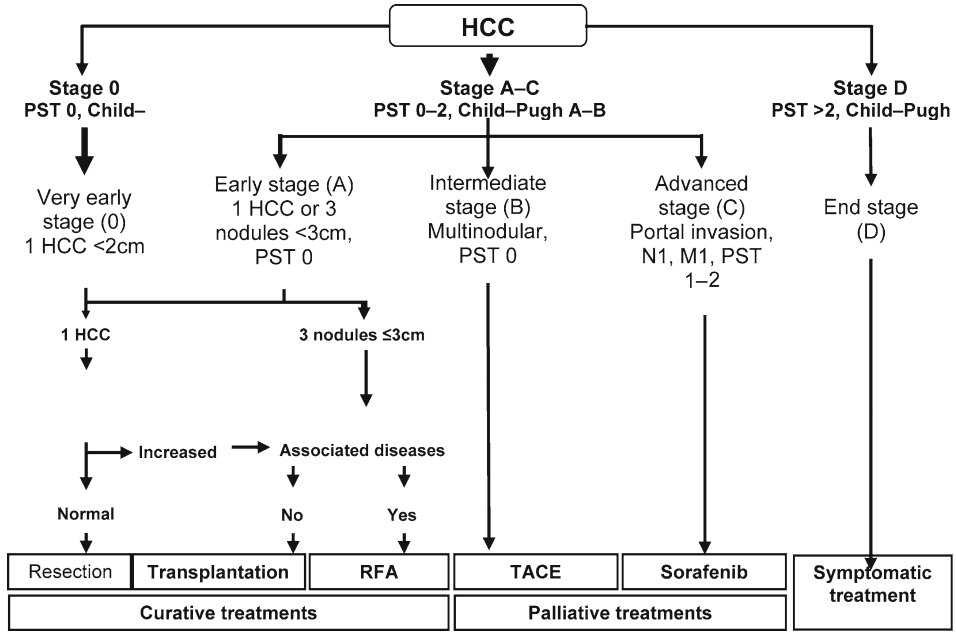

Fig. 5.2 The BCLC liver cancer staging system

Treatment today is guided by staging. The most widely used HCC staging system is that proposed by the Barcelona Cancer of the Liver Clinic (Fig. 5.2) [75]. This scheme combines a traditional staging system with treatment allocation and provides a useful guide as to what forms of treatment are appropriate to each stage.

Surgical Resection

Two decades ago resection seldom achieved long-term survival. Today however, the 5-year survival after resection can exceed 50% [76–78]. In part this is due to treatment of earlier stage disease. However, several major advances have increased the long-term survival figures. More accurate evaluation of the underlying liver function has identified those in whom resection cannot be tolerated. A normal bilirubin concentration and the absence of clinically significant portal hypertension as measured by hepatic vein catheterization (hepatic vein pressure gradient <10 mmHg) are the best predictors of excellent outcomes after surgery, with minimal risk of postoperative liver failure [78]. In contrast, the majority of patients with significant portal hypertension will develop postoperative decompensation (mostly ascites), with a 5-year survival of less than 50%. If both portal hypertension and elevated bilirubin or multifocal disease is present survival is less than 30% at 5 years, regardless of their Child–Pugh stage [76]. Clinically significant portal hypertension is also indicated by the presence of thrombocytopenia (platelet count below 100,000/mm^3), varices, or significant splenomegaly. Although resection can be performed in some of these patients, mortality is higher and they might be better served by liver transplantation or local ablation.

Most groups restrict resection to patients with a single tumor in a suitable location for resection. The size of the tumor is not a clear-cut limiting factor. The risk of vascular invasion and dissemination increases with tumor size [79], but some tumors may grow as a large single mass with no vascular invasion. In these, surgery may be safely performed and the risk of recurrence is not significantly increased as compared to smaller tumors [80].

Since patients with hepatitis C who develop HCC mostly have fully developed cirrhosis, and since the incidence of HCC increases with advancing liver disease resection of HCC is uncommon in chronic hepatitis C.

Liver Transplantation

Selection of patients with HCC for liver transplant is largely still based on criteria developed in 1996. The seminal paper by Mazzaferro demonstrated that as long as the there was only a single lesion smaller than 5 cm or no more than three lesions, each no more than 3 cm in diameter transplantation could be performed with results no different than for any other indication [72]. These criteria became known as the Milan criteria and have remained in force in most liver transplant units ever since.

Although patients with hepatitis C make up the single largest indication for transplantation in the West, the results may not be as good as for other indications, and this is true for hepatitis C-related HCC as well. The reason is the recurrence of hepatitis C in the transplanted liver that contributes to mortality. Furthermore, hepatitis C is associated with the metabolic syndrome, in particular diabetes, which in turn also contributes to mortality. Treatment of hepatitis C after transplant is possible, but once again, the outcomes are not as good as for treatment in the nonimmunosuppressed.

Local Ablation

Two techniques have been widely applied, injection of absolute alcohol or RFA, a procedure in which radio waves are used to heat up a needle tip placed within a lesion, resulting in coagulation of tissue. Percutaneous ablation is usually performed under ultrasound guidance.

Ethanol injection can achieve complete necrosis in 90–100% of HCC smaller than 2 cm, but is less effective with tumors between 2 and 3 cm and lesions between HCC between 3 and 5 cm will only achieve complete necrosis in about 50% [81–86]. Child–Pugh A patients with complete tumor necrosis may have a 50% survival at 5 years [81, 87]. This compares well with the outcome of resection.

The efficacy of percutaneous ablation is assessed by dynamic CT 1 month after therapy [17]. Although not entirely reliable, the absence of contrast uptake within the tumor reflects tumor necrosis, while the persistence of contrast uptake indicates treatment failure. The recurrence rate after ablation is as high as for resection. Some recurrences will occur in the vicinity of the treated nodule and are due to the presence of microscopic satellites not included in the ablation zone. The efficacy of RFA in tumors <2 cm is similar to that of ethanol but requires fewer treatment sessions [88, 89]. The efficacy in tumors >2 cm is better than with ethanol [90].

Currently, RFA should be the first choice for local ablation, but ethanol injection remains useful under certain circumstances.

Transarterial Chemoembolization

HCC is mostly dependent on the hepatic artery for blood supply. This feature provides the basis for the radiological characteristics that are used to diagnose the disease. It also provides the rationale for arterial obstruction as an effective therapeutic option. Acute arterial obstruction induces ischemic tumor necrosis. The procedure requires the advancement of the catheter into the hepatic artery and then to lobar and segmental branches aiming to be as selective as possible so as to induce only minimal injury to the surrounding nontumorous liver. The artery is then obstructed with one of several possible agents, Gelfoam particles, polyvinyl alcohol [91], starch microspheres [92], and metallic coils [93]. When this procedure is combined with the prior injection of chemotherapeutic agents, usually mixed with lipiodol into the hepatic artery, the procedure is known as TACE. Lipiodol is an oily contrast agent used for lymphographic studies and is selectively retained within the tumor. Several chemotherapeutic agents have been used for TACE, but the most common is to inject adriamycin or cisplatin [94].

TACE is considered for patients with nonsurgical HCC who are not eligible for percutaneous ablation, provided there is no extrahepatic tumor spread. The main contraindication is the lack of portal blood flow (because of portal vein thrombosis, portosystemic anastomoses, or hepatofugal flow). Patients with lobar or segmental portal vein thrombosis are poor candidates for TACE. First, TACE has not been adequately tested for safety or efficacy in these patients. Second, prognosis in patients with macroscopic vascular invasion is much worse than without portal vein invasion, so that data from patients without portal vein tumor thrombus cannot be extrapolated to those with tumor thrombus. Patients with advanced liver disease (Child–Pugh class B or C) and/or clinical symptoms of end-stage cancer should not be considered for these treatments as they have an increased risk of liver failure and death.

Response to treatment is associated with a significant improvement in survival, as demonstrated in two randomized controlled trials [69, 70] and a meta-analysis [71]. The improvement in survival ranges from 20 to 60% at 2 years [71].

Sorafenib

For patients who have either failed TACE or who present with more advanced HCC, sorafenib has been demonstrated in two randomized controlled trials to prolong life [74, 75]. Sorafenib is a multikinase inhibitor with reported activity against Raf-1, B-Raf, VEGF-R2, PDGF-R, c-Kit receptors, and other tyrosine kinase receptors. As a result, sorafenib is now established as first-line treatment in patients with HCC who can no longer be treated with potentially more effective therapies. These trials included only patients with preserved liver (Child-Pugh A). Data in Child-Pugh B are scarce.

Future

Until the recent past there was little research into new treatment modalities for HCC. However, the increasing incidence of HCC and the advent of sorafenib have stimulated interest in this disease, with the result that there are dozens of new treatments in the pipeline.

Laparoscopic resection of HCCs confined to one or two segments is now fairly common. This will decrease morbidity and the length of hospital stay, but is unlikely to allow resection in patients who could not tolerate resection by laparotomy, because the limiting factor remains the amount of liver to be removed and the functional status of the remnant.

Some of the immunosuppressive agents, such as sirolimus have anticancer effect, and this might be used to reduce the rate of recurrence after transplantation for HCC. This has still to be proven.

Additional methods of local treatment will become available. There are several modalities under study. High frequency focused ultrasound might allow noninvasive ablation of HCC. Laser has also been used to ablate tumors. However, the improvement that might come soonest is microwave ablation, when instead of radio waves producing heat microwaves are used. This technique lacks many of the drawbacks of RFA. Proximity to a large blood vessel decreases the efficacy of RFA because of a heat sink effect. For some reason this does not appear to be a problem with microwave ablation. It is also supposed to be much quicker and applicable to larger lesions.

Another intriguing possibility is to use thermolabile liposomal encapsulated doxorubicin in conjunction with RFA. The thermolabile liposomes breakdown in the presence of heat, i.e., in the region of the RFA probe. The doxorubicin is then released locally, with the hope of increasing the kill zone, in particular killing microsatellites.

There are any number of molecular targeted agents currently in various stages of testing for activity against HCC. Some have already failed, e.g., sunitinib. However, others, such as brivanib are in phase 3 testing. These are being investigated as second-line therapy in sorafenib failures, as first-line therapy in place of sorafenib, and as adjuvant and neo-adjuvant therapy. These are small molecule inhibitors of various protein kinases, thought to be essential to the maintenance of cell

growth, and in some cases to angiogenesis. Monoclonal antibodies, such as bevacizumab, another antiangiogenic agent are also being tested. Although it is likely that some of these will fall by the wayside, there are so many being tested that some at least are likely to be found to be clinically useful.

Summary

HCC remains a common cause of cancer mortality worldwide, with a significant contribution due to chronic hepatitis C infection. The new direct-acting antiviral agents are likely to decrease the incidence of HCC in parts of the world that can afford them. For those who already have advanced disease or in whom the direct-acting agents will be ineffective, the risk of HCC remains. The good news is that with early detection the likelihood of cure is substantial, perhaps as high as 90%. However, this requires regular surveillance with ultrasonography and aggressive investigation of any focal lesions so identified. For those whose HCC is missed at the early stage noncurative therapy is improving, although short of destruction of the tumor cure is still unlikely. Some of the many new agents being tested will likely improve survival, although none is likely to be a cure. The new mantra is to induce stability, and this is possibly achievable with the new agents or combinations of new agents.

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Extrahepatic Manifestations of Chronic HCV

6

Vincenza Calvaruso and Antonio Craxì

Keywords
Chronic HCV infection • Mixed cryoglobulinemia • B-cell • Non-Hodgkin's lymphoma • Direct-acting antiviral agents

Introduction

While the liver is the primary organ affected by chronic hepatitis C virus (HCV), a broad clinical spectrum of extrahepatic complications and diseases are associated with this viral infection [1–3]. These include mixed cryoglobulinemia, non-Hodgkin's lymphoma (NHL), cutaneous vasculitis, glomerulonephritis, neuropathy, lymphoproliferative disorders (LPDs), and other less common manifestations. A recent classification of HCV extrahepatic manifestations (EHMs-HCV) is provided in Table 6.1. The EHMs-HCV are classified into four main categories: (A) EHMs-HCV characterized by strong epidemiologic and pathogenetic evidence; (B) disorders for which an association with HCV infection is supported through data; (C) associations that have been linked to HCV but require confirmation and/or a more detailed characterization; and (D) those disorders where only an anecdotal link has been reported [4]. The most well characterized of the EHM are HCV-related LPDs, especially cryoglobulinemia (MC) and its complications.

V. Calvaruso, MD (✉) • A. Craxì, MD
Department of Dibimis, Sezione Di Gastroenterologia & Epatologia, DIBIMIS, Policlinico Paolo Giaccone, Università di Palermo, Piazza delle Cliniche n. 2, Palermo 90127, Italy
e-mail: vcalvaruso@libero.it

Immune Manifestations of HCV

Mixed Cryoglobulinemia (MC)

Mixed cryoglobulinemia (MC) is the most well-documented extrahepatic manifestation of HCV infection [5, 6]. MC is characterized by the presence of circulating immunocomplexes produced by a benign proliferation of B-cells. MC is defined by the presence of immunoglobulins (Ig) that precipitates from serum at low temperature (under 37 °C)and dissolves upon re-warming. The classification of cryoglobulins is summarized in Table 6.2. Type II MC (MC-II) is characterized by polyclonal IgG and monoclonal IgM with rheumatoid factor (RF) activity; type III MC (MC-III) by polyclonal IgG and IgM [7].

The association between HCV and MC has been confirmed by serological, pathological, and molecular testing [7–9]. MC represents the link between HCV and various autoimmune LPDs. Serum cryoglobulins (CGs) are frequently present

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Table 6.1 Classification of extrahepatic manifestations of HCV infection

A: Association defined on the basis of high prevalence and pathogenesis	MC (complete or incomplete syndrome)
B: Association defined on the basis of higher prevalence than controls	B-cell NHL Monoclonal gammopathies Porphyria cutanea tarda Lichen planus
C: Associations to be confirmed	Autoimmune thyroiditis Thyroid cancer Sicca syndrome Alveolitis lung fibrosis Diabetes mellitus Noncryoglobulinemic nephropathies Aortic atherosclerosis
D: Anecdotal observation	Psoriasis Peripheral/central neuropathies Chronic polyarthritis Rheumatoid arthritis Polyartheritis nodosa Bechet's syndrome Poly/dermatomyositis Fibromyalgia Chronic urticaria Chronic pruritus Kaposi's pseudo sarcoma Vitiligo Cardiomyopathies Mooren corneal ulcer Erectile dysfunctions Necrolytic acral erythema

Table 6.2 Classification of cryoglobulins

	Clonality of immunoglobulins	Associated disease
Type 1	Monoclonal immunoglobulins (IgG or IgM)	Lymphoproliferative diseases
Type 2 (mixed)	Polyclonal immunoglobulins (mainly IgG) plus monoclonal immunoglobulins (IgM, IgG, IgA)	Mixed cryoglobulinemia
Type III(mixed)	Polyclonal IgG and polyclonal IgM	Mixed cryoglobulinemia

in patients with chronic HCV (from 19 to 50% according to different studies) [7–11]. However, in many patients CGs are present at low levels and symptoms are often absent or very mild. Only about 5% of HCV-infected subjects have clinically overt MC syndrome. MC syndromes as well as other HCV-EHDs represent an important spectrum of HCV-related diseases and a good model for the study of viral-driven immunologic and neoplastic disorders [7–10].

Pathogenesis of MC

HCV is both hepato- and lymphotropic [6–9, 12]. Infected lymphoid tissue is a "reservoir" of the virus in the host and a potential site for the persistence of the infection [6–9, 12]. The mechanisms responsible for the expansion of autoantibody-producing B-cells and chronic lymphoproliferation in HCV infection remain controversial [7–9, 12]. Chronic lymphoproliferation may be enhanced by the interaction of the E2 viral envelope protein and the CD81 receptor of infected cells, which may increase genetic rearrangements in antigen-reactive lymphocytes [7–9, 12], as shown by the presence of the t(14;18) translocation [13]. The subsequent activation of the protooncogene Bcl2, with antiapoptotic activity, is responsible for the prolonged survival of lymphocytes. This hypothesis may explain how HCV could be responsible for different autoimmune and LPDs, in genetically predisposed subjects, under the influence of environmental factors that remain not yet well known [7–9, 12].

Clinical Features of MC

The skin, kidney, nerves, and joints can be affected by cryoglobulins. The skin is involved in 95% of cases. The appearance of cutaneous vasculitis includes palpable purpura, hemorrhage which includes purplish spots or patches in the lower extremities and large necrotic ulcerations. Biopsy of skin lesions shows immune-complex vasculitis of small vessels with mononuclear infiltration. These lesions are caused by plugging of the dermal capillaries with MC. HCV antigens are detected in skin lesions in 40% of cases [14]. A sensation of cutaneous burns occasionally

precedes its appearance and is usually self-limited with resolution of symptoms within a week. The vasculitic process can progress to necrotizing skin lesions with ulcerations in approximately 10% of patients. The severity of vasculitis correlates with the level of HCV viremia but not with the level of serum cryoglobulins. MC may also involve the nervous system. Sensorimotor neuropathy arises from cryoglobulin deposition in the vasa vasorum. Painful paresthesias and concomitant weakness, particularly in the lower limbs may occur [15]. Mononeuritis can be manifested by foot or wrist drop.

The isolation of serum CGs is complex and can lead to false negative results. Whole blood is obtained at warm temperature and then centrifuged for 2–3 min at 37°C; the serum is then collected and incubated at 4°C for 1 week for evidence of protein precipitates. Due to the fact that some mixed CGs are present in low concentrations, the differentiation between type II and III CGs often requires a more sensitive method for immunochemical characterization [16]. RF is detected in nearly all patients with MC. Thus, it is often helpful to assess for the presence of both RF and CGs. If RF is present but MGs are not detected in a patient with the clinical appearance of MC the test for CG is likely to be a false negative. In contrast, it is extremely rare to have MC in the absence of RF.

Non-Hodgkin's Lymphoma (NHL)

A frequent reported association of HCV infection is NHL [17, 18]. MC may be the intermediary disorder and has been observed to proceed NHL in up to 11% of cases [19]. The association is typically associated with low-grade NHL.

HCV viremia has been reported in up to 35% of patients with B-cell lymphoma and almost 90% of NHL patients with cryoglobulinemia [20]. There is contradictory evidence regarding the presence of HCV in malignant cells [21, 22]. Some studies did not detect HCV in the lymphoma cells; others have repeatedly demonstrated HCV-RNA in lymphoid organs and bone marrow cells.

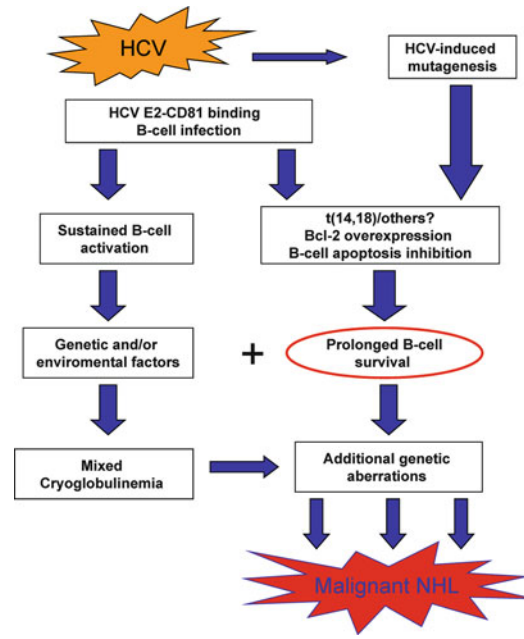

Fig. 6.1 Possible mechanisms involved in the pathogenesis of MC and/or other HCV-related LPDs (modified from Zignego et al. [111], © 2008 with permission from Elsevier)

Pathogenesis of Non-Hodgkin's Lymphoma

The pathogenetic mechanism of LPDs may be due to long-term HCV infection, resulting in clonal B-cell expansion of immunoglobulin (cryoglobulin)-secreting lymphocytes, and ultimately a combination of genetic and environmental factors resulting in a mutational event with activation of oncogenes and resulting in NHL (Fig. 6.1).

Another possibility is the inhibition of apoptosis of HCV-infected lymphocytes by t(18;14) translocation, which results in an overexpression of the bcl2 oncogene, and a second mutation (myc oncogene) leading to the development of lymphoma [23, 24].

The most common types of NHL associated with MC are follicular lymphoma (CFL), B-cell chronic lymphocytic leukemia/small lymphocyte lymphoma (B-CLL), lymphoplasmocytoid/immunocytoma (LPL) and marginal zone lymphoma (MZL).

Approximately 65% of HCV-related NHL show extranodal involvement (particularly salivary glands and liver) compared with 19% of non-HCV-related lymphomas [25]. This characteristic is related to the hypothesis that B-cell NHL arises selectively from the marginal zone B-cell. The extranodal marginal zone cell lymphomas seem to derive from organized lymphoid tissue which develops in response to an infection or as a component of an autoimmune disease. Several studies showed a strong link between HCV infection and mucosa-associated lymphoid tissue (MALT) lymphoma [26, 27]. HCV RNA has been isolated in the gastric mucosa of patients with MALT lymphoma, suggesting the possibility that HCV may be involved in its pathogenesis [27].

Gammopathies of Uncertain Significance (MGUS)

Other hematologic disorders in the course of HCV infection are gammopathies of uncertain significance (MGUS): usually they are gammopathies IgM/Kappa.

MGUS are present in up to 11% of patients with HCV infection without cryoglobulins. Some authors reported an association with HCV genotype 2a/c [28]. These monoclonal gammopathies have to be monitored in order to exclude the possibility of an evolution to multiple myeloma. Diagnosis may be missed over a long period, due to the occult presentation and/or similarity of symptoms to those of chronic HCV infection.

Renal Diseases

The association between chronic HCV infection and glomerulonephritis has been clearly shown both in native and transplanted kidney [29, 30]. The most common form of HCV-associated glomerulonephritis is membranoproliferative glomerulonephritis (MPGN) which can occur with or without MC. Membranous nephropathy is observed less commonly [31, 32]. Nephropathy is observed in 20% of patients with MCS [30]. These patients have a worse prognosis compared to patients with MCS and no nephropathy [32].

Mechanisms of HCV-Induced Kidney Injury

HCV-induced nephropathy is secondary to mixed cryoglobulins that are deposited in the mesangium of the glomerulus. Their nephrotoxicity is attributed to particular affinity of the IgM-k-RF for cellular fibronectin in the mesangial matrix. Cryoglobulins are deposited in the glomerular capillaries and appear as eosinophilic material on histologic analysis that stain densely with antisera to IgM, C3, and fibrin by immunofluorescence [33]. This is usually associated with other histologic signs of vasculitis and fibrinoid necrosis of the glomeruli. Cryoglobulins may also induce endothelitis via antiendothelial antibody activity and complement activation leading to overexpression of VCAM-1 and subsequent platelet aggregation [33]. Besides MPGN, other forms of glomerular disease have been associated with HCV infection including IgA nephropathy, postinfectious glomerulonephritis, membranous nephropathy, thrombotic microangiopathies, focal and segmental glomerulosclerosis [34], and fibrillary or immunotactoid glomerulopathy [35].

Diagnostic Criteria

Renal involvement is reported to occur in one-third of patients with MC [34], but the reasons this only occurs in a limited proportion of patients remain unknown. Renal signs of cryoglobulinemia include proteinuria (sometimes in nephritic range, i.e., >3 g/24 h) and microscopic hematuria with mild-to-moderate renal insufficiency [34]. Glomerular disease can manifest itself as acute oliguric renal failure in 5% of cases [34]. The majority of these patients develop severe hypertension that is often difficult to control. However, in the vast majority of patients the glomular disease is indolent. Histological analysis of the kidney demonstrates typical immune complex deposition within glomeruli characteristic of MPGN, infiltration of the glomerular capillaries, and expansion of mesangial matrix [31, 34, 36].

The long-term outcome of HCV-associated nephropathy remains ill defined. In a recent retrospective cohort study involving over 470,000 adult veterans, patients with nephropathy and HCV

infection were more likely to develop end-stage renal disease (4.3 vs. 3.1/1,000 person years) than HCV-seronegative patients [37]. In a cross-sectional study, HCV-positive patients were found to have a 40% higher likelihood for developing renal insufficiency compared to subjects without HCV [38]. In patients with an estimated glomerular filtration rate (GFR) ≤30 mL/min, the presence of HCV was associated with a nearly threefold increased risk of progression to end-stage renal disease.

The diagnosis of an HCV-related nephropathy should be strongly suspected in a patient with HCV infection, proteinuria, hematuria, and low serum concentrations of complements [34]. All patients with chronic HCV should therefore have urinalysis performed as part of their initial evaluation, particularly if they have hypertension or any other signs of renal dysfunction.

HCV-Related Arthritis

Arthritis in the course of chronic HCV infection can be seen either as part of the autoimmune processes (e.g., associated with cryoglobulinemia) or independently.

HCV arthritis unrelated to cryoglobulinemia is far less common but represents an independent entity. The incidence of HCV in patients with rheumatoid arthritis (RA) ranges from 0.65%, similar to that observed in the general population, to 7.3%. Whether arthritis is specifically attributable to HCV infection or rather to the nonspecific result of a chronic inflammatory process remains undefined in many patients. Some authors suggest that arthritis and HCV infection coexist by chance; others suggest that HCV might act as a trigger of the immune disease in individuals genetically predisposed to RA. Others feel that HCV may cause a distinct infectious arthritis.

Two subsets of articular involvement have been identified in patients with chronic HCV. A polyarthritis involving small joints that resembles RA is the most common. A nonerosive oligoarthritis involving the medium-sized and large joints is less common and appears to be associated with MC [39]. RF is positive in 50–80% of cases.

Antibodies anticyclic citrullinate peptide (anti-CCP) are positive in less than 6% of patients with HCV-associated arthritis which may help to differentiate the two conditions. Cryoglobulins may or may not be detectable. Other incidental antibodies can be detected (ANA 10–38%, anti-DNA 5%, acL 25%, anti-SSA 1%) [40]. HCV infection should therefore be considered in the differential diagnosis of patients with atypical arthritis.

Sjögren Syndrome (SS)

Several studies describe chronic lymphocytic sialoadenitis similar to sialoadenitis associated with idiopathic SS in approximately 50% of patients with HCV infection [41]. Up to 6% of patients with SS are HCV positive as opposed to ~1% in the general population. Studies in mice have shown that HCV can lead to an exocrinopathy similar to human SS, and suggest that a cross-reactivity between the HCV envelope and host salivary tissue can lead to an immune reaction directed against salivary glands [42]. HCV antigens are not detected in affected glands, but HCV RNA is present in the saliva of patients with HCV-associated sicca syndrome. SS related to HCV may evolve into a B-cell malignant lymphoma, especially if it coexists with MC [43].

There are some substantial differences between the primary SS and the HCV-related SS. The pseudo-SS associated with HCV infection is not associated with anti-SSA and anti-SSB antibodies. In contrast, RF is positive in the majority of patients with HCV-induced SS and is frequently associated with MC. Clinically, xerophthalmia and xerostomia are mild or absent in 90% of patients, whereas arthritis, cutaneous vasculitis, and neuropathy beyond alteration of liver function are more frequent [44]. Histological samples show a milder lymphocytic pericapillaritis. The lymphocytic type of the infiltrate in the minor salivary gland shows a predominance of CD8 lymphocytes which is not observed in primary SS [45]. Patients with SS and HCV should be considered to have HCV-induced SS as opposed to primary SS.

Lichen Planus

Lichen planus is a recurrent pruritic eruption characterized by flat-topped violaceous papules that can develop on any skin site (arms, trunk, genital, nails, and scalp), including mucosal membranes (oral). Skin biopsy reveals lymphocytic infiltration with CD4+ cells in the upper dermis, with vacuolar degeneration of basal epithelium and the presence of acidophilic bodies, probably represented by apoptotic keratocytes [46]. HCV seems to replicate in the epithelial (skin and mucosal) cells. Some studies suggest an association between HCV-induced cirrhosis and lichen planus, not infection alone. The prevalence of HCV in patients with oral lichen planus (OLP) is estimated around 27% [47]. HCV-RNA has been found in oral mucous membrane biopsy supporting the association between OLP and HCV [48].

Available Data on the Current Treatment of Extrahepatic Manifestations Related to the Immune Stimulus of HCV

Mixed Cryoglobulinemia

The realization that HCV was the etiologic agent responsible for most cases of MC led to concerns that potential adverse effects could occur if MC was treated with immunosuppressive therapy. Alternatively, the link between HCV and MC provided the opportunity to control HCV-MC with antiviral therapy [49]. The usefulness of IFN therapy for HCV-related MC is now firmly established. Antiviral therapy has been shown to reverse bone marrow monoclonal B-cell expansion and in patients with HCV-MC [50] and to reverse cutaneous vasculitis [51]. However, additional therapy may be needed in MC patients with severe organ involvement and in those patients who do not respond to peginterferon and ribavirin therapy [51, 52]. In these patients, rituximab a chimeric monoclonal antibody that binds to the B-cell marker CD20 has been utilized [53]. Several studies have shown that cryoglobulins disappear from serum in two-thirds of the treated patients and this is associated with a clinical improvement [54–58]. Unfortunately, symptoms usually reappear with reconstitution of peripheral B-cells. Although rituximab is generally safe and well tolerated [59, 60], its use has been associated with modestly elevated (up to twofold) levels of HCV viremia [54, 61]. The safety and efficacy of multiple courses of rituximab in the setting of HCV MC remain undefined. The combination of Peg-IFN plus ribavirin (SoC) with rituximab has been shown to shorten the time to clinical remission, improve renal response rates, and yield higher rates of cryoglobulin resolution [62]. Persistence of MCS stigmata has been described after successful antiviral therapy and without evidence of occult HCV persistence [63, 64].

Prednisone 1–1.5 mg/kg/day has successfully been used to treat vasculitic symptoms. There are extremely limited data on the palliative use of plasmapheresis to treat HCV MC vasculitic symptoms. However, case reports suggest that it is safe and may result in improvement in glomerulonephritis [65], neuropathy [66], and purpura [67, 68]. An integrated approach to the treatment of HCV-related MC with the current Standard of Care (SoC) is shown in Fig. 6.2.

Non-Hodgkin's Lymphoma

Although antiviral therapy appears to be an attractive therapeutic tool for low-grade HCV-positive NHL [51, 69], in intermediate and high-grade NHL, chemotherapy is expected to be necessary and antiviral treatment may be suggested as maintenance therapy after chemotherapy is completed and patients are in remission. Remission of LPD after HCV eradication in some patients with splenic and gastric MALT lymphomas has been reported [70]. However, it has been observed that, even in cases of responsive SLVL, the rearrangement of the monoclonal Ig genes was still detectable [71]. In some patients these types of LPDs may be independent of HCV infection. Immunotherapy with rituximab is widely

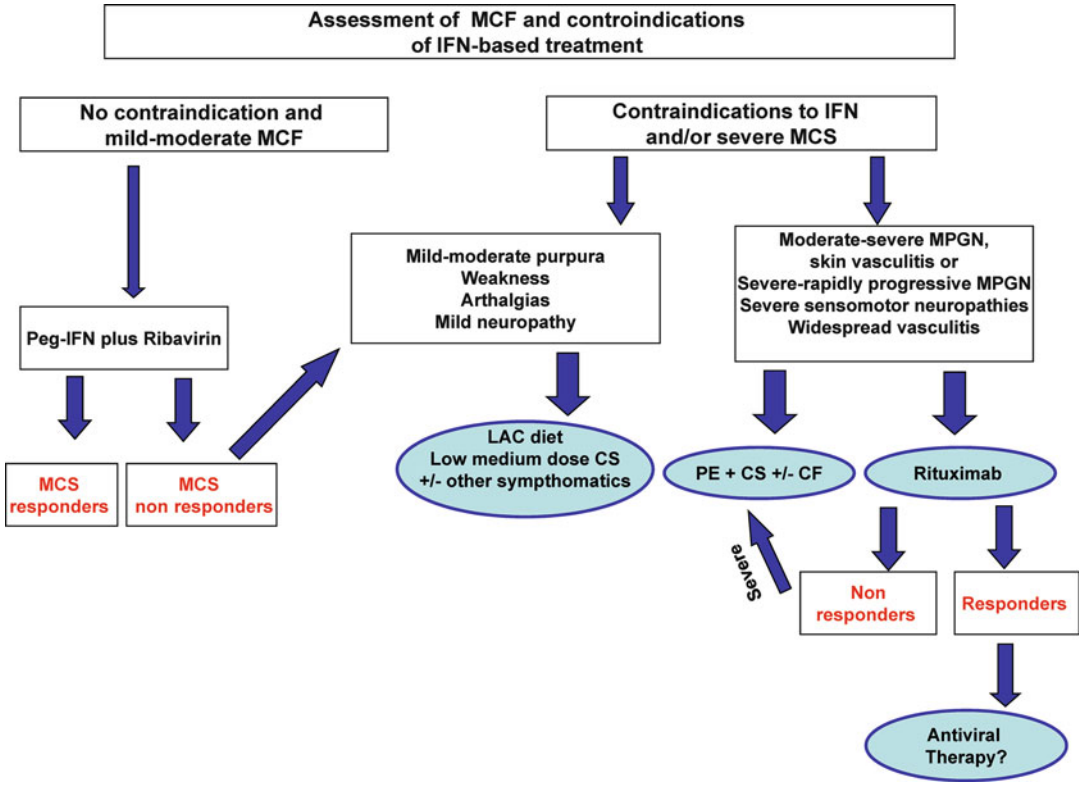

Fig. 6.2 Algorithm for the treatment of HCV-positive mixed cryoglobulinemia patients (modified from Craxì et al. [112]. © 2008, with permission from Elsevier)

utilized in NHL [72]. Rituximab in addition to cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) chemotherapy represents the gold standard for the treatment of nodal aggressive NHL, since it improves response rate, overall survival (OS), and event-free survival vs. chemotherapy alone. Patients who were not treated for chronic HCV prior to chemotherapy should be treated after they have achieved remission and recovered from the side effects of chemotherapy.

Antiviral therapy with peginterferon and ribavirin alone does not achieve remission of NHL in a large percentage of patients. The use of rituximab has been associated with the development of viral reactivation [73]. Preliminary data suggests the combination of Peg-IFN plus ribavirin (SoC) with rituximab can achieve complete response of B-cell NHL in nearly all patients [66].

HCV-Related Glomerulonephritis

Hypertension, proteinuria, and progressive renal failure are the main clinical manifestations of HCV-associated chronic kidney disease (CKD). Thus, renal protection with blood pressure-lowering and antiproteinuric agents should be utilized [74, 75]. Diuretics, renin–angiotensin system inhibitors (either angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers), and eventually lipid-lowering agents have also been proven to be beneficial in patients with CKD resulting from HCV [76].

Given the link between HCV infection and the immune response targeting the glomerulus, both antiviral and immunosuppressive therapies have been utilized in patients with HCV-induced GN [77]. The goals of these agents are to eliminate HCV and/or to reduce the production of HCV-related

antibodies and immune complexes. Patients who achieve a sustained virologic response following treatment with peginterferon and ribavirin have a marked reduction or resolution of proteinuria and improvement in serum creatinine. The best results are achieved in patients with lower values for serum creatinine and prior to the development of irreversible morphologic changes to the glomeruli. A meta-analysis has compared the efficacy and safety of interferon-based therapy to corticosteroids alone or in combination with cyclophosphamide. Proteinuria improved to a greater extent and in more patients with interferon-based therapy as opposed to treatment with immune suppressive agents with an odds ratio of 3.9 and proteinuria improved in all patients when HCV RNA became undetectable [78]. However, both treatments failed to significantly improve renal dysfunction. These data strongly suggest that interferon should be the primary treatment in patients with HCV-induced GN. Uncontrolled studies have suggested that corticosteroids can achieve remission in acute GN, but was poorly tolerated [79, 80]. Caution should be taken when using immunosuppressive agents in HCV-associated glomerulonephritis because of the concern for increasing viral replication which could possibly exacerbate GN [81]. Plasma exchange has also been utilized with limited success [82–84]. Since HCV-induced GN is a manifestation of MC, rituximab has also been utilized. In one case series, 22 patients with MC and GN had a marked reduction in proteinuria and renal function stabilized following treatment with 2–6 weekly doses of rituximab [85].

HCV-Related Arthritis

Few data exist regarding the treatment of HCV-associated arthritis. As a consequence, the therapeutic approach for this disorder is still largely empirical. Nonsteroidal anti-inflammatory drugs (NSAIDs), hydroxychloroquine, and low doses of corticosteroids are the cornerstones of the treatment of HCV-related arthritis, but some authors describe an incomplete relief of symptoms, especially in the rheumatoid-like subset. Low doses of corticosteroids and NSAIDs are more effective in subjects with mono-oligoarthritis. In patients with severe arthralgias immunosuppressive agents have been utilized. Methotrexate should be utilized with caution and not without monitoring liver histology as this agent is known to cause hepatic fibrosis. Although successful interferon-based therapy may lead to resolution of myalgias and arthralgias, interferon may exacerbate these symptoms and many patients cannot tolerate therapy.

Sjögren's syndrome

Few authors have described the evolution of SS associated with HCV when chronic hepatitis C is treated with antiviral therapy. Doffoel-Hantz et al. [86] described a high incidence (more than 50%) of immunological IFN-mediated complications in patients with SS and HCV infection. However, several anecdotal reports have described an improvement in sicca symptoms following successful HCV treatment with peginterferon and ribavirin.

Lichen Planus

Successful treatment of HCV with peginterferon and ribavirin has been shown to lead to resolution of lichen planus. However, treatment with interferon may also lead to the appearance of lichen planus. In patients who do not respond to interferon, lichen planus has been successfully treated with topical immune suppressive agents.

Future Perspective in the Treatment of Extrahepatic Manifestations of HCV

Potential Role of DAAs

The treatment of extrahepatic manifestations of HCV is likely to improve significantly with the approval and availability of protease inhibitors. When combined with peginterferon and ribavirin

a sustained virologic response of approximately 70% has been reported [87–89]. It is yet unknown if resolution of immune-related manifestations of HCV will occur in all patients who achieve an SVR with these medications. Future studies will explore these data when DAAs are available. As additional, direct antiviral agents are developed, the potential exists to treat HCV with several STAT-C agents alone, without interferon and/or ribavirin. Studies to address this possibility will likely be performed soon after these agents are available.

These observations suggest that suppression of HCV with protease and polymerase inhibitors may be an important avenue for treatment for many extrahepatic manifestations of HCV in the future.

HCV-Related Endocrinological Disorders

The most frequent and clinically important endocrinologic manifestations of HCV are thyroid disorders and type 2 diabetes mellitus (T2D).

Thyroid Disorders and HCV

A spectrum of clinical thyroid disorders may be observed in patients with HCV including hypo- or hyperthyroidism, Hashimoto's thyroiditis, or the presence of antithyroid autoantibodies in the absence of overt thyroid disease [90–94]. The prevalence of thyroid disease is more frequently observed in HCV-positive patients compared to patients with viral hepatitis B or D [93, 95, 96] or normal subjects [94, 97]. The most frequent thyroid disorder in patients with chronic HCV is simply the presence of circulating antithyroid antibodies. This is most commonly seen in female subjects [93]. Subclinical hypothyroidism was observed in 2–9% of patients with chronic HCV infection, particularly in those patients with MC [92, 95, 98]. It has been suggested that these patients may be susceptible to develop Hashimoto's autoimmune thyroiditis and Grave's disease when treated with interferon.

Approximately 5–12% of HCV patients receiving interferon therapy appear to develop thyroid disease [92–94, 99, 100]. These disorders resolve in the majority of cases 6 months after therapy is discontinued [101, 102]. Patients with chronic HCV have also been found to have a higher prevalence of papillary thyroid carcinoma [100, 103].

Because of the high prevalence of thyroid disorders in patients with chronic HCV, thyroid function studies to include free T4 and TSH should be performed in all patients. Since interferon-based therapy could exacerbate thyroid dysfunction, patients with abnormal thyroid function tests should be fully evaluated and treated if necessary prior to initiating HCV treatment. Patients should also be monitored at periodic intervals during treatment for either the onset of new thyroid dysfunction or an exacerbation of a previously recognized thyroid disorder. Hypothyroidism which develops or is exacerbated by interferon can be treated by adjusting the dose of thyroid replacement and only rarely requires HCV treatment to be discontinued. In contrast, hyperthyroidism which develops during interferon therapy may be more difficult to manage and may require that HCV treatment be discontinued.

Diabetes Mellitus

In several studies, a high prevalence of diabetes mellitus type 2 has been observed in patients with chronic HCV infection. In contrast, no association with diabetes mellitus type 1 has been identified [11, 101, 102, 104–106]. Among 9,841 subjects, the risk of diabetes mellitus type 2 was nearly three times higher in patients over the age of 40 years with HCV than in HCV-negative subjects. In addition, a higher incidence of type 2 diabetes mellitus is found in patients with chronic HCV when compared to patients with other liver disorders [101, 106]. The association between chronic HCV and DM is independent of the severity of the liver disease. In patients with HCV infection, the appearance of diabetes type 2 is associated with insulin resistance, but not with

the presence of pancreatic anti-insulin antibodies [107]. In contrast, interferon treatment of HCV has been associated with the development of anti-pancreas autoimmunity and the appearance of diabetes mellitus type 1 [108–110].

In patients with HCV, the risk factors for diabetes include older age, HCV genotype 3, severe liver fibrosis, family history of diabetes, and liver/kidney transplantation [105]. The blood sugar should therefore be monitored in patients with chronic HCV infection.

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Chronic HCV and HIV Coinfection

7

Patrick Ingiliz, Maud Lemoine,
and Yves Benhamou

Keywords
HIV–HCV coinfection • ART • Liver fibrosis progression • Drug toxicity • Steatohepatitis • Directly acting agents • Liver transplantation • HCC

Historical Perspective

The acquired immunodeficiency syndrome (AIDS) was described in the early 1980s. As no treatment options existed at that time, most patients eventually died from infections following the impaired immune system. So-called opportunistic infections, such as pneumocystis pneumonia, cryptococcal meningitis, and mycobacterial infections or AIDS-related malignancies (e.g., lymphoma) were the main cause of mortality in these patients. Some of these infections have the ability to affect the liver and these conditions as well as AIDS-associated malignancies infiltrating the liver determined the hepatologist's focus in this setting.

P. Ingiliz, MD (✉)
Department of Infectious Diseases, Medical Center for Infectious Diseases, Seestr. 13, Berlin 13353, Germany
e-mail: p_ingiliz@web.de

M. Lemoine, MD, PhD
Hepatology Department, St. Antoine Hospital, 184, rue du Faubourg Saint-Antoine, Paris 75012, France

Y. Benhamou, MD, PhD
Hepatology Department, Pitié-Salpêtrière Hospital, 87, boulevard de l´Hôpital, Paris 75013, France

With the introduction of combination antiretroviral therapy (ART) life expectancy for human immunodeficiency virus type 1 (HIV-1)-infected patients has dramatically improved and liver diseases have emerged as an important factor of non-AIDS morbidity and mortality in HIV patients [1, 2]. Huge observational cohorts were able to show that many of those who developed liver failure or died had controlled HIV infection and an established immune system [1–3]. This indicated that these patients would have probably not died otherwise.

Hence, liver-related pathologies have become one of the key issues in HIV patient care with viral hepatitis coinfection (especially hepatitis C virus [HCV]) being the cornerstone due to high coinfection rates in some parts of the world and certain patient groups [4, 5]. Chronic coinfection with HCV is common in the HIV-infected population: Of the 35 million people currently living with HIV worldwide around 20% (~7 million) have chronic hepatitis C. This population is mainly represented by individuals with a past history of intravenous drug use, hemophiliacs, and recipients of contaminated blood [6]. Moreover, outbreaks of hepatitis C among HIV-positive homosexual men have been reported in several large European and North American cities

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since the year 2000 [7–9] making HCV a sexually transmitted disease in this setting.

As HIV outcomes are likely to improve with better tolerated treatment options, HCV-associated mortality and morbidity is likely to increase.

The Natural Course of HCV in the Setting of HIV Coinfection

HIV coinfection has a negative impact on the course of chronic hepatitis C: HIV coinfected patients have an increased risk to develop liver cirrhosis compared to non-HIV-infected patients with other liver diseases [10]. Several groups found a faster progression of liver fibrosis in this setting [11, 12]. Factors associated with an increased risk of advanced fibrosis in coinfection include male gender, older age, and alcohol consumption >50 g/day [13]. In coinfected patients, a low CD4 count, alcohol consumption, age at HCV infection, and hepatic necroinflammation at liver biopsy are associated with a higher liver fibrosis progression (LFP) rate [11, 14].

Despite its potential hepatotoxicity [15, 16] (see below) the prevalence of cirrhosis and mortality seems to be lower in patients with effective antiretroviral treatment [17–19]. This is reflected in recent guidelines for the treatment of HIV-infected patients that recommend earlier ART start (e.g., a CD4 lymphocyte count below 500/mm^3 or even above) in patients with viral hepatitis coinfection [20–22].

The "real" estimation of LFP in clinical practice underlies several limitations. First, "liver fibrosis progression" expresses a rate between a nominal variable expressed as a number and a continuous variable: the time between two biopsies. It's value to analyze the natural history of hepatitis C and its validity are somewhat controversial. It has been suggested that fibrosis progression is not linear especially in HIV–HCV-coinfected patients: thus, e.g., Metavir fibrosis stage 4 is not two times Metavir stage 2. In fact the time needed to progress from stage 1 to stage 2 is not the same needed to progress from stage 3 to stage 4. Moreover, only few studies examined paired (or serial) liver biopsy samples over a sufficient period of time and in most studies the number of patients is limited. Another problem is the inability to determine the exact point of time at which HCV infection occurred. Very few patients with HCV infection had only one risk event that can be identified as the source of infection. In injection drug users, for example, most investigators usually consider the year of the first needle use as the year of HCV infection which hardly reflects the "real" event. Consequently, liver fibrosis is assumed to be absent at HCV infection. This approach does not take into account several concomitant factors that may lead to liver fibrosis in the setting of HIV infection (see below).

Macias et al. [23] performed paired biopsies in HIV–HCV-coinfected patients and observed a fibrosis progression of one or more fibrosis stages in 44% of their patients over a period of 3 years. This is consistent with other paired-biopsy reports [24]. Progression of fibrosis was predicted by the degree of necrosis and inflammation at the first biopsy. Response to anti-HCV therapy and suppression of HIV replication with potent ART were associated with slower or no fibrosis progression. Regression of liver fibrosis has been observed in HIV-infected and uninfected subjects with chronic hepatitis C who achieve sustained virological response (SVR) [25, 26] and in HCV-monoinfected patients treated with pegylated interferon plus ribavirin without SVR [27].

In a more recent report, Sterling et al. found similar fibrosis progression in 59 HIV–HCV-coinfected patients matched to HCV-monoinfected patients by age and fibrosis stage [28]. HIV patients had higher rates of piecemeal necrosis and lobular inflammation at baseline. However, the only factor associated with fibrosis progression (in both groups) was an increased body mass index (BMI). Of note, the median CD4 lymphocyte count in their study was 515/mm^3 and all patients were nondrinkers. It has to be hypothesized that in a well-controlled HIV population and in the absence of comorbidities, fibrosis progression might not be increased compared to HCV monoinfection.

Hypothetically, the order of infection may be clinically relevant. In "classical" HIV–HCV collectives, HCV usually occurs before HIV infection due to higher transmissibility from a small inoculum (e.g., needlestick). In more recently observed sexually transmitted HCV infection in MSM, HIV infection has already been established which may lead to a qualitative change of adaptive immunity [7, 29]. High rates of chronic evolution and fast fibrosis progression have been reported in this setting where HIV infection antedates HCV infection [30, 31]. However, these observations remain to be confirmed. Nevertheless, this subgroup of patients will allow a more sophisticated estimation of fibrosis progression as most patients attend regular HIV checkups including liver function tests before being diagnosed with hepatitis C. Therefore, the estimation of the transmissive HCV event is very accurate.

HIV infection of CD4 lymphocytes probably contributes to the greater HCV persistence seen in HIV-infected persons compared to those without HIV [32]. The nadir of CD4 lymphocyte depletion has been shown to correspond with the magnitude of reduction in HCV-specific CD8+ lymphocyte responses [33, 34]. Similarly, HCV-specific humoral responses appear to be indirectly correlated with CD4 lymphocyte suppression [35].

Hepatic Particularities in HIV Infection (Fig. 7.1)

HIV infection adversely affects all phases of the natural history of chronic hepatitis, increasing the frequency of viral persistence after acute infection, the level of viremia among persistently infected persons, the rate of progression to cirrhosis, and the proportion of persons who will ultimately develop end-stage liver disease [36–39].

The reason why HIV–HCV-coinfected patients tend to faster disease progression is not entirely understood. Factors that have been associated with fibrosis progression in HCV-monoinfected patients are older age, male sex, alcohol consumption, obesity, insulin resistance (IR), hepatic steatosis, immunosuppression, nonresponse to interferon treatment, and necroinflammatory activity in liver biopsy (Table 7.1). In the HIV-infected population, some of these factors deserve special consideration as they may develop independently due to the chronic inflammatory state or the antiretroviral treatment.

Extensive Alcohol Intake

Excessive alcohol consumption and alcoholic-related liver injuries are not well documented in HIV patients. Studies regarding the rate and the impact of alcohol use disorders are rare and contradictory. Higher alcohol intake has been reported in this population [40]. In contrast, in the Swiss cohort study [41], an excessive alcohol consumption (>40 g/day for women and >60 g/day for men) was seen in only 4% of patients. Several studies have also shown an impact of moderate-to-severe alcohol consumption on unprotected sexual behavior and HIV transmission.

Insulin Resistance and Metabolic Steatosis

In the HIV-negative population, nonalcoholic fatty liver disease (NAFLD) has become a common cause of chronic liver disease that is related to the increasing prevalence of obesity in industrialized countries. NAFLD has been identified as the hepatic manifestation of the metabolic syndrome and it commonly encompasses two histopathological entities: the most common benign "simple" steatosis and its necroinflammatory form, nonalcoholic steatohepatitis (NASH). NASH can be associated with significant liver fibrosis in about one third of patients leading to cirrhosis and its complications [42]. The pathological mechanisms that lead to steatohepatitis and liver fibrosis remain unclear. However, there is no doubt that IR, the hallmark of the metabolic syndrome, plays a central role in the development of fatty liver disease.

In HIV-infected patients, IR was found to be associated with HIV viral load, the lipodystrophy syndrome, HCV coinfection, and antiretroviral treatment. Assessment using the HOMA index

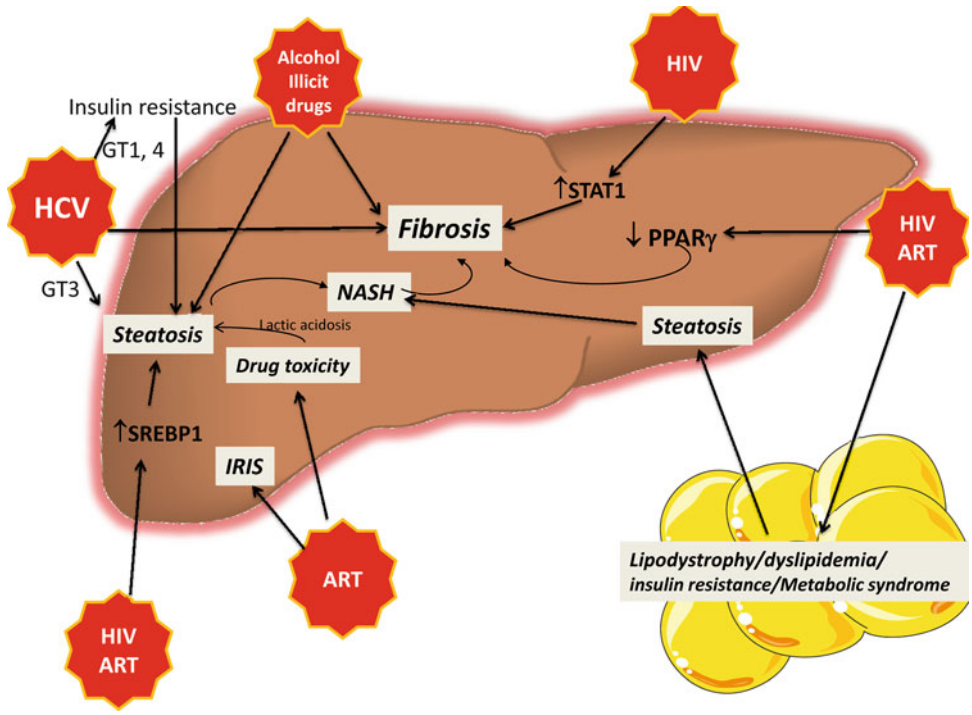

Fig. 7.1 Liver damage caused by HIV infection, antiretroviral treatment, and HCV coinfection. *HCV* hepatitis C virus; *GT* genotype; *HIV* human immunodeficiency virus; *ART* antiretroviral treatment; *SRBP* sterol receptor element binding protein; *PPAR* peroxisome proliferator-activated receptor; *NASH* nonalcoholic steatohepatitis; *STAT* signal transducer and activator of transcription factor

Table 7.1 Factors associated with fibrosis progression in HCV monoinfection and HIV–HCV coinfection

Fibrosis progression increased by
Age
Male sex
Alcohol consumption
HIV infection, low CD4, HIV viral load
Immunosuppression
Insulin resistance
High BMI/Severe Steatosis
Necroinflammatory activity in liver biopsy
Nonresponse to interferon therapy

(homeostasis model assessment: fasting serum glucose (mmol/L) × fasting serum insulin (mU/L)/22.5) showed that 12.4% of treatment-naïve HIV patients [43] were insulin resistant compared to 55% of treated patients with and without HCV coinfection [44]. Palacios et al. described an incidence of IR of 13% in HIV patients 1 year after the initiation of antiretroviral treatment [43]. In this study, HCV coinfection and indinavir exposure were independent risk factors for IR. Grunfeld et al. compared 927 HIV patients with 258 non-HIV patients [45]. This study showed that the rate of HOMA index >4 was significantly higher in the HIV group (37 vs. 27%, $p = 0.005$). A HOMA index >4 was associated with a higher volume of thoracic subcutaneous adipose tissue and visceral adipose tissue.

The reasons for the changes in insulin sensitivity in these patients are not well understood but are without doubt promoted by the virus itself or its antiretroviral treatment. Several experimental studies have suggested direct interaction between the virus and molecules involved in insulin signaling and indirect mechanisms by changes in the adipose tissue due to antiretroviral drugs. Experimental models demonstrated interactions between viral proteins (Vpr and Nef) and PPARγ (peroxisome proliferator-activated receptor gamma), a transcription factor that plays a crucial

role as well in insulin sensitivity as in fibrogenesis. These viral proteins could be able to inhibit the expression and the activity of PPARγ [46, 47]. The reduced PPARγ expression may also promote fibrosis development in these patients [48]. High rate of IR could explain the high prevalence of metabolic disorders in HIV-infected patients. Metabolic syndrome can be observed in 19.4–41.6% of HIV patients on antiretroviral treatment [49]. These metabolic disorders participate in the development of NASH which has been described in several studies. Sutinen et al. have demonstrated an increased fat accumulation in the liver by proton spectroscopy in 25 HIV patients with lipodystrophy compared to healthy controls [50]. By using the same technique, Hadigan et al. found steatosis in 42% of cases among a small cohort of 33 HIV-infected nondrinkers [51]. More recently, NAFLD was diagnosed in 36.9% of 225 HIV-monoinfected nondrinkers [52]. However, no histological analysis was performed in this study. Hepatic steatosis was independently associated with sex (OR 2.49), ALT activity (OR 4.59), waist circumference (OR 1.07), and duration of exposure to reverse transcriptase inhibitors (OR 1.12). Accordingly, in another study steatosis was diagnosed by ultrasound in 31% of 216 HIV-monoinfected patients [53]. Waist circumference, low HDL cholesterol, and hypertriglyceridemia were identified as risk factors for sonographic steatosis. In this study, patients with radiological steatosis or elevated transaminases had a liver biopsy and the diagnosis of NASH was histologically confirmed in 30% of the cases. In two different studies, NASH was common in HIV patients on antiretroviral treatment with persistent elevated transaminases without coinfection or excessive alcohol use [48, 54]. Lemoine et al. found histological proof of NASH in 57% and even a third had concomitant significant fibrosis [48]. Ingiliz et al. performed liver biopsies in 30 HIV-monoinfected patients. NASH was found in 53% of the biopsies and was associated with markers of IR, leading to significant fibrosis in 20% and even liver cirrhosis in 10% of the patients. Of note, the mitochondrial analysis in this study did not demonstrate any evidence of mitochondrial toxicity [54].

The potential steatogenic role of protease inhibitors (PI) has also been an issue of investigation. PIs have been shown to increase the levels of activated SREBP-1 (sterol receptor element binding protein), a transcription factor involved in lipid metabolism and lipogenesis [55]. Ritonavir inhibits in vivo the activity of lipoprotein lipase that causes hypertriglyceridemia [56]. Riddle et al. confirmed that ritonavir induces an increase of SREBP-1 maturation leading to steatosis [57]. Consequently, Lemoine et al. have shown that HIV patients with nonalcoholic steatosis have a liver overexpression of SREBP-1 compared to non-HIV patients with NAFLD and healthy controls [48]. Increased endoplasmatic reticulum stress and the activation of the UPR (unfolded protein response) by PIs further contribute to these metabolic alterations [58]. Thus, HIV patients on antiretroviral treatment are at high risk to develop metabolic hepatic injuries irrespective of viral coinfections or obesity. As these liver injuries can lead to severe liver damage, the diagnosis of NASH in HIV patients with persistent abnormal liver tests should be systematically discussed. The natural history of ART-associated NASH and its possible therapeutic interventions are not well established. Studies are needed to assess the efficiency of treatments that are under investigation in non-HIV patients (ursodeoxycholic acid, vitamin E, glitazones). The treatment of metabolic abnormalities (high blood pressure, hyperlipemia, diabetes), physical exercise, and dietary measures should be taken when applicable.

Drug Toxicity

Hepatotoxicity of antiretroviral treatment occurs in 5–10% of persons given a new antiretroviral regimen. This occurs more often if the person is coinfected with HCV or HBV [15, 59, 60]. However, there is not an internationally accepted definition of drug hepatotoxicity or drug-induced liver injury. Most studies focus on aminotransferase elevation reflecting hepatocellular injury [61], although other parameters (e.g., alkaline phophatase) may also reflect liver damage. The

AIDS Clinical Trials Group criteria [62] grades ALT or AST elevation according to the following score system: grade 1 (1.25×–2.5× upper limit of the normal range [ULN]); grade 2 (2.6×–5× ULN); grade 3 (5.1×–10× ULN); and grade 4 (>10× ULN). Apart from steatosis, HIV drugs can lead to hepatotoxicity by four other mechanisms: hypersensitivity reactions, direct mitochondrial inhibition, direct cell stress, and immune reconstitution in the presence of viral hepatitis coinfection [63].

HIV Infection of the Liver

HIV infects the liver but the degree to which replication occurs in hepatocytes, Kupffer cells, and stellate cells is largely unknown. In vitro data has shown that HIV envelope proteins may induce hepatocyte apoptosis, increase STAT1 (signal transducer and activator of transcription factor) activation and Fas ligand expression have been proposed as the underlying mechanism [64, 65]. HIV infection may also enhance liver fibrosis by increasing translocation of microbial products from the gut. HIV infection of gut lymphoid tissues is associated with enhanced microbial translocation and these products are taken up near the portal vein by Kupffer cells [66, 67]. This process could lead to increased toll cell 4 activation of stellate cells, especially if the Kupffer cell ability to take up bacterial products is diminished by HIV infection [68].

The amount of liver disease in the HIV-monoinfected population is unknown as liver biopsy is not routinely performed in this setting. The use of noninvasive markers and devices to assess liver fibrosis in HIV patients without hepatitis coinfection could yield important insight. To date only a few studies have been undertaken. A study utilizing APRI score (Aspartate-to-platelet-ratio-index) suggested that 8.3% of 432 patients with HIV had significant liver fibrosis [69]. Excessive alcohol consumption, type 2 diabetes mellitus, and a detectable HIV viral load were independently associated with liver fibrosis in this study. Sulkowski et al. described a similar prevalence of 7% using the APRI score in 1,845 HIV-monoinfected patients [70]. One study using transient elastography (Fibroscan®, Echosens, Paris) conducted in 1,307 HIV-monoinfected patients, another one using the APRI score in 533 patients showed a prevalence of significant fibrosis in only 1 and 3.9%, respectively [71, 72].

The Current Treatment for HCV Infection in the Setting of HIV Coinfection

The treatment for HIV–HCV-coinfected patients is not different from that of the HCV-monoinfected population. Due to faster fibrosis progression, treatment is generally more urgent and may be limited by drug interactions. Response rates in coinfected populations are, however, lower and treatment duration is longer. The current standard of care is also the combination of pegylated interferon alpha 2a (180 μg s.c. per week) or 2b (1.5 μg/kg s.c. per week) and ribavirin (1,000–1,200 mg p.o. per day).

Three key studies of HCV treatment in coinfected persons – the Adult AIDS Clinical Trials Group's ACTG 5071, Roche's APRICOT, and the ANRS HC02 (RIBAVIC) – have reported that pegylated interferon-based regimens are more effective than standard interferon-based regimens, although SVR rates were lower in these coinfection treatment trials than in HCV monoinfection treatment trials [73–76]. In APRICOT, the pegylated interferon/ribavirin SVR rate was 29% for genotype 1 infected persons and 62% for those infected with genotype 2 or 3 infection. Moreover, the APRICOT trial showed that coinfected patients with HCV genotype 2 or 3 have better SVR rates when treated longer (48 weeks) than the recommended duration in HCV-monoinfected patients. As in monoinfected patients, virologic response rates to pegylated interferon and ribavirin varied by baseline HCV viral load, and SVR rates in genotype 1 patients were >60% with pretreatment HCV RNA levels below 800.000 IU/mL [75].

In addition, the administered dose of ribavirin is an independent predictor of SVR and the highest response rates in monoinfected patients are

achieved with a daily dose of at least 10.6 mg/kg [27]. Consequently, in a Spanish multicenter trial (PRESCO), 389 HIV–HCV-coinfected patients with stable immune system (patients with a CD4 lymphocyte count below 300/mm^3 were not included) were allocated to receive higher weight-based doses of ribavirin (1,000 mg/day for body weight <75 kg and 1,200 mg/day >75 kg) than in the trials before. The overall SVR rate in PRESCO was higher (49.6%) than in the previous pivotal trials, but still only 35% in genotype 1 and 32% in genotype 4 infected patients [77]. Another important finding of this study was the positive predictive value (PPV) of becoming HCV RNA negative at week 4 (rapid virological response – RVR) [78]. Low baseline HCV RNA levels below 500.000 IU/mL and RVR were the strongest predictors of SVR in the PRESCO trial [79]. Consecutively, the PPV for RVR was high for all patients in this study except for genotype 1 patients with high viral load.

The higher level of serum HCV-RNA in coinfected vs. HCV-monoinfected patients (1 log on average) has been postulated to be one of the main reasons for the lower SVR rate to that of HCV therapy in coinfected vs. monoinfected patients [80].

Extension of treatment duration to 72 weeks in genotype 1 has shown promising results in difficult-to-treat (slow viral decline) HCV-monoinfected patients [81]. However, this treatment strategy has been hampered by higher dropout rates in coinfected patients [77]. In the PRESCO trial, treatment extension to 72 weeks did not lead to a significant increase in SVR rates in genotype 1 patients, but a trend towards a better response was seen in the multivariate analysis [78]. In a more recent analysis, extension to 72 weeks in genotype 1 coinfected patients led to significantly higher SVR and lower relapse rates [82].

Although the duration of treatment in HIV-positive patients coinfected with HCV genotype 3 has been established in 12 months [83, 84], recent reports have shown that treatment may be shortened to 6 months, at least for the subset of patients with RVR [85, 86]. It should be emphasized, however, that weight-based ribavirin (1,000/1,200 mg/day) dosing was used in all these trials.

These data have led to American and European guidelines suggesting a 48-week treatment duration with either pegylated interferon alpha-2a 180 μg or pegylated interferon alpha-2b 1.5 μg/kg s.c. weekly and weight-based ribavirin 1,000/1,200 mg/day p.o. for all genotypes in coinfected patients. In patients with RVR and genotype 2/3, treatment can be shortened to 24 weeks if the baseline HCV RNA was below 400.000 IU/mL and in the absence of significant liver fibrosis. In patients with genotype 1/4 without RVR, treatment should be extended to 72 weeks (Fig. 7.2).

Influenceable and Uninfluenceable Factors of Treatment Response

The Optimal Dose of Interferon

HCV viral kinetics in the first month of treatment are highly predictive of SVR and experimental studies have shown a viral decline in a dose-dependent matter in the beginning of interferon treatment [87–92]. In HCV-monoinfected patients, high induction doses of interferon failed to improve SVR rates [93–95]. Likewise, induction with pegylated interferon alpha-2a 270 μg weekly in HIV–HCV-monoinfected patients did not lead to favorable treatment outcomes [96] and an augmentation of interferon doses cannot be recommended to date.

The Optimal Dose of Ribavirin

The role of ribavirin dose has been an issue of discussion in recent years [97]. As it had been previously shown that SVR rates improve with higher doses of ribavirin (see above), and increasing the dose above the recommended thresholds is therefore a tempting approach in difficult-to-treat patients [98, 99]. The IDEAL study showed significantly higher SVR rates in HCV-monoinfected patients who had a decline in hemoglobin to below 10 g/dL [100] during treatment.

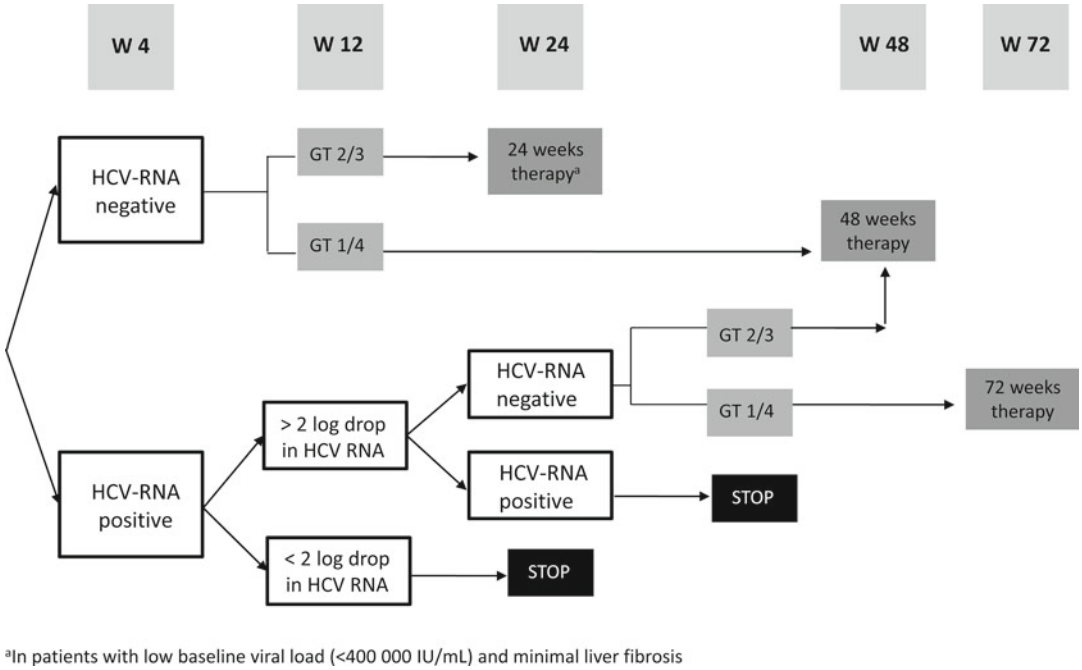

Fig. 7.2 European guidelines for the treatment of HIV–HCV coinfection (EACS guidelines, version 5-2)

In HIV–HCV coinfection high-dose ribavirin has not been systematically studied and is likely to lead to a significant increase in side effects, especially anemia. However, in the Coral-2 study [101], a ribavirin induction dose of 1,600 mg/day over 4 weeks was associated with higher response rates.

A ribavirin dose of 15 mg/kg/day might achieve the best balance between efficacy and a manageable safety profile and is currently recommended [102].

IL28B in HIV–HCV Coinfection

A recent study showed that genetic variation in the interleukin 28B (IL28B) gene, which encodes interferon lambda (IFN-λ), is associated with spontaneous HCV clearance [103], and three genome-wide association studies reported that a single nucleotide polymorphisms (SNP) in IL28B was associated with a higher response to antiviral therapy [104–106] in individuals infected with HCV genotype 1.

The association between IL28B gene and SVR has also been observed in patients with HIV–HCV coinfection [107]. Pineda et al. confirmed that IL28B gene variations independently predict SVR in HIV–HCV-coinfected patients with HCV genotype 1 and nongenotype 1 HCV infection [108]. The results have been confirmed by other groups [109, 110]. Its impact on spontaneous clearance in acute HCV infection remains an issue of controversy [110]. Of note, the association between IL28B genotype and plasma low-density lipoprotein cholesterol in their study suggests that the low-density lipoprotein ligand/receptor might be involved in the effect of this genotype [108].

Insulin Resistance in HIV–HCV Coinfection

In HCV monoinfection, IR is associated with advanced fibrosis and impaired treatment responses to pegylated interferon and ribavirin

[111, 112]. Effective treatment, however, has been shown to reverse IR [113].

Among 238 coinfected patients treated with pegIFN-a-2b and RBV, Cacoub et al. reported that a HOMA-IR >2.5 was a negative predictor of SVR [114]. In a cohort of 134 coinfected patients, Ryan et al. similarly reported that a HOMA-IR >3.8 was a negative predictor of SVR [115]. A third retrospective study of 74 HIV/HCV coinfected patients reported that a HOMA-IR >3.0 was a negative predictor of RVR [116].

In HCV-monoinfected patients, the evaluation of the impact of insulin sensitizing agents on SVR at the time of starting pegIFN and RBV treatment in patients with IR is under investigation. Two studies showed positive results at least in certain subgroups of patients [117, 118], and one did not [119].

In HIV-coinfected patients, no data has been published so far. The effect of insulin sensitizing agents is of major interest in this setting, even more because IR may develop by multiple mechanisms in HIV coinfection (see above).

Interactions of Antiretroviral Treatment and Anti-HCV Therapy

Hepatitis C coinfection is an indication for earlier anti-HIV therapy as a stable immune function slows down fibrosis progression and improves response rates to anti-HCV therapy. It is therefore of utmost importance to choose the optimal antiretroviral regimen to reduce drug–drug interactions. The best ART regimen in the setting of coinfection is not defined, but certain agents demand specific considerations while some others are even contraindicated. This accounts mainly for the combination of ribavirin with nucleoside reverse transcriptase inhibitors (NRTI).

An interaction between abacavir and ribavirin has been described, resulting in an increased risk of nonresponse to anti-HCV therapy [120]. An inhibitory competition for phosphorylation is the suspected mechanism [121] as both drugs are guanosine analogues and have metabolic pathways in common, but this effect was overcome by weight-based dosing [122, 123].

The use of zidovudine has been identified as an independent factor contributing to hematological adverse events in patients undergoing ribavirin and PEG-IFN treatment; the combination is not recommended [124].

The use of didanosine (ddI) alongside ribavirin is contraindicated. Ribavirin increases exposure to the active metabolite of didanosine, dideoxyadenosine 5′-triphosphate, which may lead to severe mitochondrial toxicity and fatal cases of pancreatitis and lactic acidosis have been described [125]. Mitochondrial toxicity has also been observed with combinations of stavudine and ribavirin. In vitro data have shown that ribavirin can inhibit phosphorylation of zidovudine and stavudine. The clinical significance is not clear; however, close monitoring of HIV RNA with this combination is recommended [126].

It has been reported that in HIV/HCV-coinfected patients, serum bilirubin increases following initiation of PEG-IFN and ribavirin was 1.9-fold higher in patients taking an atazanavir-containing regimen [127]. Atazanavir is a known inhibitor of UGT1A1 [128], and the elevation of bilirubin is not a sign of liver disease but may complicate clinical evaluation in some cases.

With HIV NNRTI, if patients receive efavirenz alongside PEG-IFN, monitoring of CNS effects is important, as the incidence of depressive symptoms in patients with HIV/HCV coinfection treated with IFN is reportedly high [129].

HIV and Hepatocellular Carcinoma (HCC)

In HIV-infected patients the incidence of liver cirrhosis and its complications including HCC has risen dramatically in the last years. Liver-related mortality has increased from 1.5% in 1995 to 15% in 2005 [130]. HCC is responsible for one quarter of liver-related deaths in HIV patients and in most cases these patients are coinfected with viral hepatitis. In an Italian and Spanish cooperative study, Puoti et al. compared 41 HIV patients with HCC to 384 non-HIV patients with HCC. HIV patients were younger (mean age 47 vs. 65 years), had more severe liver

disease, and presented with more advanced HCC which was often multifocal and with extrahepatic lesions [131]. This data has been confirmed by the study of Brau et al. comparing 63 HIV patients with HCC to 223 non-HIV patients with HCC [132]. In this study, patients presented at a younger age (52 vs. 64 years), with more advanced HCC and higher alpha-fetoprotein (AFP) levels. Several studies have seen a relationship between the degree of immunosuppression and liver-related mortality in HIV patients. A study from the Swiss cohort showed a significant relationship between the risk of developing HCC and a CD4 cell count below 500/mm^3 a year before diagnosis of HCC [133]. However, the CD4 nadir does not seem to be a predictive marker for the occurrence of HCC in these patients. Of note, several studies have shown that in HIV-HCC patients viral hepatitis (and especially HCV) is more frequently the underlying cause than in non-HIV-HCC patients [131, 132]. The antiretroviral drug class does not seem to have an impact on HCC development. Whereas advanced HIV disease accelerates fibrosis progression, its role in the emergence of HCC is not well described. It remains an issue of investigation if IR may play a specific role in the occurrence of HCC in HIV patients. While in the study of Puoti et al. survival was lower in the HIV-HCC group (1-year survival about 30 vs. 60%), this was not seen by Brau et al. (6.9 vs. 7.4 months). However, more of the HIV patients were classified as palliative at the time of diagnosis (19 vs. 4%). This fact raises the question of accuracy of HCC screening methods in HIV patients. Hepatic ultrasound every 6 months remains the best validated screening method for HCC so far. It is usually combined with AFP serum levels, however this marker has been criticized for lack of sensitivity. Considering the importance of early diagnosis in HIV patients, we still consider AFP as part of an HCC screening program. Whether shorter surveillance intervals (e.g., ultrasound every 3 months) or additional imaging techniques improved the outcome in this population remains to be investigated. Patients with advanced fibrosis (F3 Metavir), severe immunosuppression, or untreated viral hepatitis B and C should also be included in screening protocols. Treatment of HCC in HIV does not differ from that in HCV monoinfection.

HIV and Liver Transplantation

While liver transplantation is associated with excellent results in HIV-HBV coinfected patients it remains an issue of extreme controversy in the setting of HIV–HCV coinfection. To date, several hundred coinfected patients have received a liver graft and the results are conflicting [134].

A very recent analysis showed that HIV coinfection was an independent predictor of mortality on the waiting list, with an estimated survival at 24 weeks of 52 vs. 74% ($p = 0.001$). However, after a median follow-up of 91 weeks after LT, HIV coinfection did not lead to a higher mortality [135]. A study of 79 consecutive patients transplanted between 1999 and 2005 (35 with HCV-HIV coinfection and 44 with HCV infection alone) reported 2- and 5-year survival rates of 73 and 51% in HCV-HIV coinfected patients compared with 91 and 81% in HCV-monoinfected patients ($p = 0.004$) [136].

One major challenge in this setting is potentially severe drug–drug interactions between immunosuppressive drugs such as tacrolimus and HIV PIs [137].

Overall, outcomes seem to be worse in coinfected patients and HCV recurrence more severe while infections due to immunosuppression are not increased [138]. However, liver transplantation appears to be an option in the setting of coinfection and additional studies are needed to determine its applicability.

The Treatment of HCV Infection in the Setting of HIV Infection in the Future

Directly Acting Agents (DAA)

The high rates of nonresponse and relapse to the current standard of care in HIV–HCV-coinfected patients indicate the need for new treatment options in this setting. Several agents are in clinical

development to date, including PIs [139–141] and nucleoside/nonnucleoside polymerase inhibitors [142]. Other agents under investigation include novel analogues of ribavirin [143], modified interferons [144], cyclophilin inhibitors [145], alpha-glucosidase inhibitors [146], oligonucleotides [147], and immune modulators [148].

To date HIV patients have not had access to most of these investigational agents. However, with the anticipated approval of PIs for treatment of chronic HCV studies in the HIV coinfected population will be forthcoming. As seen in patients with HCV monoinfection, treatment of HCV with a PI, peginterferon, and ribavirin in patients with HIV coinfection is expected to profoundly increase SVR rates [140, 141, 149]. The most common side effects in previous clinical trials of PIs were pruritus, rash, and anemia for telaprevir, and dysgeusia and anemia for boceprevir. Interactions of these drugs are likely when coadministered with anti-HIV drugs. In human liver microsomes, the metabolism of telaprevir and boceprevir was substantially inhibited in the presence of ritonavir [150]. In addition, codosing either telaprevir or boceprevir with ritonavir in rats led to an increase in the plasma concentration of both HCV agents. These findings suggest that telaprevir and boceprevir may be primarily metabolized by cytochrome P450 3A (CYP3A). Most anti-HIV PIs are inhibitors of this enzyme, whereas the NNRTIs efavirenz, nevirapine, and etravirine are inductors. These interactions could be utilized to boost anti-HCV PIs with ritonavir or new boosters such as cobicistat to reduce administration frequency. In conclusion, drug–drug interactions between many HIV medications and HCV PIs are likely and studies are urgently needed to investigate their importance.

Although the antiviral efficacy is likely to be increased in coinfected patients treated with new DAAs, the development of drug resistance by HCV will be of greater concern in the future. Likewise antiretroviral drugs, anti-HCV polymerase, and PIs select for resistance mutations when administered as monotherapy [151] due to the high genetic diversity and rapid mutation and turnover rates of HCV.

Cross-resistance occurs when resistance mutations are selected that are common to more than one drug within each class and has been described for DAAs [152].

As HIV patients have higher HCV viral loads compared to HCV monoinfected and nonresponse to interferon/ribavirin is more common, it is likely that the development of resistance will develop more often in this setting. The implications of this for treating HCV-HIV coinfection in the future remain to be determined.

Conclusions

HIV infection can be controlled by large number of medications. This control has led clinicians and patients to focus on comorbidities in patients with HIV, of which liver disease plays a pronounced role. This is mainly due to HCV coinfection. With HCV being a potentially curable disease, it is of crucial importance to accurately diagnose the disease in patients with HIV and to implement effective treatment. Clearance of the HCV is eventually the only way to prevent end-stage liver disease and HCC. Unfortunately, an SVR is achieved in only about 35–40% of patients with HCV in the setting of HIV coinfection. New drugs are eagerly awaited. Clinical trials with these new substances are necessary to develop new treatment strategies and to determine the optimal ART backbone, treatment duration, and drug combination. This is even more important when treatment combinations will be eventually accessible for those patients who do not tolerate interferon due to side effects or ineffectiveness.

Unfortunately, many HIV patients will not benefit from treatment with new HCV drugs and will remain at risk to develop cirrhosis. These patients depend on the collaboration of their infectious disease physician and hepatologist to provide adequate HCC screening and management of liver cirrhosis. Finally, liver transplantation might be an option for selected patients.

HIV infection has become a manageable chronic disease in the Western world. More aggressive management of HCV in the coinfected

population may lead to a further improvement in outcomes for these patients as well.

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Chronic HCV in Patients with Renal Disease

8

Paul Martin and Fabrizio Fabrizi

Keywords
HCV • Chronic kidney disease • Dialysis • Kidney transplantation • Interferon • Ribavirin

Historical Perspective

Shortly after its identification [1], HCV was recognized to be highly prevalent in patients with chronic kidney disease (CKD), including dialysis populations. Introduction of serologic tests, enzyme-linked immunoassays (ELISAs) for detecting antibodies to various HCV antigens [2], and molecular techniques for detecting HCV viremia (HCV RNA) has facilitated the study of the epidemiology and clinical significance of HCV infection in patients on maintenance dialysis.

The prevalence of HCV in maintenance dialysis populations ranges from below 5% in northern Europe, around 10% in southern Europe and the United States, and between 10 and 70% in developing countries (including North Africa, Asia, and South America) [3]. The prevalence of HCV is highly variable from unit to unit within the same country, with recent reports from some dialysis units in the United States still reporting prevalences around 25–30% [4].

Several studies from European countries demonstrated a major decrease in the prevalence of anti-HCV-positive dialysis patients during the 1990s. In contrast, the prevalence of anti-HCV seropositivity recorded by the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, USA has not appreciably changed over the last 10 years in the United States, remaining around 8–10% [4]. Recent data indicate that the incidence of anti-HCV seroconversion has decreased to less than 1–2% in many developed countries in patients with CKD on maintenance HD [4]. The evolution of the epidemiology of HCV in dialysis patients in the developing world is poorly defined; high incidence rates have been recently described in several countries such as Sudan (17.6%/year) and Morocco (9.4%/year) [3].

Natural History of HCV in CKD Population

The treatment of HCV in patients with CKD is predicated on the premise that HCV is associated with decreased patient survival. The association

P. Martin, MD (✉)
Division of Hepatology, University of Miami, Miami, FL, USA
e-mail: PMartin2@med.miami.edu

F. Fabrizi, MD
Division of Nephrology, Maggiore Hospital, IRCCS Foundation, Milan, Italy

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between anti-HCV-positive status and diminished survival in CKD population is already established even if an accurate assessment of the natural history of HCV in dialysis patients and renal transplant recipients has been difficult to determine. HCV infection in dialysis patients and renal transplant recipients is typically asymptomatic with an apparent indolent course. HCV infection evolves over decades rather than years, whereas CKD patients generally have greater morbidity and mortality compared to the general population due to age and comorbid conditions, making the long-term consequences of HCV infection difficult to establish. Accurate evaluation of HCV infection is further complicated in this setting by the observation that aminotransferase values are typically lower in the dialysis than in nonuremic populations. Dialysis patients who have detectable serum HCV RNA have aminotransferase levels greater than those who do not, although values are typically within the "normal" range. In addition, recent advances in antiviral therapy for hepatitis C support antiviral treatment of HCV in the CKD population; this will hamper implementation of adequate trials on the natural history of HCV in this population.

A large meta-analysis on the impact of HCV on mortality (seven observational studies enrolling 11,589 unique patients on maintenance dialysis) showed that the summary estimate for adjusted relative risk (RR) of all-cause mortality with anti-HCV was 1.34 with a 95% confidence interval of 1.13–1.59 [5]. As a cause of death, hepatocellular carcinoma and liver cirrhosis were significantly more frequent among anti-HCV-positive than -negative dialysis patients. The summary estimate for liver-related mortality was 5.89 (95% CI, 1.93; 17.99, $P<0.001$), according to a random-effects model. These data are consistent with other reports. Kalantar-Zadeh et al. evaluated a database of 13,664 chronic dialysis patients in the United States who underwent HCV antibody serology testing at least once during a 3-year interval (July 2001–June 2004) and observed that the mortality hazard ratio was strongly associated with HCV infection, 1.25 (95% CI, 1.12–1.39, $P<0.001$) [6].

Similarly, HCV-infected kidney transplant recipients have diminished long-term graft and/or patient survival compared to uninfected controls. Studies in renal transplant recipients suggest that posttransplant immunosuppressive therapy has a permissive effect on viral replication potentially accelerating liver injury after transplantation. A recent meta-analysis of observational studies (eight clinical trials, 6,365 unique patients) reported that the presence of anti-HCV antibody was an independent and significant risk factor for death and graft failure after RT [7]. The summary estimate for adjusted relative risk (aRR) was 1.79 (95% CI, 1.57–2.03), and 1.56 (95% CI, 1.35–1.80), respectively. The higher mortality observed in HCV-positive recipients has been linked to hepatocellular injury. Posttransplant HCV infection has been implicated in an increased incidence of graft glomerulonephritis as well as a higher rate of serious infections, diabetes mellitus after renal transplantation, and chronic allograft nephropathy.

Treatment of HCV in CKD Population

Evaluation of HCV-Infected CKD Patients for Antiviral Treatment

The recent KDIGO guidelines for the clinical management of hepatitis C in CKD recommended that all CKD patients with HCV infection be evaluated for antiviral treatment with the decision to treat based on potential benefits and risks of therapy (including life expectancy, candidacy for kidney transplantation, and comorbidities) [8]. As an example, given the generally indolent progression of HCV, treatment is not recommended for the patient with less than a 5-year estimated survival due to comorbidities such as cardiovascular disease.

Patients should be appropriately informed of the risks and benefits of antiviral therapy and should also participate in the decision-making process. The decision to treat an HCV-infected patient with CKD must be made in the context of the patient's clinical situation. In some patients, for

example in the pretransplant patient, information in support of antiviral therapy exists. The rationale is that the achievement of a sustained viral response before transplantation is durable and reduces the risk of both hepatic and extra-hepatic manifestations of HCV viremia after transplantation. To date, three controlled clinical studies have been published. Overall, the quality of evidence is low as the patient allocation was not randomized. Of 15 HCV-positive recipients who received pretransplant IFN therapy, 10 (67%) had sustained virological response (SVR); only 1 (7%) of these 15 treated patients, who had remained viremic, developed de novo GN. Among the 63 untreated HCV-positive allograft recipients, all of whom were HCV RNA viremic at the time of transplantation, 12 (19%) developed de novo GN ($P<0.0001$) [9].

Pretransplant antiviral therapy of HCV-infected transplant recipients appears to lower the incidence of new-onset diabetes after transplant (NODAT). The frequency of NODAT was higher in the group of HCV-positive recipients who had not received IFN than in those who were treated with IFN before transplantation, 25% (10/40) vs. 7% (1/14), $P=0.009$ [10].

In a relatively large cohort ($n=50$) of kidney transplant recipients, a higher proportion of non-treated controls developed chronic allograft nephropathy compared with IFN-treated patients, 41% (13/32) vs. 6% (1/18), $P=0.009$. In the logistic regression analysis, the absence of IFN therapy before RT was a risk factor for chronic allograft nephropathy with an odds ratio of 12 ($P=0.02$) [11].

The treatment of HCV-infected renal transplant recipients, according to the KDIGO guidelines, is recommended only when the benefits of treatment clearly outweigh the risk of allograft rejection due to IFN-based therapy (e.g., fibrosing cholestatic hepatitis) as graft dysfunction and failure are common consequences of IFN therapy after renal transplant. Drop-outs during antiviral therapy are mostly related to IFN-induced acute rejection, which is frequently steroid-resistant and irreversible. Controlled and cohort (prospective or retrospective) studies have addressed this issue in kidney transplant recipients.

In patients with well-compensated cirrhosis, the decision to treat is a difficult one. The presence of even compensated liver cirrhosis before kidney transplantation has the potential to increase the risk of recipient mortality in terms of operative procedure, marginal posttransplant reserve and nutritional state, and increased susceptibility to posttransplant infectious and metabolic complications, as well as evolution to decompensated liver disease. HCV-infected kidney transplant candidates with liver cirrhosis on biopsy should be only considered for kidney transplantation under investigational protocols as only very limited outcome data about transplantation of a kidney alone in HCV-infected recipients with preexisting compensated cirrhosis of the liver exist. Mathurin et al. reported that patients with liver cirrhosis before transplant had a 10-year rate of survival of only 26% [12]. In addition, no data are available to establish whether patients with early cirrhosis on liver biopsy yet well-compensated clinical disease do better if they are transplanted or remain on dialysis. Successful antiviral therapy can improve liver histology as a recent report demonstrated that 5 (7.8%) of 64 patients with normal kidney function and three of four HCV-infected dialysis patients with cirrhosis achieved SVR [13]. Thus, if improvement in liver histology can be documented after antiviral therapy achieving SVR in a patient with advanced fibrosis or cirrhosis, it is suggested that the patient's candidacy for renal transplant be reevaluated in the context of the most recent liver biopsy. If the patient with well-compensated cirrhosis remains viremic, kidney transplantation alone is not recommended.

HCV-infected patients with evidence of decompensated liver disease should be evaluated for simultaneous liver–kidney transplantation. Transplantation of a kidney alone in this situation is not recommended.

Type of Antiviral Therapy and CKD Stage

The current standard of care for HCV infection in patients with intact kidney function is the

combination of weekly subcutaneous injection of pegylated interferon (IFN) and oral ribavirin, as demonstrated in three large randomized trials [8]. No data exist to guide therapy for HCV in patients with CKD stages 1 and 2. However, in patients with a GFR >60 mL per min per 1.73 m^2, this degree of impairment of kidney function does not have a major impact on the efficacy and safety of combined IFN and ribavirin therapy and therefore results in patients with normal kidney function treated with pegylated IFN plus ribavirin should apply to CKD stages 1 and 2.

Importantly, literature on the antiviral therapy of hepatitis C in CKD stages 3–5 including dialysis patients is limited. Clinicians have been generally reluctant to initiate IFN-based therapy for hepatitis C in dialysis population as it was felt to be too toxic in this setting. The immune modulatory activity of IFN causes a variety of side-effects even with normal renal function including alopecia, bone marrow depression, fever/flu-like syndrome, and possibly bacterial infections. Dialysis patients were typically older and often with comorbidities (including cardiomyopathy, malnutrition, and gastrointestinal abnormalities). However, the available data do support monotherapy with standard interferon in patients on maintenance hemodialysis. SVR to monotherapy with standard IFN in maintenance hemodialysis patients (summary estimate of 37%), as demonstrated in a recent meta-analysis, is higher than that observed in patients with HCV and intact kidney function (7–16%) treated with standard IFN monotherapy [14]. Several mechanisms may account for the relatively higher response to IFN in patients receiving maintenance hemodialysis. Dialysis patients with HCV usually have a lower viral load; the infection is frequently associated with milder forms of histologic liver disease; clearance of IFN is lower in dialysis patients than in non-CKD patients [15]; and an increase in endogenous IFN release from circulating white blood cells during hemodialysis sessions has been reported [16]. A marked and prolonged release of hepatocyte growth factor (or other cytokines) caused by hemodialysis may play an additional role [17].

Although response rates to conventional IFN are better in the dialysis population, tolerance to IFN monotherapy is lower in patients on maintenance hemodialysis than in non-CKD individuals [14]. The summary estimate of drop-out rate was 17% in dialysis patients who received standard IFN monotherapy, whereas the frequency of side-effects requiring IFN discontinuation ranged between 5 and 9% in non-CKD patients with chronic hepatitis C treated with standard IFN monotherapy (3 MU thrice weekly for 6 months). Altered pharmacokinetics of IFN in the hemodialysis population [15], older age, and comorbid conditions is reflected in a higher frequency of side-effects leading to IFN discontinuation. The IFN half-life was longer in dialysis than in normal controls, 9.6 vs. 5.3 h ($P=0.001$) and the area under the curve was twice that of patients with normal kidney function.

Even more limited data exist for monotherapy with pegylated IFN in HCV-infected patients on long-term hemodialysis. The pharmacokinetic profile of pegylated IFN-α 2a in patients on regular hemodialysis is similar to that in healthy individuals with only a 30% relative reduction in clearance. The pharmacokinetic of pegylated IFN-α 2a during hemodialysis may vary in dialysis patients, depending on the permeability and dialyzer pore size. In a separate analysis, it was shown that hemodialysis had negligible effects on pegylated IFN-α 2b clearance.

It appears that pegylated IFN does not provide an added benefit in terms of virologic response in comparison with standard IFN monotherapy in the hemodialysis population, treatment discontinuation due to adverse events was also similar. A recent meta-analysis (254 unique patients on regular hemodialysis with chronic hepatitis C) found that the summary estimate for SVR and drop-out rate was 33% (95% CI, 24–43) and 23% (95% CI, 14–33), respectively. The most frequent side-effects requiring interruption of treatment were hematological (18%) and gastrointestinal (14%) [18].

For HCV-infected kidney transplant recipients in whom the benefits of antiviral treatment clearly outweigh the risks, monotherapy with standard

Table 8.1 Pegylated interferon in combination with ribavirin in patients with chronic hepatitis C on maintenance hemodialysis: clinical trials

Authors	SVR	Antiviral agent
Bruchfeld et al. [24]	50% (3/6)	Peg-IFN alfa-2a ($n=2$) or peg-IFN alfa-2b ($n=4$) plus ribavirin
Rendina et al. [25]	97% (34/35)	Peg-IFN alfa-2a plus ribavirin
[a]Schmitz et al. [26]	50% (3/6)	Peg-IFN alfa-2b plus ribavirin
Carriero et al. [28]	29% (4/14)	Peg-IFN alfa-2a plus ribavirin
Van Leusen et al. [27]	71% (4/7)	Peg-IFN alfa-2a plus ribavirin
Hakim et al. [29]	5% (1/20)	Peg-IFN alfa-2a plus ribavirin

Results have been calculated according to an intention to-treat (ITT) analysis
[a]This study concerned liver/kidney transplant recipients

IFN is suggested [8]. The development of HCV-related fibrosing cholestatic hepatitis may be an indication for IFN use after kidney transplantation, as fibrosing cholestatic hepatitis has an ominous course [19]. Alternative regimens based on drugs other than IFN are not recommended after kidney transplant, as no proof of their efficacy has been provided. No impact on viral response was seen with ribavirin monotherapy even though a biochemical response was observed [20]. Similarly, amantadine monotherapy after kidney transplantation has been reported to have no impact on either HCV viremia or liver histology [21].

Although genotype does not predict the outcome of infection, it predicts the probability of response to determine the necessary duration of therapy. Infections with HCV genotypes 1 and 4 are less responsive to IFN-based therapy and require 48 weeks of treatment. In contrast, genotype 2 is more responsive to treatment and requires only 24 weeks of therapy to achieve SVR. Genotype 3 is also more responsive to therapy although treatment needs to be extended to 48 weeks if viral load is high. HCV genotype 5 appears to have a response similar to genotypes 2 and 3 but also requires 48 weeks of therapy. Genotype 6 responds better than genotype 1 but not so well as genotypes 2 and 3. These results have been obtained in patients with HCV and normal kidney function. In a systematic review of patients on maintenance hemodialysis, the overall summary estimate for SVR with standard IFN monotherapy was 37% in the whole group and 30% in those patients with HCV genotype 1 [14]. In another review, the pooled SVR rate was 33% in the whole group and 26% with HCV genotype 1 [22].

Ribavirin Use in CKD Population

Extensive data do not exist about the use of combination antiviral therapy (conventional or pegylated interferon plus ribavirin) in CKD stages 3–5 patients. Data on combined therapy derive mostly from studies of patients on maintenance hemodialysis. Impaired excretion of ribavirin occurs in CKD patients as ribavirin is mostly eliminated by kidneys. Very little ribavirin is removed via dialysis so there is a propensity for drug to accumulate, exacerbating hemolysis in the dialysis population already at significant risk for anemia as well as other comorbidities (e.g., cardiac ischemia) at baseline. A "Black Box" warning has been made from the FDA on the use of ribavirin in this setting. The use of ribavirin in patients with a glomerular filtration rate (GFR) <50 mL per min per 1.73 m^2 is not recommended in AASLD guidelines. Limited recent data do support ribavirin use in CKD patients with a GFR <50 mL per min per 1.73 m^2 in a carefully well-monitored setting. Ribavirin should be used only after the implementation of several precautions, including: (1) very low ribavirin dose (about 200 mg daily or 200 mg thrice weekly); (2) weekly monitoring of hemoglobin levels; and (3) high doses of erythropoietin to treat anemia [8].

In a recent systematic review including 151 unique dialysis patients with chronic hepatitis C receiving IFN plus ribavirin, the summary estimate for SVR and drop-out rate was 56% [95% confidence intervals (95% CI) 28–84] and 25% (95% CI, 10–40), respectively [23]. Some of the trials included in this review have been reported in Table 8.1 [24–29]. The most frequent side-effects

requiring interruption of treatment were anemia (26%) and heart failure (9%). These results occurred irrespective of the type of interferon (conventional or pegylated IFN, pegylated IFN alfa-2a or alfa-2b), the trial design (controlled or cohort study), or the clinical characteristics of patients (naïve, nonresponders, or relapsers). The authors concluded that the addition of ribavirin led to a significant improvement in SVR rates in comparison with IFN monotherapy in dialysis populations.

Monitoring the Response to HCV Treatment in CKD Patients

CKD patients who have been treated with antiviral therapy for chronic HCV infection must have their response to therapy monitored. It is recommended that the guidelines available for the general population be applied to the CKD populations. Viral response to therapy, defined by the occurrence of SVR (clearance of viremia at 6 months after completion of antiviral treatment), remains the gold standard to evaluate the efficacy of antiviral therapy in patients with hepatitis C and normal kidney function. Achieving SVR may improve clinical outcomes (improved survival, lowered rate of hepatocellular carcinoma) in patients with HCV and normal kidney function. No data are available yet to confirm that achieving SVR translates into improved survival in the CKD population with HCV infection. However, there are reports that successful antiviral therapy can improve other outcomes (e.g., liver histology). Pretransplant SVR after IFN therapy is associated with improved liver histology in patients who remain on dialysis and in those who go on to receive a kidney transplant. If SVR is achieved, it is suggested that testing with nucleic acid testing be performed annually to ensure that the patient with CKD remains nonviremic. For patients on maintenance hemodialysis, repeat testing with nucleic acid testing every 6 months is suggested.

HCV and Kidney Transplantation

HCV Infection Should not be a Contraindication for RT

Although HCV-infected kidney transplant recipients have inferior patient and allograft survivals after transplantation when compared to uninfected kidney transplant recipients there is evidence that, compared to remaining on dialysis, kidney transplantation confers a survival advantage to HCV-infected patients. Kidney transplantation should therefore be considered the treatment of choice for patients with CKD stage 5 and HCV infection [8]. All potential kidney transplant recipients should be tested for HCV. HCV infection should not be considered a contraindication to transplantation for various reasons. At least three retrospective studies have demonstrated that survival is improved with transplantation compared to the remaining waitlisted on dialysis in HCV-infected patients with kidney failure [30–32]. Liver disease does not progress in many patients after kidney transplantation. Whereas progressive liver disease does impact patient outcomes, it usually occurs over many years and thereby affects long-term survival. Finally, it is extremely unlikely that a randomized controlled trial comparing kidney transplantation to dialysis for long-term treatment of HCV-infected CKD stage 5 patients will ever be performed.

Use of Kidneys from HCV-Infected Donors

There is clear evidence that HCV can be transmitted from infected donors to recipients by solid organ transplantation, including kidney transplantation and therefore renal grafts from HCV-positive donors should not be transplanted into HCV-negative recipients. In fact, this is absolutely prohibited in Europe. A completely restrictive

policy however, whereby all kidneys from HCV-positive donors are discarded, aggravates the organ shortage. Therefore, numerous organ transplant organizations (OPOs) have introduced a policy of accepting kidneys from HCV-positive donors for HCV-positive recipients, even if the safety on the use of kidneys from HCV-infected donors remains unclear. Some reports have addressed this topic. No adverse effect on short-term patient or graft survival was observed, and the waiting time for RT of these patients was shortened. However, many of these reports had retrospective design, the follow-up was rather short (up to 60 months) in small single-center studies [33–35]. More convincing results have been given by a registry analysis derived from the United States Renal Data System (USRDS) with recipients of kidneys from HCV-infected donors were associated with a higher rate of mortality, regardless of the anti-HCV antibody status of the recipient [36]. In conclusion, some risks with the use of kidneys from HCV-positive donors cannot be excluded.

A large study from the USRDS indicated that the transmission of HCV infection via transplantation was associated with an increased risk of post-transplant diabetes mellitus and a reduction in recipient life expectancy [37]. To avoid these potential but major complications in uninfected recipients, it is suggested that kidneys from HCV-infected donors should not be used in potential recipients without HCV viremia. The risks and effects of super-infection with an HCV genotype from the donor that is different from the genotype of the potential HCV-infected recipient are unknown. Two single-center investigations reported aggressive hepatitis C related to HCV genotype mismatch after renal transplant [38, 39].

Role of Liver Biopsy Before Kidney Transplantation

All HCV-infected kidney transplant candidates should undergo liver biopsy before RT as recommended by the KDIGO Work Group. Liver biopsy is crucial to determine the severity of hepatic injury and thereby to assess the prognosis of the patient. Biochemical liver tests do not adequately reflect the histologic severity of liver damage in this patient group, and liver disease has the potential to deteriorate after RT in HCV-infected patients. As reported above, some studies with sequential liver biopsies after RT have documented the progressive nature of chronic hepatitis C in this setting; however, studies with pre and posttransplant sequential liver biopsies have not been published so far. One report has shown that presence of cirrhosis on pretransplant liver biopsy is associated with a 10-year survival of only 26% [12]. The utility of noninvasive studies (e.g., Fibroscan) for assessing liver injury in HCV-infected CKD patients is not yet established. For HCV-infected dialysis patients who are kidney transplant candidates, antiviral therapy is suggested to prevent extra-hepatic complications even in those with a pattern of histologic injury that does not meet the recommended stage of fibrosis to qualify for therapy in the general population (i.e., Metavir score <2 and Ishak score <3) [8]. Staging of disease severity may guide considerations for antiviral therapy as patients identified with advanced fibrosis should be considered for a liver–kidney transplant only.

Limitations and Research Recommendations

Concern persists about application of antiviral therapy of HCV in CKD population, as most of the subjects included were on the waiting list for kidney transplantation and were younger and probably healthier than the general dialysis population. Furthermore, only a few studies were from North America where many CKD patients are African-American. This is of special relevance, as there are racial differences in the response to IFN therapy in subjects with normal kidney function.

Early virologic response (i.e., virologic response obtained 12 weeks after initiation of antiviral therapy with at least a 2 log fall in the HCV viral titer) has been demonstrated to be

highly predictive of SVR in HCV-infected patients with normal kidney function. There are no studies which have formally addressed the predictive value of early viral response in evaluating the response of HCV-infected CKD patients to antiviral therapy. Many dialysis patients who receive antiviral therapy are potential renal transplant candidates but they cannot be wait-listed for transplant while receiving antiviral therapy [8]. Thus, the failure to achieve a virologic response 12 weeks after the initiation of antiviral therapy can support discontinuation of antiviral treatment, and placement in the active waiting list for transplant. Prospective studies on the clinical utility of early changes in the viral load, including rapid virologic response, measured as absolute viral loads or change in viral load from baseline, are required in CKD-infected patients.

Information about adverse effects during IFN therapy in dialysis patients is unsatisfactory. It remains unclear whether the adverse effects in dialysis patients with HCV are related to IFN activity per se or to the high prevalence of comorbid conditions typical of dialysis patients. Prospective, controlled studies in dialysis patients are required to establish the rate of adverse effects during IFN-based therapy.

Prospective trials involving the treatment of HCV-infected patients on peritoneal dialysis are needed. Essentially, all information available on the treatment of dialysis patients comes from studies in hemodialysis patients.

The higher efficacy of combined antiviral therapy as compared to IFN monotherapy for hepatitis C in patients with normal renal function is likely related to the synergistic activity played by ribavirin. However, the activity of ribavirin is dose-dependent, and the effective role of low-dose ribavirin in enhancing the antiviral activity of IFN in dialysis patients remains to be determined. Controlled studies designed to answer this question should be performed.

Prospective studies are needed to assess whether the benefit of therapy in terms of lower mortality is realized in a patient population with significantly reduced long-term survival.

Conclusions

Recent evidence has unequivocally shown that HCV infection has a detrimental effect on patient and graft survival in patients with CKD. Patients on a kidney transplant waiting list should be evaluated for HCV infection and all kidney transplant candidates with persistent HCV viremia should undergo a liver biopsy before transplantation. Monotherapy with standard interferon is recommended in HCV-infected patients evaluated for kidney transplantation. Antiviral treatment of HCV-infected kidney transplant recipients should be considered only when HCV has an ominous course with severe liver disease. HCV infection should not be considered a contraindication for kidney transplantation. Transplantation of kidneys from donors infected with HCV should be restricted to viremic recipients. There is encouraging evidence supporting the durability of pre-transplant SVR after renal transplantation but this needs to be confirmed in prospective studies.

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Does Treatment Alter the Natural History of Chronic HCV?

9

Fasiha Kanwal and Bruce R. Bacon

Keywords
Hepatitis C • Natural history • Cirrhosis • Hepatocellular cancer • Treatment • Effectiveness

Introduction

Chronic hepatitis C virus (HCV) infection is a chronic infectious disease caused by an RNA virus and is predominantly transmitted through parenteral (i.e., blood transfusion prior to 1992, intravenous drug use) exposure. Sexual transmission has also been reported to occur but this is relatively uncommon except in select situations (i.e., having multiple sexual partners or in the setting of HIV co-infection) [1]. HCV is a common condition and estimates are that up to 4 million persons (1.3%) in the United States have HCV infection [2]. Because the majority of HCV patients are thought to have acquired their infection as young adults in the 1970s [1, 3], the number of patients chronically infected for more than 20 years continues to rise [4]; a decrease in the newly acquired infections in recent years has not yet resulted in any discernable change in the prevalence of HCV [5].

Defining the natural history of HCV has been challenging for several reasons. First, it is usually difficult to precisely define the onset of infection. Acute infection can be asymptomatic in over 60% of patients and often goes unnoticed. Second, the disease course is slow and may span several decades, during which time most patients do not experience any symptoms related to their infection. Third, progression from mild to advanced disease may be quite heterogeneous due to a variety of host factors such as age at infection, gender, degree of alcohol use, and presence of steatosis. Thus, given these issues, an ideal study to assess the natural history of HCV requires a large population, with a discrete and definable onset of infection, and a comprehensive long-duration follow-up. Such studies are hard to conduct due to issues of cost and feasibility.

Despite these difficulties, it is important to define and understand the natural history of HCV. These data can provide necessary information to both the patient and the treating clinician regarding the stage of liver disease, the intermediate as well as long-term prognosis, and thus the urgency

F. Kanwal, MD, MSHS
Department of Internal Medicine,
Division of Gastroenterology and Hepatology,
John Cochran VA Medical Center, St. Louis, MO, USA

B.R. Bacon, MD (✉)
Department of Internal Medicine,
Division of Gastroenterology and Hepatology, Saint Louis University School of Medicine, 3635 Vista Avenue at Grand Boulevard, St. Louis, MO 63110-0250, USA
e-mail: baconbr@slu.edu

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of antiviral treatment. Furthermore, these estimates can provide a critical insight into the magnitude of the disease burden associated with HCV and therefore guide the health care system to develop strategies and capacity targeted toward providing timely and effective care to patients with HCV. The natural history estimates from untreated cohorts can also serve as the reference standard against which to compare the potential impact of antiviral treatment on cirrhosis and its complications in this highly vulnerable group of patients. Therefore, the natural history data can not only guide our everyday clinical practice but can also help shape public policy as it relates to individuals with HCV.

In light of these considerations, we have organized this chapter in three sections. The first section discusses the available data on the natural history of untreated HCV, the second section discusses the potential impact of current treatments on the natural history of HCV, and the last section discusses the impact of future antiviral treatments on the natural history of HCV.

Section 1

Natural History of Chronic Untreated Hepatitis C Virus Infection

Definition of CHC

Chronic hepatitis C (CHC) is defined as persistence of serum HCV RNA for ≥6 months. In the first months following infection, the serum HCV RNA level often reaches a stable level between 4 and 6 $\log_{10}$ in many infected individuals, and the RNA level remains fairly constant over subsequent decades. Liver enzymes (i.e., ALT, AST) can fluctuate [6–8].

Clinical Course

The disease course in CHC is slow and usually spans many decades. During its prolonged chronic phase, CHC patients are either completely asymptomatic or may experience nonspecific symptoms that are difficult to link to CHC. Yet, CHC is a progressive condition and given the clinically silent course, many patients with CHC remain undiagnosed until the disease has advanced to cirrhosis, decompensation, or hepatocellular cancer (HCC).

How many patients with CHC will, if at all, progress to these potentially life-threatening complications of advanced liver disease? Several factors limit our understanding of the clinical course and thus the natural history of CHC in individual patients. For example, acute infections are generally asymptomatic and the high-risk exposure resulting in CHC may occur on more than one occasion and over a long period of time. Thus, it is hard to define the precise onset of infection in many patients. However, there have been a few "natural experiments" – where the onset of infection can be precisely defined – that shed light on the natural history of CHC.

One such case was the unfortunate iatrogenic outbreak of HCV related to contaminated Rh immune globulin in Ireland [9]. In 1994, batches of anti-D immune globulin used in Ireland during 1977 and 1978 to prevent Rh iso-immunization were found to be contaminated with HCV. Of the 62,667 women who were screened, 704 (1.1%) had evidence of past or current HCV infection, and 390 of those 704 (55%) had positive tests for serum HCV RNA. Ninety-six percent of these women were evaluated for liver disease. After a mean of 17 years follow-up post exposure, serum alanine aminotransferase concentrations were above the limits of normal in 55%. Although the liver biopsies showed inflammation in 98% of women, the inflammation was mild (41%) or moderate (52%) in most patients. Fifty-one percent of women had some evidence of fibrosis, but only 2% had probable or definite cirrhosis.

Similar data were reported in a 25-year follow-up of 2,867 women in East Germany who received contaminated Rh immune globulin in 1978–1979 [10]. The investigators were able to trace and then follow 1980 women representing 70% of the exposed cohort. Of 1,718 untreated subjects, 49% (836 patients) had spontaneous recovery from their HCV infection as evidenced by normal ALT and the absence of HCV RNA. Of the 868 (51%) patients who developed CHC, 683 were untreated allowing for assessment of natural history over the 25-year duration of the

Table 9.1 Estimates of disease progression in patients with chronic hepatitis C virus (HCV) infection

Study design	Exposure interval, mean (years)	Age at assessment, mean (years)	Steatosis, % patients (*N* studies)	HAI, mean (*N* studies)	Cirrhosis, % (range)
Retrospective or cross-sectional [33]	17.0	43.1	47 (26)	6.3 (21)	18 (15–21)
Retrospective–prospective [33]	21.1	42.6	19 (3)	8.7 (1)	7 (4–14)

study. Over this prolonged span, only 9 (1.3%) developed cirrhosis, 1 (0.1%) developed HCC and 30 (4.4%) had significant fibrosis that might evolve to cirrhosis; HCV-related mortality was 0.35%.

Seeff et al. [11] also reported follow-up on individuals with acute transfusion-related hepatitis C. Twenty-five years after exposure, 21 (23%) of 90 patients infected at the time of transfusion had spontaneously recovered. Among the 69 patients who were HCV-RNA-positive during follow-up, 49% (38% of the total of 90 cases) had biochemical evidence of chronic hepatitis. Seventeen percent (13% of the total of 90 cases) of HCV-RNA-positive patients were observed (or projected) to have cirrhosis.

Collectively, these data show that women infected with HCV in their mid-20s have an approximately 50% chance of spontaneous recovery and in those with persistent infection, there is only a 5% probability of developing bridging fibrosis, cirrhosis or HCC during the first 25 years of infection [9, 10]. The risk of progression may be slightly higher in individuals who are exposed later in their lives (mean age at exposure was 49 ± 13 years in the study by Seeff et al.), with 15% progressing to cirrhosis [12]. These relatively benign outcomes are quite encouraging. However, these populations may represent the best-case scenario, because of the young age and general good health at the onset of infection in the first two reports [9, 10] and rarity of co-morbid factors such as alcohol use in the report by Seeff et al. [12].

Our understanding of the natural history of CHC is further limited by the fact that the course of disease progression can be variable and affected by an interplay of factors such as age at infection and duration of infection [13–16], gender [13, 15, 17], race [18, 19], and co-existence of other hepatic injuries including excess alcohol consumption [20–23], nonalcoholic steatohepatitis [24, 25], and co-infection with hepatitis B [26–28] or HIV [29–32].

In addition to the patient population studied, disease progression estimates are also highly dependent on the design employed by the individual studies. This limitation – engendered by the studies themselves – is compounded by the sheer number of published reports presenting natural history data, thus further limiting the ability to collate estimates across different studies. For example, a recent meta-analysis of fibrosis progression in CHC found 111 unique reports involving 33,121 individuals with chronic infection [33]. Of these 111 studies, 100 studies were either cross-sectional or retrospective in nature. These studies generally included patients with liver disease presenting for clinical care, often at large tertiary care centers, where efforts were made to track their liver disease back to the presumed time of infection based on the history of receipt of blood or blood product or of the first use of injection drugs [33]. Only 11 studies had a retrospective–prospective design. These studies included patients in whom the precise time of infection with acute hepatitis C could be defined retrospectively and the subjects were then subsequently followed prospectively [9, 10, 34–42]. Table 9.1 summarizes key estimates from this meta-analysis. The data show that after 20 years of infection, the cumulative prevalence of cirrhosis ranges anywhere from 7% (estimate from retrospective–prospective studies) to 18% (estimate from cross-sectional/retrospective studies).

Each set of estimates has its limitations. Estimates from cross-sectional/retrospective studies may reflect selection bias because individuals with advanced liver disease are more likely to be referred to tertiary care centers than

Table 9.2 Outcomes of compensated cirrhosis in patients with untreated chronic hepatitis C (CHC) depicted as the annual incidence of hepatocellular cancer (HCC), ascites, and death/transplantation

Study	*N* patients	Follow-up, mean (years)	HCC (%)	Ascites (%)	Death/transplantation (%)
Gramenzi et al. [43]	72	4.8	5.5	3.2	3.2
Mazzella et al. [44]	92	2.8	3.5	–	–
Okanoue et al. [45]	55	5.6	7.1	–	–
Shiratori et al. [46]	74	6.8	7.0	–	4.8
Kobayashi et al. [47]	490	8.2	–	–	6.7
Fattovich et al. [48]	136	6.8	2.5	–	3.8
Toshikuni et al. [49]	152	5.4	5.6	3.9	5.4
Bruno et al. [50]	158	14.4	2.3		3.9
Sangiovanni et al. [51]	214	9.5	3.9	2.9	4.0

those with earlier and milder disease. The estimates provided from the retrospective–prospective studies are generally lower than those with the cross-sectional/retrospective studies and may be closer to the truth; however, these studies generally fail to provide information on longer-term outcomes so relevant to patients and clinicians. However, as stated above, the available retrospective–prospective studies likely represent the best-case scenario because of the young age and general good health of the participants at the onset of infection, because of likely abstinence of alcohol, and because of rarity of other co-morbid factors. Therefore, it is plausible that the risk of cirrhosis in CHC likely falls somewhere between the two extremes presented in Table 9.1.

Given the known role of co-factors on disease progression in CHC, it is not surprising that the published estimates of disease progression also vary by the setting, by different age at the time of HCV infection, and by duration of infection. For example, the predicted estimates of cirrhosis for nonclinical setting-based studies are lower compared to clinical setting-based studies (7%, 4–12% vs. 18%, 16–21%) [33]. Individuals who acquired the infection at an older age (>30 years) are approximately 2–3 times more likely to progress to cirrhosis at 20 years than those who acquired infection at a younger age (<30 years) [33]. Estimates for individuals who had a shorter duration of infection (<10 years) are higher than those who had a longer duration of infection (>10 years) [33].

Not all individuals with HCV cirrhosis develop complications of cirrhosis, HCC, or death due to liver failure. Similar to progression from CHC to cirrhosis, the course of HCV-related cirrhosis is also generally slow and variable. The variability, once again, is probably due to the interplay with other potential host and environmental causes of liver disease. Table 9.2 describes the summary of the data from nine studies on the outcomes of patients with compensated HCV cirrhosis who did not receive any antiviral therapy for HCV [43–51]. These data, therefore, give a glimpse of the course of HCV cirrhosis independent of antiviral therapy. Based on these data, the best estimate is that patients with cirrhosis develop HCC, decompensation of liver disease, and death at rates of 2.5–7, 2–4, and 3–7% per year, respectively.

Although these data are important, it is hard to extrapolate the direct population-based estimates of the number of patients with cirrhosis and related complications in relation to overall infection with HCV. Davis et al. [5] recently developed a decision model that accounts for the heterogeneity of the U.S. HCV cohort. This multi-cohort natural history decision model followed six cohorts (based on gender and age), each with their own cohort-specific transition states for chronicity, fibrosis progression, and complications. Based on this model, the proportion of cases with advanced fibrosis will continue to rise during the next two decades, with the proportion of HCV with cirrhosis reaching 25% in

2010 and 45% in 2030. Hepatic decompensation and liver cancer will continue to increase for another 10–13 years. Moreover, the age of those with cirrhosis and its complications will continue to rise [5].

Section 2

Potential Impact of Current Treatments on the Natural History of CHC

There are three major landmarks in the natural history of HCV: development of chronic infection, development of cirrhosis, and development of cirrhosis-related complications. The goal of antiviral treatment is to eradicate chronic infection, to slow – and preferably stop – the progression of fibrosis to cirrhosis and its related complications, and thus improve survival. Several large studies have suggested that successful treatment with pegylated interferon and ribavirin may halt and even reverse hepatic fibrosis. Camma et al. [52] found that sustained virologic response (SVR) was associated with a reduction in fibrosis in 1,013 patients with HCV who had had pre- and post-treatment liver biopsies and had received interferon or pegylated interferon. Similarly, Poynard et al. [53] found that among 3,010 patients for whom pre- and post-treatment biopsy results were available, reversal of fibrosis occurred in 12% of those treated for 24 weeks with standard interferon and up to 24% of those treated with an optimal schedule of pegylated interferon and ribavirin. They also reported regression of cirrhosis in 49% of their patients after successful treatment. We recently reported data on 150 patients with SVR after treatment of HCV. Of these 150 patients, 128 had stage 2 or greater fibrosis on pretreatment biopsy [54]. Sixty of these one hundred and twenty-eight patients (47%) underwent a follow-up biopsy at least 4 years after their SVR; of these, 49 patients had their paired pretreatment and long-term follow-up biopsies blindly rescored. Forty of these patients (82%) had a decrease in fibrosis score, and forty-five (92%) had a decrease in combined inflammation score. Ten patients (20%) had normal or nearly normal livers on long-term follow-up biopsy. Two patients with pretreatment cirrhosis developed hepatocellular carcinoma (HCC), and one died. All the other patients with pretreatment cirrhosis or advanced fibrosis had improved fibrosis scores on long-term follow-up biopsy [54].

Although these results are promising, data on long-term clinical outcomes after antiviral treatment (decompensation, HCC, liver-related mortality) have been lacking; this has been one of the main reasons that a screening program for HCV has not been implemented in the United States. However, in the last few years, there have been several reports of the long-term benefit of antiviral treatment on important clinical outcomes in patients with CHC. These data – showing that eradication of virus clearly reduces risk of liver failure or HCC – may help to change attitudes toward screening persons who are at risk for HCV as well as promoting strategies for early treatment of this burgeoning patient population.

Veldt et al. [55] reported data from a retrospective cohort of 479 patients with biopsy-proven advanced fibrosis or cirrhosis that was treated with an interferon-based regimen between 1990 and 2003 at five large tertiary centers in Europe and Canada. Approximately 30% of patients had SVR and 70% did not. After a median follow-up of 2.1 years, 4 patients with and 83 patients without SVR had at least one clinical outcome (defined as liver or nonliver-related death, liver failure, or hepatocellular carcinoma). SVR was associated with a significant reduction in the hazard of events (adjusted hazard ratio, 0.21 [95% CI, 0.07–0.58]). The effect was largely attributable to a reduction in liver failure, which developed in no patients with and 42 patients without SVR (5-year occurrence, 0% vs. 13.3% [CI, 8.4–18.2%]; unadjusted hazard ratio, 0.03 [CI, 0.00–0.91]). In this study, the incidence of HCC at 5 years did not differ between patients with SVR and nonresponders; SVR was, however, associated with a trend toward lower risk of HCC in patients with virologic response (adjusted hazard ratio, 0.46 [CI, 0.12–1.70]).

Bruno et al. [56] reported data from a retrospective study of 920 patients with HCV-related histologically proven cirrhosis who were treated

at 23 centers in Italy between January 1992 and December 1997. Of these patients, 13.5% had SVR. During an average follow-up of 8 years, 7 patients with and 122 patients without SVR developed HCC (annual incidence of HCC, 0.66 among SVR vs. 2.10 in the non-SVR group, $p<0.001$). One hundred and seven patients without SVR had at least one liver-related complication (defined as ascites, upper gastrointestinal bleeding, and hepatic encephalopathy) whereas none of the patients with SVR experienced these complications (annual incidence of liver-related complications, 0.0 among SVR vs. 1.88 in non-SVR group, $p<0.001$). The annual incidence of liver-related death was 0.19 among SVR and 1.44 among non-SVR ($p<0.001$ by log-rank test).

Studies from Japan have shown a decrease in the incidence of HCV-related HCC after interferon therapy in patients with significant fibrosis and cirrhosis. In a prospective cohort study, Shiratori et al. [46] reported data from 345 patients with HCV-related cirrhosis who were enrolled in previous treatment trials. Of these 345 patients, 271 patients received nonpegylated IFN therapy. Seventy-four patients with HCV who fulfilled the inclusion criteria declined to receive IFN therapy. The treated and untreated groups were similar except for differences in age (57 vs. 61 years, $p<0.001$) and serum ALT levels (97 vs. 75 IU/L, $p<0.008$). The end-of-treatment response rate and sustained response rate for HCV genotype 1 were 35% (70 of 199 patients) and 15% (30 of 199 patients), respectively. For non-1 genotypes, the rates were 64% (46 of 72 patients) and 47% (34 of 72 patients), respectively. During a median follow-up of 6.8 years, HCC was detected in 31% (84 of 271 patients) of the treated group and in 47.3% (35 of 74 patients) of the untreated group. Eleven of sixty-four (17.2%) of the sustained virologic responders developed HCC compared with 73 of 207 (35.3%) of the nonresponders. Seventeen percent (45 patients) from the treated group and 32% (24 patients) from the untreated group died during the follow-up period. Deaths from liver disease occurred in none of the 64 sustained responders, 15% (32 of 207 patients) of the nonresponders, and 26% (19 of 74 patients) of the untreated patients. SVR was associated with a better chance of survival ($p<0.003$ compared to the untreated group) than was non-SVR ($p=0.19$ compared with the untreated group).

A recent meta-analysis summarized data from 26 cohort studies that followed patients with and without virologic response for development of clinical outcomes [57]. Of these, 20 studies included patients with all stages of fibrosis, whereas 6 were limited to patients with either advanced fibrosis or cirrhosis (F3–F4 or Ishak 4–6). HCV patients with SVR were much less likely to suffer liver-related mortality compared with patients who did not achieve SVR, and this effect was consistent in studies that included HCV patients with all stages of fibrosis (RR, 0.23; 95% CI, 0.10–0.52) or studies that only enrolled patients with advanced fibrosis (RR, 0.19; 95% CI, 0.10–0.37). Similarly, patients with SVR were much less likely to develop HCC or decompensation compared with patients who did not achieve SVR among studies that enrolled patients with any stage of hepatic fibrosis (RR for HCC, 0.21; 95% CI, 0.16–0.27; RR for decompensation, 0.16; 95% CI, 0.04–0.59) and among studies that only enrolled patients with advanced fibrosis or cirrhosis (RR, 0.32; 95% CI, 0.23–0.44; RR for decompensation, 0.13; 95% CI, 0.06–0.27).

Combined, these data demonstrate that SVR is associated with improved long-term outcomes in patients with HCV. These data also show that successful antiviral treatment has the potential of altering the endpoints in high-risk patients with HCV (i.e., those with fibrosis). Whether or not these improved endpoints in clinical studies will translate into a parallel improvement in the natural history at the level of the entire population of individuals with HCV remains to be seen. It is clear that benefit of antiviral treatment is primarily limited to patients with successful eradication of the virus. However, the percentage of patients who achieve SVR is very small compared with the absolute numbers of those infected with HCV. Therefore, a reduction in the incidence of liver-related complications and HCC may not be achieved unless an increasing number of patients

are diagnosed and treated early in the course of infection with more effective therapies.

The recent decision model by Davis et al. [5] clearly highlights these issues. As part of their model, Davis et al. estimated the potential impact of antiviral treatment on cirrhosis and its complications. Assuming that 30% of HCV cases are diagnosed, up to 25% of those are treated, and that all patients with SVR experience no further progression of liver disease (all quite optimistic assumptions), we can expect to see just a 1.0% reduction in cirrhosis by 2020 with current antiviral treatment [5].

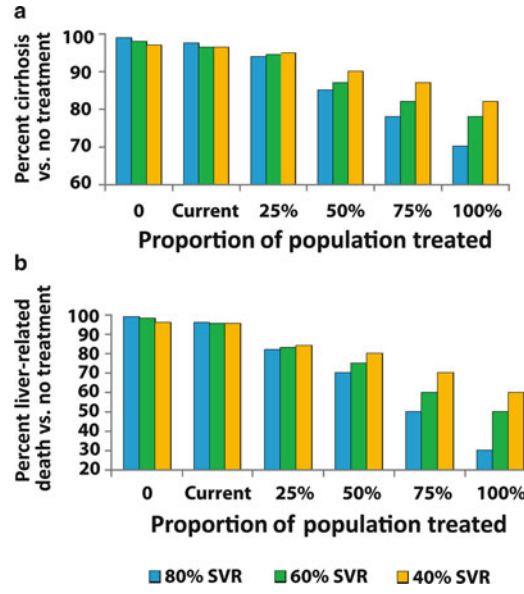

Fig. 9.1 Estimated reductions in cirrhosis (**a**) and liver-related death (**b**) by 2020 assuming incremental treatment of 0–100% of infected persons and sustained virologic response (SVR) rates of 40, 60 and 80% (reprinted from [5]. ©2010. With permission from Elsevier)

Section 3

Future Treatments and Their Likely Impact on the Natural History of CHC

New therapies for CHC are now available in clinical practice. Results from the recently reported clinical trials evaluating the most advanced compounds telaprevir and boceprevir show that the addition of these direct acting protease inhibitors to pegylated interferon and ribavirin strongly improves the chance to achieve an SVR in treatment-naive HCV genotype 1 patient (70–80%) as well as in prior nonresponders and relapsers (40–70%) to standard therapy [58–61]. Although, the effectiveness of these newer agents in clinical practice is expected to be partially offset by new challenges with viral resistance and increased adverse events, these new additions to the armamentarium against HCV are very promising and will likely change the way we treat patients with CHC.

How will this change in the treatment paradigm impact the current projection of cirrhosis and its related complications in patients with HCV? Assuming that the SVR rates in routine clinical practice are as high as those reported in the phase 2 telaprevir clinical trials (~70%), at the current level of treatment penetrance, antiviral treatment will decrease cases of cirrhosis by a mere 5% in 2020. The only way these highly effective therapies can change the trajectory of CHC will be to increase the penetrance of treatment; more patients will need to be treated with these effective therapies. For example, treatment of half or all of CHC persons with these new agents would reduce cirrhosis by 15.2 and 30.4%, respectively, after just 10 years [5]. The effects are more pronounced when looking at complications of liver disease. Indeed, treatment of half or all of infected persons in 2010 would result in decreased cases of liver failure of 39.4 or 78.9%, HCC by 30.2 or 60.4%, and liver-related deaths by 34.0 or 68.0% over the next decade [5] (Fig. 9.1).

These data show that despite the known and potentially improving effectiveness of antiviral treatment, more patients need to be diagnosed and treated in order to impact the current trajectory of disease. The low detection and treatment rates are a special concern given the rising prevalence of cirrhosis in CHC, a disturbing trend that will no doubt continue unless effective treatment can be provided to more patients in a timely manner.

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Part II

Treatment of Chronic HCV with Interferon-Based Therapy

Treatment with Interferon and Ribavirin

10

William Kemp and Stuart K. Roberts

Keywords
Hepatitis C • Interferon-α • Pegylated interferon • Ribavirin • Virological response

The Medications: Interferon and Ribavirin

Although interferon (IFN) and ribavirin have been the cornerstones of treatment of chronic hepatitis C for over a decade, the precise mechanisms by which these agents exert their antiviral effect(s) are incompletely understood. The approved IFN and ribavirin preparations are shown in Table 10.1.

Interferon-α

The IFNs belong to a diverse group of glycoproteins called cytokines and are named after their ability to "interfere" with viral replication within a host cell. IFNs are typically divided into three classes (I–III). IFN-α is one of the type-I IFNs that also includes IFN-β and IFN-ω. These IFNs are produced by lymphocytes in response to pathogens as part of the innate immune system. The type 1 IFNs exert their action through binding to a specific cell surface receptor complex known as the IFN-α receptor (IFNAR) that consists of IFNAR1 and IFNAR2 chains [1]. The two commercially available IFNs in widespread usage for the treatment of hepatitis C are IFN-α2a and IFN-α2b.

The antiviral effect of IFN-α relates not primarily to a direct action upon the virus or replicative cycle, but instead through an indirect process resulting in the transcriptional activation of hundreds of IFN-stimulated genes (ISGs) [1]. The cumulative effect of these ISGs is incompletely understood, although the downstream effect can result in a broad range of actions including inhibition of viral replication, apoptosis and inflammatory cellular responses. Perhaps the most well characterized ISGs are the protein kinase R (PKR), 2′–5′ oligoadenylate synthetase (OAS) and Mx proteins. By phosphorylating the α subunit of eukaryotic initiation factor, eIF2α, PKR inhibits translation of both viral and cellular proteins, thereby explaining the antiviral and antiproliferative effect of IFN. OAS activates a pathway leading to the cleavage of viral and

W. Kemp, MBBS, FRACP
• S.K. Roberts, MBBS (Hons), MD, FRACP (✉)
Department of Gastroenterology,
The Alfred Hospital, Commercial Road, Prahran,
Melbourne, VIC, 3181, Australia
e-mail: s.roberts@alfred.org.au

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Table 10.1 Approved medications for the treatment of chronic hepatitis C

Type-1 interferon (IFN)	Trade name	Dose
Pegylated interferon α2a	Pegasys®	180 μg once weekly
Pegylated interferon α2b	PEG-Intron®	1.5 μg/kg once weekly
IFN-α2a	Roferon®	3–4.5 million IU 3 times/week
IFN-α2b	Intron A®	3 million IU 3 times/week
Consensus IFN	Infergen®	9 μg 3 times/week

Ribavirin	Trade name	Dose
Ribavirin	Copegus®	80–1,200 mg daily
Ribavirin	Rebetol®	80–1,400 mg daily

Based on data from [97]

cellular RNA by RNase L and Mx proteins which interfere with replication of negative stranded RNA viruses such as influenza.

Other Interferons

A variety of other type 1 IFNs have been used in the treatment of hepatitis C. These include consensus IFN (CIFN) or IFN alphacon-1 (Infergen; Valeant) and Albinterferon α-2b (Alb-IFN) (Human Genome Sciences, Novartis).

CIFN is a synthetic recombinant type I IFN protein derived from a consensus sequence of the most common amino acids found in naturally occurring α IFN subtypes. It was suggested that CIFN may possess enhanced biological activity compared to the naturally occurring IFN-α subtypes such as IFN-α2a and IFN-α2b [2]. A multicenter, randomized, double-blind, controlled study of 704 patients who were treated with one of two doses of CIFN (3 and 9 μg) or IFN-α2b (3 million units [MU]) weekly for 24 weeks found similar rates of SVR for the group receiving 9 μg of CIFN and those treated with IFN-α2b [3]. More recent data suggest improved efficacy of CIFN/ribavirin combination compared with IFN-2b/ribavirin particularly in persons with genotype 1 and high viral load [4]. One of the key disadvantages of CIFN is the requirement to administer it more frequently than the once weekly dosing of the Peg-interferons and regimes up to daily dosing have been used [5, 6]. As superior efficacy has not been demonstrated for CIFN compared to either PEG-IFN-α2a or PEG-IFN-α2b it is no longer considered part of the standard of care regime.

Albuferon is a novel 85.7 kDa protein consisting of recombinant human IFN-α2b genetically fused to recombinant human albumin [7]. This results in a formulation of IFN that can be administered every 2–4 weeks due to an extension of the half-life to approximately 200 h. Two large Phase III trials have now been concluded in patients with genotype 1 infection (ACHIEVE-1) [8] and genotype 2/3 (ACHIEVE-2/3) [9]. Both of these trials demonstrated comparable clinical efficacy of albuferon 900 μg every 2 weeks in combination with ribavirin to standard dose PEG-IFN-α2a and ribavirin. Concerns regarding pulmonary toxicity were raised during the course of both of these trials that lead to a discontinuation of the higher dose albuferon treatment arm (1,200 μg). Overall, the reported adverse event profile of the 900 μg dose of albuferon appears similar to that of PEG-IFN-α2a. However, a higher incidence of alopecia, cough, weight loss and ongoing concern for pulmonary toxicity led to a decision not to seek approval for this agent for the treatment of chronic hepatitis C virus (HCV) in the USA or the European Union.

A major limitation of the current standard-of-care regimes is drug toxicity. Novel IFNs such as peg-interferon λ (IL-29) show promise in reducing the constitutional side-effects and hematological toxicities commonly associated with peg-interferon-α2a/α2b, whilst maintaining antiviral activity [10]. Peg-interferon-λ1a (ZymoGenetics)

is a type III IFN (which includes IL-28A, IL-28B, and IL-29 also known as IFN-λ2, 3, and 1, respectively) that binds to a unique receptor with a more limited distribution than the type I IFN receptor. This receptor is not found on bone marrow CD34+ progenitor cells hence explaining the decreased incidence of neutropenia observed with this type of IFN. Peg-interferon-λ1a is currently undergoing further clinical trials to establish its efficacy compared to the current hepatitis C treatment regimes and the results of these studies are eagerly awaited.

The ongoing desire to improve efficacy, reduce side-effects, and simplify drug delivery has led to a variety of novel IFN delivery systems [11]. Locteron™ is a newly developed controlled release preparation of recombinant IFN-α2b in combination with a biodegradable polymer that allows for dosing every 2 weeks by increasing the elimination half-life up to double that of PEG-IFN-α2b [12]. Furthermore, Locteron™ appeared to be associated with fewer influenza-like side-effects [13]. MicroSphere technology has also been used in the development of the polymer-based Medusa® system. This has been formulated with IFN-α2b (IFN-α2b XL) which early phase studies have suggested improved tolerability with weekly dosing [14]. In addition to these novel injectable agents, an oral formulation of IFN has also been tested in phase 1 clinical trials. Lyophilized Belerofon has been incorporated into an enteric-coated tablet. Early results indicate comparable blood levels to injectable IFN although there are no data on efficacy [11].

Ribavirin

Ribavirin is a synthetic nucleoside (guanosine) analogue with a broad spectrum of antiviral activity. Developed in 1970, ribavirin was initially approved for use in an aerosolized form against respiratory syncitial virus. However in the early 1990s, ribavirin was noted to have activity against a range of flaviviruses and it was therefore evaluated as monotherapy for chronic HCV infection. This resulted in improvement or normalization of transaminase levels but as viral loads did not decrease it was deemed ineffective as a primary anti-HCV agent. In contrast, ribavirin when used in combination with IFN substantially increased the proportion of patients clearing the virus and reduced the frequency of relapse post-therapy resulting in a doubling of SVR rates compared to IFN monotherapy. This led in 1998 to its approval in the USA for use in combination with IFN against HCV.

The precise mechanism(s) underpinning the antiviral activity of ribavirin against chronic HCV remains obscure. In general, the potential mechanisms can be thought of as either (1) indirect by effecting the host response or (2) direct inhibition of viral replication. Examples of the indirect action of ribavirin include an effect on the host immune response, altering the TH1/TH2 balance favoring a TH1 response. The TH1 response results in viral clearance whereas the TH2 response favors chronic infection. Furthermore, ribavirin monophosphate (RMP) competitively inhibits inosine monophosphate dehydrogenase (IMPDH), which results in depletion of the GTP necessary for viral RNA synthesis. Ribavirin is also thought to exert a direct inhibitory action on HCV replication via interfering with the HCV RNA polymerase through the incorporation of ribavirin triphosphate. In addition, ribavirin can act as a viral mutagen resulting in production of virions that are less fit to replicate. This would significantly impact upon the ability of HCV to escape the innate immune system and render it more vulnerable to the effects of IFN [1].

Historical Perspective

Interferon Monotherapy

Prior to the identification of hepatitis C in 1989, Hoofnagle et al. [15] published a pilot study using recombinant human IFN-α treatment for non-A, non-B hepatitis in ten subjects. Early use of IFN in this setting was hampered by the absence of diagnostic test for hepatitis C and therefore initial endpoints for therapy were improvement in liver transaminases. While hepatic transaminase levels did improve or normalize on treatment they

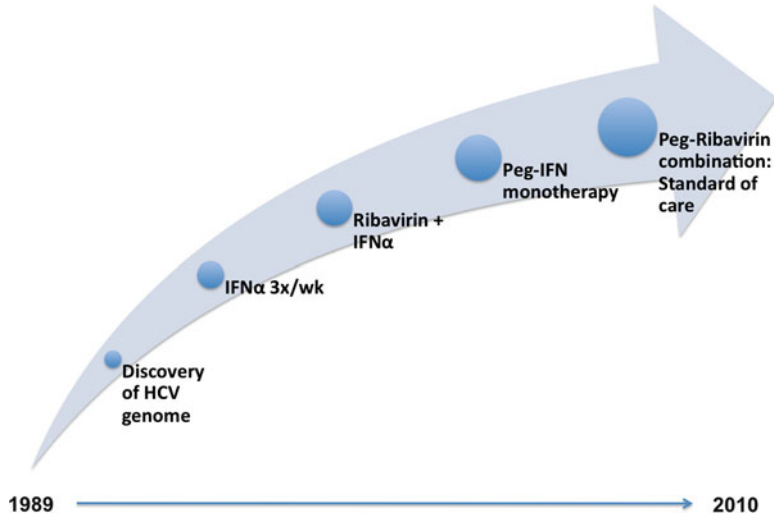

Fig. 10.1 The evolution of hepatitis C treatment

frequently increased again during follow-up. Once HCV was identified [16] and diagnostic tests were developed, the effect of IFN therapy on HCV could be studied. Reanalysis of the serum samples from Hoofnagle's original publication indicated that of the ten subjects, two were virologic nonresponders to IFN, two responded to IFN but relapsed and six remained PCR-negative after 3–6.5 years of follow-up (one of these patients was PCR-negative at baseline) [17]. In 1989 initial reports on the treatment of HCV were published [18, 19], however success was limited by high rates of relapse. Several randomized controlled trials were subsequently published using IFN-α2b [18–22]. Dosages of IFN typically ranged between 1 and 3 MU subcutaneously 3 times/week for 6 months. Responses and relapses were still defined by biochemical results although several of the early studies included liver biopsy analysis as well. These early studies confirmed Hoofnagle's original observation that treatment of non-A, non-B with IFN was able to improve serum biochemistry and completely normalize serum ALT levels in-between 6 and 52% of patients depending on the regime used. A dose–response effect was observed with complete normalization of ALT being demonstrated in 38% of subjects treated with 3 MU, 3 times/week for 6 months compared to 23% of those receiving 1 MU [23]. In 1991, IFN-α2b was approved as the first treatment for chronic HCV. Subsequent studies indicated that prolonging the duration of therapy could increase the number of patients normalizing their ALT, however biochemical relapse after treatment was stopped continued to be a common occurrence (see Fig. 10.1) [24]. Furthermore, an increasing body of evidence indicated that IFN-α monotherapy produced histological improvement in both necro-inflammatory activity and fibrosis [25, 26].

Interferon and Ribavirin

A major step forward in the treatment of chronic HCV occurred in 1998 with the simultaneous publications of the results of IFN and ribavirin combination therapy (see Fig. 10.1) [27, 28]. McHutchison and colleagues conducted a randomized double-blind, placebo-controlled trial of 912 treatment-naïve individuals with chronic hepatitis C to receive IFN-α2b either as monotherapy or in combination with weight-based ribavirin for either 24 or 48 weeks. Sustained virological response (SVR), defined as undetectable HCV RNA after 24 weeks of treatment-free follow-up (Fig. 10.2), for the 24- and 48-week IFN-α2b monotherapy groups was 6 and 13% respectively.

Combination IFN-α2b plus ribavirin therapy significantly improved SVR rates to 31 and 38% respectively. Davis and colleagues also examined the utility of IFN-α2b with or without ribavirin in 345 patients who had relapsed after a prior response to IFN therapy. In this more difficult-to-treat population, SVR rates were 48% in the combination group vs. only 5% in the IFN-α2b monotherapy group. The relatively good SVR achieved in this study of patients who had relapsed to prior IFN therapy may in part be explained by a lower proportion of genotype 1 subjects compared to McHutchison's cohort (57% vs. 73%). In the same year, Poynard et al. [29] published the results from their randomized controlled trial of IFN-α2b ± ribavirin. SVR rates in this trial of 832 subjects were 43% for IFN-α2b and ribavirin combination therapy compared with 19% for IFN-α2b monotherapy (Fig. 10.2). Reichard et al. [30] produced similar results in a trial of 100 IFN-naïve subjects treated for 24 weeks (36% vs. 18% SVR rates for combination therapy and monotherapy respectively). These studies confirmed that virological, biochemical, and histological endpoints were more frequently achieved with combination therapy compared to IFN monotherapy in both treatment-naïve subjects and patients who had previously relapsed following IFN monotherapy. Overall SVRs were now achievable in 38–49% of subjects treated for 48 weeks. A meta-analysis including 6,585 patients randomized to IFN-α plus ribavirin vs. IFN-α monotherapy concluded that combination therapy decreased the risk of treatment failure by 26% in treatment-naïve patients (relative risk 0.74, 95% confidence interval 0.70–0.78), 33% in relapsers (0.67; 0.57–0.78), and 11% in nonresponders (0.89; 0.83–0.96) [31]. Thus, the new standard of care became combination therapy with IFN and ribavirin (see Fig. 10.1; Table 10.2).

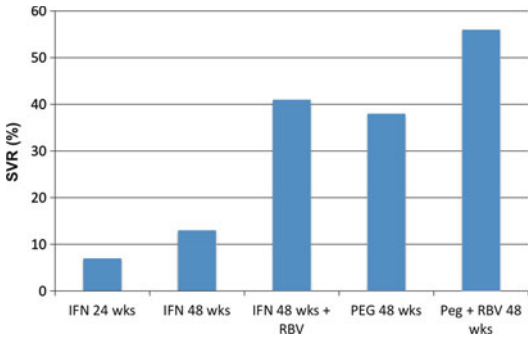

Fig. 10.2 Patterns of virological response to chronic hepatitis C treatments

Table 10.2 Accepted indications and contraindications to chronic hepatitis C according to the AALSD hepatitis C practice guidelines

Indications
Age 18 years or older
HCV RNA-positive in serum
Liver biopsy showing chronic hepatitis with significant fibrosis (bridging fibrosis or higher)
Compensated liver disease (total serum bilirubin <1.5 mg/dL; INR <1.5; serum albumin >3.4 g/dL, platelet count $>75 \times 10^9$/L and no evidence of hepatic decompensation (hepatic encephalopathy or ascites))
Acceptable hematological and biochemical indices (hemoglobin 13 g/dL for men and 12 g/dL for women; neutrophil count 1.5×10^9/L and serum creatinine <1.5 mg/dL)
Willing to be treated and to adhere to treatment requirements
No contraindications
Contraindications
Major uncontrolled depressive illness
Solid organ transplant (renal, heart, or lung)
Autoimmune hepatitis or other autoimmune conditions known to be exacerbated by peginterferon and ribavirin
Untreated thyroid disease
Pregnant or unwilling to comply with adequate contraception
Severe concurrent medical disease such as severe hypertension, heart failure, significant coronary heart disease, poorly controlled diabetes, chronic obstructive pulmonary disease
Age less than 2 years
Known hypersensitivity to drugs used to treat HCV

Adapted from [37], with permission from Wiley

Pegylated Interferons

The development of pegylated IFNs more than 15 years ago represented a major advance and today these agents still remain the standard of care in the treatment of chronic hepatitis C. The conjugation of polyethylene glycol (PEG) with either IFN-α2b or IFN-α2a allowed for a reduction in clearance compared to IFN alone. Open-labeled comparison studies demonstrated a tenfold increase in the elimination half-life of IFN-α2b [32] which allows for weekly subcutaneous dosing rather than the 3 times a week regimen used with standard IFN. There are currently two pegylated IFNs used in widespread clinical practice: peginterferon-α2a (PEG-IFN-α2a) and peginterferon α2b (PEG-IFN-α2b). Despite the apparent similarities of the two molecules there are several important differences. PEG-IFN-α2a is the product of conjugating a 40 kDa single branched PEG chain to IFN-α2a. The active molecule has a molecular weight of approximately 60 kDa (Pegasys product information. Amended Oct 2009). Peak serum concentrations are reached 72–96 h after dosing and serum concentrations are maintained throughout 7 days supporting weekly dosing. By comparison, PEG-IFN-α2b consists of a linear 12 kDa PEG chain linked to IFN. The two peg-interferon molecules therefore significantly differ in their molecular mass. Another key difference is the nature of the chemical bond between the PEG molecule and the IFN. PEG-IFN-α2b utilizes a urethane linkage that is susceptible to hydrolysis, and once injected the native IFN molecule is released from the PEG and circulates in the body. The branched chain PEG moiety used in PEG-IFN-α2a is bound by a stable, amide bond that is not subject to hydrolysis and hence the entire compound circulates intact, and interacts with the receptor. These differences mean that PEG-IFN-α2b is absorbed more rapidly (4.6 h compared to 50 h) and has a larger volume of distribution (0.99 L/kg compared to 8 L) compared to PEG-IFN-α2a [33]. The clinical implication of these differences is that PEG-IFN-α2a can be given as a fixed nonweight-dependent dose of 180 μg/week whereas weight-based dosing is required for PEG-IFN-α2b.

In 2000, Zeuzem et al. [34] reported on the first efficacy and safety of PEG-IFN-α2a (40 kDa) as monotherapy compared to conventional IFN monotherapy (see Fig. 10.1; Table 10.2). Subjects received 48 weeks of PEG-IFN-α2a treatment and a further 24 weeks of follow-up. This study was open-labeled and included mixed genotypes. At the end of follow-up 39% of the PEG–IFN group achieved an SVR compared to 19% of the standard IFN group. Equally as important as the improved efficacy of weekly pegylated IFN was that the side-effect profile was similar between in the two regimes. A similar improvement in efficacy was demonstrated in the difficult-to-treat subgroup of subjects with advanced hepatic fibrosis/cirrhosis [35].

Current Treatment

Standard of Care

The current recommendations for the treatment of chronic HCV are a combination of either PEG-IFN-α2a, 180 μg/week subcutaneously or PEG-IFN-α2b, 1.5 μg/kg/week subcutaneously in combination with ribavirin (see Fig. 10.1) [36, 37]. This strategy is based upon the results of three pivotal studies demonstrating the efficacy of this regime over standard IFN and ribavirin or Peg-IFN-α monotherapy (see Table 10.2; Fig. 10.2) [38–40]. Hadziyannis explored the utility of shortening treatment regimes from 48 to 24 weeks in addition to comparing low-dose ribavirin (800 mg/day) vs. a standard weight-based dose (1,000 or 1,200 mg/day). This pivotal study demonstrated that the optimal regime for genotype 1 subjects was standard-dose ribavirin in conjunction with 48 weeks of PEG-IFN-α2a. This regime resulted in an SVR of 52% that was significantly higher than the 41% in the group who received 800 mg of ribavirin and PEG-IFN-α2a for 48 weeks. Not surprisingly, the lowest SVR of 29% was observed in the group who received low-dose ribavirin in conjunction with 24 weeks of PEG-IFN-α2a. In contrast, 84% of genotype 2 and 3 subjects included in this study achieved an SVR that remained high even with

low-dose ribavirin and treatment with PEG-IFN-α2a for only 24 weeks. In the current standard of care regimens, ribavirin dosing depends on both body weight and HCV genotype. There is evidence supporting the use of ribavirin at a dose >10.6 mg/kg [39]. For patients infected with an HCV genotype 1 or 4 weight-based ribavirin doses of 800–1,200 mg/day (1,400 mg/day for patients who weigh >105 kg receiving PEG-IFN-α2b) are recommended. For patients with an HCV genotype 2 or 3 infection, the recommended dose of RBV is 800 mg/day. The duration of therapy is also influenced by the hepatitis C genotype. Both genotype 1 and 4 are treated for 48 weeks and genotypes 2 and 3 treated for 24 weeks. The recommendations for patients infected with genotypes other than 1–4 are also for 48 weeks of treatment (see Table 10.3).

Patient Selection

All patients with chronic hepatitis C are potential candidates for treatment with peginterferon plus ribavirin in the absence of major contraindications (see Table 10.2). Important factors typically considered by both the treating physician and patient during the evaluation for therapy include an estimate of the likelihood of response, the severity and/or estimated risk of progression of liver disease, patient motivation, presence of any active contraindications, and response and/or tolerability to prior IFN. Ultimately, the decision to proceed with treatment should be based on an informed assessment that there is a favorable benefit to risk ratio for the patient.

Predictors of Response

HCV genotype is the strongest predictor of therapeutic efficacy to combination peginterferon plus ribavirin. Using standard of care regimes, SVR rates are around 50% for genotype 1, 80–90% for genotype 2 and 65–80% for genotype 3. Genotype 4 patients have an SVR in-between the genotype 1 and 3 subjects. There are however a number of other important virological, disease, and host factors that substantially influence treatment response. These include: viral load, hepatic fibrosis severity, HIV co-infection, race, age, gender, body mass index, insulin resistance, and more recently recognition of the importance of the IL-28 genotype [41–43].

Adherence with therapy is another critical determinant of treatment response. The initial evaluation of adherence by McHutchison and colleagues [39] divided patients into two groups – those that received at least 80% of the planned total cumulative dose of ribavirin and IFN. Patients with HCV genotype 1 who achieved this dose had SVR rates of 51% compared to only 34% for patients who received lesser amounts of these medications. The greatest impact was observed in patients who received less than 80% of the total cumulative dose of ribavirin and peginterferon during the first 12 weeks of treatment. In contrast, no significant difference in SVR was observed for patients with HCV genotypes 2 and 3 who received more or less than 80% of the total cumulative doses of ribavirin and peginterferon.

Comparison of Peginterferons

Both PEG-IFN-α2a and PEG-IFN-α2b are generally thought to have similar clinical efficacy in the treatment of chronic HCV infection and that drug selection predominantly relates to patient and physician preference. However, several recent data have emerged that challenges this assumption. The largest head-to-head trial (see Table 10.3) [44] was the IDEAL study conducted only in patients with genotype 1. This study included 3,070 treatment-naïve participants that were randomized to one of three different regimes (PEG-IFN-α2b at a standard dose of 1.5 μg/kg/week or a low dose of 1.0 μg/kg/week, plus ribavirin at a dose of 800–1,400 mg/day, or PEG-IFN-α2a at a dose of 180 μg/kg/week plus ribavirin at a dose of 1,000–1,200 mg/day). End-of-treatment responses (ETR) were higher in the PEG-IFN-α2a recipients compared to the low- and standard-dose PEG-IFN-α2b (64.4% vs. 49.2 and 53.2%) although the overall SVR rates were

Table 10.3 Summary of pivotal randomized PEG-interferon based trials in chronic hepatitis C

References	*N*	Intervention	Duration (weeks)	Genotype	SVR (%)
Heathcote et al. [35]	271	IFN-α2a 3 MU 3×/week	48	Mixed (cirrhosis or bridging fibrosis)	8
		Peg-IFN-α2a 90 μg/week			15
		Peg-IFN-α2a 180 μg/week			30
Zeuzem et al. [34]	531	IFN-α2a 6 MU 3×/week for 12 weeks then 3 MU 3×/week for 36 weeks	48	Mixed	19
		Peg-IFN-α2a 180 μg/week			39
Manns et al. [39]	1,530	IFN-α2b 3 MU 3×/week + RBV 1,000–1,200 mg	48	Mixed	47 (33% G1, 79% G2/3)
		Peg-IFN-α2b 1.5 μg/kg/week + RBV 800 mg			54 (42% in G1, 82% G2/3)
		Peg-IFN-α2b 1.5 μg/kg/week for 4 weeks then 0.5 μg/kg/week + RBV 1,000–1,200 mg			47 (34% in G1, 80% G2/3)
Fried et al. [38]	1,121	IFN-α2b 3 MU 3×/week + RBV 1,000–1,200 mg	48	Mixed	44 (36% G1, 61% G2/3)
		Peg-IFN-α2a 180 μg/week			29 (21% G1, 45% G2/3)
		Peg-IFN-α2a 180 μg/week + RBV 1,000–1,200 mg			56 (46% G1, 76% G2/3)
Hadziyannis et al. [40]	1,311	Peg-IFN-α2a 180 μg/week + RBV 800 mg	24	G1	29
				G2/3	84
		Peg-IFN-α2a 180 μg/week + RBV 800 mg	48	G1	41
				G2/3	79
		Peg-IFN-α2a 180 μg/week + RBV 1,000–1,200 mg	24	G1	42
				G2/3	81
		Peg-IFN-α2a 180 μg/week + RBV 1,000–1,200 mg	48	G1	52
				G2/3	80
Mangia et al. [57]	283	Peg-IFN-α2b 1.0 μg/kg/week + RBV 1,000–1,200 mg	24	G2/3	76
			12 if RVR		85
		Peg-IFN-α2b 1.0 μg/kg/week + RBV 1,000–1,200 mg	24 if no RVR		64
Shiffman et al. [59]	1,469	Peg-IFN-α2a 180 μg/week + RBV 800 mg	16	G2/3	62 (79% if RVR)
		Peg-IFN-α2a 180 μg/week + RBV 800 mg	24		70 (85% if RVR)
McHutchison et al. [44]	3,070	Peg-IFN-α2a 180 μg/week + RBV 1,000–1,200 mg	48	G1	40.9
		Peg-IFN-α2b 1.5 μg/kg/week + RBV 800–1,400 mg			39.8
		Peg-IFN-α2b 1.0 μg/kg/week + RBV 800–1,400 mg			38
Roberts et al. [64]	896	Peg-IFN-α2a 360 μg/week for 12 weeks then 180 μg/week + RBV 1,000–1,200 mg	48	G1	53
		Peg-IFN-α2a 180 μg/week + RBV 1,000–1,200 mg			50

similar (40.9% vs. 38.0 and 39.8%). A number of prospective randomized studies have since been published comparing PEG-IFN-α2a to PEG-IFN-α2b [44–51]. Most of these reports support the findings of the IDEAL study [49, 50]. In an investigator-initiated study, Rumi et al. compared standard-dose PEG-IFN-α2a and PEG-IFN-α2b in a moderately large ($n=431$) group of mixed genotype treatment naïve subjects. SVR rates were higher in PEG-IFN-α2a compared to the PEG-IFN-α2b patients (66% vs. 54%, respectively, $P=0.02$), being 48% vs. 32% in the 222 HCV-1 and -4 patients ($P=0.04$), and 96% vs. 82%, respectively, in the 143 HCV-2 patients ($P=0.01$). No differences were observed in serious adverse events (1% respectively) or treatment discontinuation rates for adverse effects (7% vs. 6%, respectively). Ascione et al. also reported similar findings in a study of 320 treatment-naïve patients infected with HCV genotypes 1–4. A subsequent systematic review that included data on over 5,000 patients concluded that PEG-IFN-α2a significantly increased the number of patients who achieved an SVR compared to PEG-IFN-α2b (47% vs. 41%) [52]. Still both PEG-IFN-α2b and PEG-IFN-α2a are currently considered appropriate first-line treatment options for chronic hepatitis C. However, there may be important implications of these findings in relation to the preferred choice of peginterferon as we enter the new era of combination therapy with direct acting antiviral agents and peginterferon.

Variations to Standard of Care

A number of variations to the standard of care have been explored in order to optimize treatment outcomes and/or minimize exposure to medication in order to reduce side-effects and encourage compliance.

Response-Guided Therapy

Subgroup analysis of the registration trial by Hadziyannis et al. demonstrated that genotype 1 subjects, those who achieved an RVR, could reduce the duration of therapy from 48 to 24 weeks without significantly compromising efficacy [53]; this finding has since been confirmed by several prospective open-label and randomized controlled trials [45, 54, 55]. Concerns have been raised, however, about applying this strategy to all RVR patients, and in particular those who have high viral loads and/or advanced hepatic fibrosis. [56] Because of this, a shortened duration therapy has not been universally embraced for all patients with RVR. Despite these caveats, both PEG-IFN-α2a and PEG-IFN-α2b are approved in the European Union for a shortened treatment duration of 24 weeks in HCV genotype 1 patients with a low viral load and an RVR.

Similarly, strategies to reduce the duration of the therapy have been explored in those infected with genotype 2 or 3 (see Table 10.3). [57, 58] The largest of these, the Accelerate study, [59] was a randomized noninferiority trial that included 1,469 subjects with either genotype 2 or 3. In this trial, SVR rates were significantly lower in patients treated for 16 weeks compared to those treated for 24 weeks (62% vs. 70%). Again, certain subpopulations were identified in whom a shorter duration did not significantly impact on SVR including persons with low baseline viral load. However, even among those with an RVR, SVR rates were higher in the 24-week treatment group compared to those treated for 16 weeks (85% vs. 79%, $P=0.02$). This customization of treatment according to on-treatment response has been termed "response-guided therapy" and will be reviewed in-depth in subsequent chapters.

Induction Therapy

A variety of alternative higher dosing schedules have been examined in an attempt to increase the number of patients achieving an SVR. Intensified therapy utilizing higher and/or more frequent doses of (peg)interferon during the initial 12 weeks of treatment has been evaluated in several studies [60–64]. The rationale for this so-called induction strategy is that high-dose IFN may improve SVR by inducing a more rapid initial decline in HCV

RNA. Earlier studies using IFN monotherapy had attempted to maximize viral suppression and minimize trough levels of IFN when viral rebound is most likely to occur [24]. While induction dosing with PEG-IFN-α2a 360 μg/week for 12 weeks followed by standard dosing did indeed increase rapid (RVR) and the early virological response (EVR) in genotype 1 subjects, the ETR and SVR remained unchanged [64]. Hence this regime cannot be recommended at the current time (see Table 10.3). Using a higher dose of peginterferon for the duration of therapy has also been examined. Higher rates of SVR are achievable in the difficult-to-treat genotype 1 subjects (high viral load and weigh >85 kg) using 270 μg/week compared to the standard of care 180 μg/week, however the increased IFN dose also increases the incidence of adverse events and premature drug withdrawal [65].

Special Patient Populations

Nonresponders

Nonresponders to previous IFN-based therapy represent a heterogeneous patient population that can be categorized into the following subgroups according to the virological response achieved on therapy: (a) relapsers, (b) partial responders, and (c) null responders (see Fig. 10.3). Virological relapsers achieve an undetectable viral load on treatment, however develop a detectable HCV viremia once treatment is ceased. This is distinct from having a virological breakthrough which occurs when HCV RNA becomes detectable again while still on therapy. Partial responders are defined as those with a greater than 2-$\log_{10}$ decline in viral load between baseline and week 12 on treatment but who remain HCV RNA-positive on therapy. Null responders are defined as those patients with either a less than 1-$\log_{10}$ decline in viral load after 4 weeks of therapy or less than 2-$\log_{10}$ decline in viral load after 12 weeks of treatment.

Retreatment of Relapsers

Subjects who relapse after previous standard IFN, either as monotherapy or in combination with ribavirin, are potentially suitable candidates for retreatment with peg-interferon-based therapies. Between 40 and 50% of relapsers to IFN plus ribavirin achieve an SVR with combination peginterferon plus ribavirin therapy for 48 weeks. HCV clearance rates improve to >50% in subjects who are HCV RNA-negative at the 12-week point of retreatment and are significantly better in genotype 2/3 than genotype 1 patients (67% vs. 32% respectively) [66, 67]. Therefore, retreatment with the current standard of care needs to be

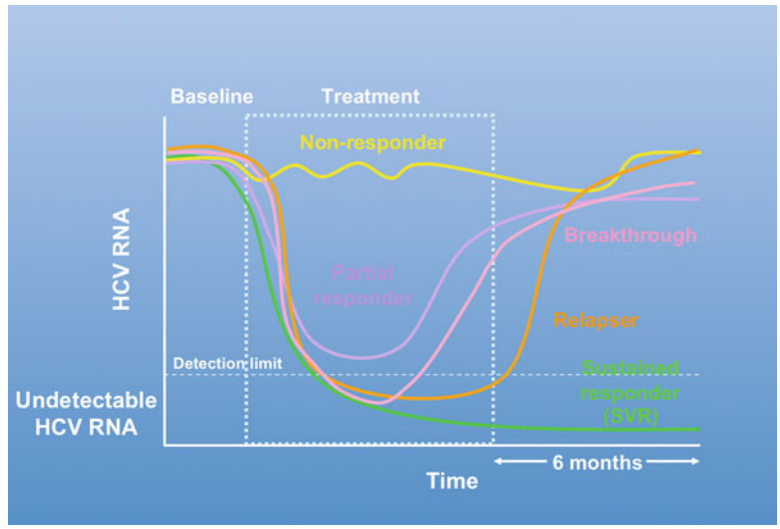

Fig. 10.3 Sustained virological response (SVR) rates (all genotypes) according to treatment regime

considered in all subjects who have relapsed after treatment with conventional IFN-based therapies.

Retreatment of relapsers to peginterferon and ribavirin is more problematic with limited data available and no consistent consensus opinion available about the appropriate retreatment strategy. However for appropriately selected subjects, acceptable SVR rates are achievable. In patients who relapse after a 24-week course of peg-interferon and ribavirin, retreatment with 48 weeks of peginterferon and ribavirin achieves SVR rates of 51 and 63% in genotype 1 and non-1 subjects respectively [68]. The results are less impressive in genotype 1 subjects who relapse after 48 weeks of peginterferon and ribavirin therapy; 29% obtain an SVR following retreatment with the same dose and duration. Limited clinical data in this group suggest that SVR rates may be increased to 50% by prolonging therapy duration to 72 weeks [5, 69].

Retreatment of Nonresponders

The results achieved with retreatment of nonresponders are generally lower than that seen in patients who relapse to previous therapy, and are particularly poor in those who fail peginterferon plus ribavirin therapy. While 18% of nonresponders to conventional IFN-based therapy achieve an SVR following retreatment with a 48-week course of peginterferon plus ribavirin, only 6–9% of nonresponders to peginterferon-based treatment obtain an SVR with the same regimen [66, 70]. Treatment outcomes in this latter group are however genotype-dependent; 20–36% of genotype non-1 subjects achieve an SVR compared to 4–6% of genotype 1 patients. Considering the low likelihood of achieving an SVR, retreatment of nonresponders with the same regime cannot be recommended, particularly those with HCV genotype 1 [37]. The options for this subset of patients are therefore maintenance therapy designed to control viral replication and/or reduce the risk of disease progression, or treatment using alternative regimens or strategies. However, the failure of maintenance therapy to improve clinical endpoints [71] has meant that this strategy is rarely adopted in clinical practice. The large REPEAT study in peginterferon α-2b nonresponders demonstrated that the SVR rate doubles to 16% by extending the duration of retreatment to 72 weeks [70]. Complete viral suppression (HCV RNA <50 IU/mL) at week 12 identified patients most likely to respond to extended therapy with 57% of these achieving an SVR. Intensified therapy with 12 weeks of high-dose peginterferon 360 μg/week was also explored in this trial but this strategy failed to improve treatment outcomes. Because of the overall low response rates and concern regarding the tolerability of prolonged therapy, many nonresponders to peginterferon and ribavirin elect to undergo monitoring or wait until direct acting antiviral agents become available either via clinical trials or as part of an approved treatment regime. Those with advanced fibrosis should be considered for HCC surveillance programs and assessed for liver transplantation if appropriate.

Cirrhosis

Despite the increased difficulty in achieving an SVR in patients with compensated cirrhosis [38–40, 64] there are clear benefits for those who do. A reduction in mortality [72–74], a lower risk of progression to advanced liver disease complications [72, 75] including hepatocellular carcinoma [76], portal hypertension [77], and development of esophageal varices [78], and an improvement in liver fibrosis and/or reversal of cirrhosis [79, 80] are all described in subjects with cirrhosis who achieve an SVR. Several treatment studies have focused exclusively on subjects with severe fibrosis demonstrating a poor 15% SVR to peginterferon monotherapy [35] that improves to 44–52% with the addition of standard-dose ribavirin to peginterferon α-2a/2b for 48 weeks (see Table 10.3) [81–84]. Results however remain poor in genotype 1 patients with only 10–33% of those with cirrhosis

achieving an SVR [64, 81–86]. In contrast 57–73% of HCV genotype 2/3 subjects with cirrhosis achieve an SVR when treated with 24 weeks of combination peginterferon plus ribavirin [81–85]. There appears to be no overall benefit to extending therapy to 48 weeks [40]. Important factors contributing to the poor SVR in subjects with cirrhosis/advanced fibrosis include a lower rate of rapid and early viral responses and higher relapse rates [85, 87]. Notably, the low SVR is not explained by inadequate therapeutic dosing of either peginterferon or ribavirin in cirrhosis patients even though dose reductions due to neutropenia and or thrombocytopenia are more common [82, 87].

The presence of portal hypertension and hepatic decompensation also has a significant negative impact on SVR. Everson et al. [86] found that SVR rates in those with cirrhosis and thrombocytopenia were 8% for genotype 1 and 25% for non-1 genotypes. Moreover, patients with decompensated cirrhosis tolerate IFN-based treatment poorly and have increased side-effects which could lead to further hepatic decompensation. As a result, treatment of patients with a history of hepatic decompensation should either not be treated or only after they have been considered candidates for hepatic transplantation [37]. Those with compensated Child–Pugh A liver disease who have not previously had complications of cirrhosis can be treated but require close monitoring for adverse events [37]. The recommended treatment duration for HCV genotype 1/4 patients is 48 weeks while genotype 2/3 patients should receive 24 weeks. During treatment, growth factors may be useful to reduce the severity of anemia and leucopenia and to improve quality of life and limit dose reductions particularly in those with decompensated cirrhosis. The use of the thrombopoietin receptor agonist, eltrombopag olamine (GlaxoSmithKline, Collegeville, PA) before and/or during peginterferon therapy may be useful in patients with thrombocytopenia to both initiate and remain on treatment [88]. Additional studies will determine if this strategy could enhance SVR.

Children

There are an estimated 7,200 new cases of HCV infection in children per year in the United States with mother-to-child (vertical or perinatal) transmission the most common mode of HCV acquisition [89]. The natural history of HCV infection in childhood appears more favorable with acutely infected children more likely to spontaneously clear HCV compared to adults [90], and those chronically infected likely to have minimal liver disease progression and infrequent cirrhosis after 10–20 years of infection [91–94]. HCV-infected children aged 2–17 years are nevertheless considered suitable candidates for treatment using the same selection criteria as those applied to adults [37]. The approved regimen in the United States is peginterferon α-2b 60 μg/m^2/week + RBV 15 mg/kg/day for 48 weeks; this achieves an overall SVR of 59 and 48% SVR in genotype 1 subjects [95].

Future Directions

Notwithstanding the significant improvements in the treatment of chronic hepatitis C over the past two decades, there is still considerable room for improvement, both in terms of increasing SVR rates as well as reducing drug-related toxicities. The combination of peginterferon and ribavirin is likely to remain the basis of future strategies in treating chronic hepatitis C in the short to medium term. The rapidly growing and encouraging literature regarding the use of HCV polymerase and protease inhibitors holds great promise to deliver improved outcomes for relapsers/nonresponders and treatment-naïve subjects in the coming years particularly those infected with HCV genotype 1. In the interim period, the recognition of both virological factors (genotype, viral load) and patient factors (ethnicity, liver fibrosis severity, gender, insulin resistance, etc.) influencing SVR rates has resulted in improved identification and stratification of patients suitable for standard therapy.

Directly relevant to this is the recent recognition of the importance of the IL-28B genotype

in predicting SVR by several independent research groups [41–43]. For a Caucasian subject infected with genotype 1 and a low viral load (<600,000 IU/mL) in conjunction with F0–F2 hepatic fibrosis (METAVIR staging system), the probability of obtaining an SVR ranges from 52 to 86% depending on the IL-28B genotype [96]. Along with viral load, ethnicity and fibrosis stage, IL-28B genotype is an independent predictor of SVR in genotype 1 patients. Pretreatment assessment of the IL-28B genotype will almost certainly become part of routine clinical practice in the future and provide a key basis on which decisions are made regarding treatment with peginterferon therapy.

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Immune Modulators and Other Agents

11

Edgar D. Charles and Ira M. Jacobson

Keywords
Hepatitis C virus • Immune modulators • Innate immunity • Adaptive immunity • Toll-like receptors • Interferon α • Interferon λ • Vitamin D3

Historical Perspective

This chapter considers the treatment and prevention of HCV with immune modulators, either as stand-alone treatments, or in combination with other antiviral therapies. The human immune system balances the need to recognize and eradicate pathogens, both familiar and novel, with the imperative to not unduly harm the host. Over the past 30 years, the development of immunologic-based therapies has been one of the most exciting success stories in medicine. Notable examples of widely used immunotherapeutics include interferon (IFN) and monoclonal antibodies (mAbs) directed against tumor necrosis factor (e.g., infliximab, etanercept, adalimumab), α4 integrin (nataluximab), and B-cell-associated CD20 (rituximab). However, while these agents have high therapeutic indices, enthusiasm for their use must be tempered by their potential for immunosuppression and/or induction of autoreactivity. In fact the elicitation of near-fatal systemic inflammatory responses in healthy volunteers who were administered TGN1412, a T-cell activation-inducing, superantagonist anti-CD28 mAb [1] has vividly demonstrated the potential adverse clinical consequences of therapeutic immune system activators.

Given the historical difficulty in treating HCV, there has been considerable interest in pharmacologically leveraging the immune system to help combat HCV infection. With HCV, the same immunological reactions that cause hepatocellular damage are also necessary for successful viral clearance. Thus, while novel immunomodulators may prove to be invaluable agents, their use for HCV infection will involve a meticulous weighing of their costs and benefits compared to other alternatives. As direct acting anti-viral (DAA) agents capable of clearing infection in 70% or more of patients become part of standard of care, therapeutic equipoise may be increasingly difficult to establish with immunomodulatory therapies as stand-alone agents. Thus, in the future, it is likely that immunomodulatory agents will be developed either as add-on therapies, as replacements

E.D. Charles, MD
Laboratory of Virology and Infectious Disease,
The Rockefeller University, New York, NY, USA

I.M. Jacobson, MD (✉)
Department of Medicine, Weill Cornell Medical College,
New York Presbyterian Hospital, 1305 York Avenue,
4th floor, New York, NY 10021, USA
e-mail: imj2001@med.cornell.edu

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Table 11.1 Anti-HCV immunomodulatory therapies in development

Class	Agent	Phase (late 2010)
Immune stimulants, unclear MOA	Nitazoxanide	3
	Vitamin D3	3
Cyclophilin B inhibitors	Debio-025	2
	SCY-635	1
HMG CoA reductase inhibitors	Statins, multiple	3
Oligonucleotide	SPC3649	1
	ISIS-14803	Halted
TLR agonists	IMO-2125	1
	ANA773	1
	SD-101	1
	CPG 10101	Halted
	ANA245	Halted
Immunomodulatory peptide	SCV-07	2
Anti-PD-1 antibodies	CT-011	1
IL-7	Human recombinant IL-7	1
Anti-phosphatidylserine mAb	Bavituximab	1
MMP inhibitors	CTS-1027	1
Caspase inhibitors	PF-03491390	1
Therapeutic vaccines	GI-5005	2
	IC41	2
	TG4040	1
	ChronVac-C	1
Prophylactic vaccines	HCV E1E2/MF59	1
Passive immunotherapy	Hyperimmune globulin	0
	Anti-HCV antibody cocktail	0

for IFN, or for use in patients who fail to reach SVR after receiving directly acting antiviral agents with or without IFN.

Immune modulation and emerging HCV therapies are rapidly moving twin targets. This chapter will focus on anti-HCV immune modulators that are in active preclinical or clinical development (Table 11.1). To put these agents into context, the chapter begins with a broad overview of HCV biology and the immune response. More comprehensive reviews of HCV immunology have recently been published [2, 3]. We hope that our overview will not only aid the reader in considering the relative merits and drawbacks of immune modulators in clinical development, but will also highlight immune targets that may be appropriate for pharmacologic intervention in the near future. The chapter will then continue by discussing immunomodulatory agents individually, and it will conclude with a brief survey of HCV vaccines and passive immunotherapy.

Implications of HCV Biology on the Host Immune Response

HCV is an enveloped positive-strand RNA virus in the genus *Hepacivirus* and the family *Flaviviridae.* HCV is physically associated with VLDL, LDL, and HDL in the blood of infected patients. Hepatocellular entry of HCV is dependent upon interaction with an array of receptors (CD81, SR-B1, claudin-1, and occludin, reviewed in [4]) that plays a major role in species and tissue tropism. The 9.6 kb HCV RNA genome contains large 5′ and 3′ untranslated regions (UTR) that are essential for translation and replication (reviewed in [5]). Virions undergo clathrin-mediated endocytosis and pH-dependent release from endosomes. It is thought that HCV translation and replication occurs in association with a cytosolic membranous web containing lipid rafts derived from the endoplasmic reticulum. Translation of the single, long open-reading frame yields a polyprotein of approximately 3,000 amino acids. This polyprotein is cleaved by host and viral proteases to release the ten individual proteins that comprise the viral particle and replication machinery.

The viral genome is transcribed in the cytoplasm to yield a complementary negative-strand RNA that serves as a template for positive-strand RNA molecules. HCV has no reverse-transcriptase activity; NS5B, an error-prone RNA-dependent RNA polymerase lacking proofreading capacity, synthesizes both negative- and positive-strand RNA. This error-prone polymerase, combined with the high replication rate of HCV, allows for rapid viral evolution in response to immune pressure or nonsuppressive antiviral therapy. This

diversification is contingent upon mutations that variably, but often negatively, affect viral "fitness." To date, six major HCV genotypes and more than 100 subtypes have been identified, and the viral population within individual patients contains a quasispecies "swarm" of distinct, yet clonally related HCV virions [6–8].

Infectious particles, thought to be comprised of HCV RNA associated with the structural proteins core, E1, and E2 and surrounded by a lipid envelope, leave the cell via the secretory pathway. The nonstructural proteins, p7-NS5B, are not likely to be present in viral particles. The major site of HCV replication is the liver, but the high rate of extrahepatic manifestations during chronic HCV infection has led some to suggest that other tissues may also be infected. Other cell types reported to contain HCV RNA include B cells, dendritic cells, monocytes, and gut mucosal and sperm cells. It has been proposed that latent infection may persist in many of these cells after elimination of virus from the peripheral blood [9]. However, the vast majority of accumulating clinical data suggest that such an infectious reservoir, if it does exist, does not pose a significant risk for either reinfection or transmission of virus.

The Host Response to HCV Infection: The Acute IFN Response

Because the acute infection is usually asymptomatic, much of our knowledge of it is derived from studies in chimpanzees. As early as 2 days after infection, HCV RNA levels rise, followed by a plateau within 2 days, concomitant with the induction of intrahepatic type I IFN [10, 11]. This response is mediated by the pattern recognition receptors, toll-like receptor 3 (TLR3), which senses double-stranded RNA in endosomes, and retinoic acid-inducible gene I (RIG-I), which recognizes the polyuridine motif of the HCV 3′ UTR in the cytoplasm. TLR3 recruits the adapter molecule Toll-IL-1 receptor domain-containing adaptor inducing IFN-β (TRIF) and RIG-I recruits IFN-β promoter stimulator protein (IPS-1). Both adaptors effect downstream signaling, resulting in the activation and translocation of latent IFN regulatory factor-3 (IRF-3), which activates the IFN-β promoter. Secreted IFN-β acts in both an autocrine and paracrine fashion to induce an antiviral state in neighboring uninfected cells. IFN-β binds to the IFN-α/β receptor, activating the JAK/STAT pathway, resulting in the induction of IRF-3 (as part of a positive feedback loop) and other IFN-stimulated genes (ISGs), which have pleiotropic effects, such as the degradation of viral and cellular RNA (e.g., *OAS1*/RNase L), mutation of double-stranded RNA (*ADAR1*), and inhibition of translation of viral and host RNAs (e.g., *PKR*). As effective as intrinsic and exogenous IFN are, clinical evidence suggests that certain patients have preactivated IFN pathways that are refractory to further stimulation with IFN. In fact, these patients are less likely to upregulate ISGs and reach SVR after therapy with pegylated IFN [12].

In vitro protein overexpression studies have suggested that HCV is capable of attenuating the host IFN response through multiple mechanisms. The serine protease inhibitor HCV NS3/4A can cleave TRIF [11] and IPS-1 [13], blocking TLR3 and RIG-I signaling, fueling speculation that HCV protease inhibitors not only have a direct antiviral effect, but may also restore innate IFN responsiveness. HCV core may interfere with JAK/STAT signaling [14]. PKR may additionally be inhibited by HCV NS5A and E2 [15, 16].

IL-28B Promoter Polymorphisms and the Response to IFN

Our understanding of how host genetics affects resolution of HCV infection has advanced rapidly with genome-wide association studies of HCV patients. A single nucleotide polymorphism (SNP) 3 kb upstream of the IL-28B gene on chromosome *9*, which encodes the type III IFN, IFN-λ-3, has been associated with HCV clearance. The C/C, compared to the C/T and T/T, genotypes, is a predictor of spontaneous clearance [17] and confers a twofold improvement in the rate of SVR upon treatment with peg-IFN-α [18, 19]. Interestingly, T allele frequency is highest

among Africans, explaining in at least part of the clinical recognition that African-Americans have lower HCV clearance rates compared to other ethnic groups. Other SNPs in the region of the IL-28B gene have similarly been associated with likelihood of response [20, 21]. The mechanisms by which IL-28B SNP polymorphisms mediate antiviral response to exogenously administered IFN-α are currently unclear, but are an active area of ongoing research. IFN-λ-3 is one of the three members of the type 3 IFN family (the IL-29, IL-28A, and IL-28B genes encode IFN-λ-1, 2 and 3, respectively). IFN-λ-1 has been shown to have an additive antiviral effect with IFN-α [22], and has been shown to have independent anti-HCV activity in a phase 1 clinical trial [23] with phase 2 trials ongoing.

Cellular Mediators of Innate Immunity

The innate immune cells, natural killer (NK) cells, natural killer T (NKT) cells, and Kupffer cells (liver-resident macrophages) are known to populate the liver. NK cells in particular are abundant in the liver, and are early responders to viral infection. Genetic polymorphisms affecting the threshold of NK cell activation have been shown to influence the outcome of HCV infection [24]. Activated NK cells stimulate dendritic cell (DC) maturation, providing a direct link between innate and acquired immunity. DCs patrol for infection and sense pathogens by pattern recognition receptors, and once they come into contact with suitable antigens, they migrate to lymph nodes, where they present antigens to and activate $CD4^+$ and $CD8^+$ T cells and B cells. DCs can be classified as monocyte-resembling "myeloid DCs" or "plasmacytoid DCs," which can produce large amounts of IFN-α upon TLR7 (which recognizes single-strand RNA) or TLR9 engagement. TLR stimulation, among other things, leads to increased expression of MHC class II antigens, as well as cytokine and chemokine release. Given the crucial roles of DCs as a bridge between innate and adaptive immunity, there is considerable interest in the development of DC-based immunotherapies or vaccines. Plasmacytoid and myeloid DCs may be reduced in patients with HCV, but appear to be fully functional [25, 26]. Several pharmacologic approaches to enhance DC function in HCV patients are being pursued. Bacterial DNAs containing unmethylated CpG dinucleotides in specific sequence contexts (CpG motifs) are potent stimulators of TLR9, and synthetic oligodeoxynucleotides (ODNs) stabilized by a phosphorothioate backbone have been developed as therapeutic mimics of bacterial DNA (see below). Also, DC-based vaccines for HCV are currently in preclinical development. In a recent phase 1 dose-escalation study, genotype 1 CHC treatment failures were injected intradermally or i.v. with autologous monocyte-derived DCs pulsed with six HLA A2 (representative of 50% of Caucasians)-restricted, conserved, cytotoxic T-cell epitopes of HCV core, NS3, and NS4B. Although DC immunotherapy was safe, it disappointingly failed to decrease HCV RNA levels [27]. For future DC immunotherapy trials, optimization of epitopes, route, and timing of administration will be necessary.

The Adaptive Immune Response to HCV Infection

Although HCV RNA and IFN responses occur within days of infection, the adaptive response, for unclear reasons, is delayed. Potent, broad, and sustained activation of HCV-specific cytotoxic $CD8^+$ T cells is essential for HCV clearance (reviewed in [28]), but HCV-specific $CD8^+$ T cells are typically not detected until 5–9 weeks after infection [29, 30]. HCV-specific antibodies may not be as essential for clearance as $CD8^+$ T cells are, but they too are delayed, as they cannot be detected until 4–20 weeks after infection [31, 32]. Helper $CD4^+$ T cells are necessary for the generation and maintenance of protective $CD8^+$ T cells and memory B cells. They are detectable at the time of clinical presentation in spontaneous resolvers, but not in those who develop chronic infection [33]. The low rate of spontaneous HCV clearance among individuals co-infected with HIV may be due to weak memory $CD4^+$ cell responses in co-infected individuals [34]. HCV-specific

neutralizing antibodies peak at the time of viral clearance in spontaneous resolvers, whereas they are absent or weak in patients who go on to chronic infection [35].

The reasons for inadequate immune system clearance of HCV are incompletely understood. Significant contributing factors are HCV's ability to dampen innate immune responses, as described above, as well as its ability to rapidly diversify and escape immune pressure. Analysis of HCV mutations in chimpanzees shows that the highest level of selective pressure occurs during the acute phase of infection and decreases during the course of infection [36]. Persistent infection is associated with the emergence of cytotoxic $CD8^+$ T-cell escape mutations [37, 38]. These escape mutations may affect binding of HCV peptides to MHC molecules, binding of antigens to the T antigen receptor, or they may affect efficiency of antigen processing. Moreover, during the course of acute and chronic infection, anti-HCV neutralizing antibodies increase in titer and exert pressure on viral variants, driving mutations that allow for escape from neutralizing antibodies [39]. Anti-HCV neutralizing antibodies are likely to be isolate-specific, and they do not prevent reinfection [40], but provide partial protection, in persons who have previously cleared infection [40, 41].

Chronic HCV infection is also associated with functional T-cell "exhaustion," as exhibited by impaired function and reduced breadth of HCV-specific $CD8^+$ cytotoxic T cells [42]. Dysfunctional HCV-specific $CD8^+$ T cells express the inhibitory receptor, programmed death 1 (PD-1) [43], which upon interaction with its ligand, PD-L1 (expressed on hematopoietic and parenchymal cells) undergo apoptosis [44]. Blocking of PD-1/PD-L1 interactions has considerable therapeutic appeal. Anti-PD-L1 antibodies have been shown to reverse dysfunction of HCV-specific $CD8^+$ T cells [45]. Moreover, dual PD-1 and cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) blockade may have synergistic effects on reversing CD8 T-cell exhaustion [46]. Despite the attractiveness of this therapeutic response, since hepatic inflammation in HCV is due to the $CD8^+$ T-cell response, not the virus itself, such a strategy may have adverse hepatic inflammatory consequences. In fact, mice with a genetic deficiency in PD-1 expression develop lethal immunopathogenic damage upon LCMV infection [47].

There certainly exist additional mechanisms for the failure of effective anti-HCV $CD8^+$ T-cell responses. For example, immunosuppressive $CD4^+Foxp3^+$ regulatory T cells (Tregs) may be increased in patients with chronic HCV, and indeed, they have been found to localize to the liver, where they may limit the extent of $CD8^+$ T-cell-mediated hepatocellular injury [48]. Reversing Treg-mediated $CD8^+$ T-cell suppression with TLR agonists may be a promising therapeutic avenue [49], although such an approach may pose a risk of hepatitis flares.

Notable Previously Studied Immunomodulatory Agents

TLR agonists represent the main class of immunomodulatory agents that have been studied as anti-HCV agents in humans. Unfortunately, as a class, these agents have previously demonstrated an unfavorable side-effect profile, owing to their properties as potent, nonspecific, activators of DCs and B cells.

CpG 10101 (Actilon, Coley Pharmaceuticals) is a synthetic "C-class" CpG ODN that stimulates TLR9 in both B cells and plasmacytoid DCs. In a phase 1a trial, CpG 10101 appeared safe and well tolerated in healthy volunteers. A phase 1b dose-escalation trial of CpG 10101 administered subcutaneously (s.c.) weekly (q.w.) or twice-weekly (b.i.w.) for 4 weeks in patients with chronic genotype (GT) 1 HCV infection showed initial promise. At week 4, 22 of 40 patients in the 1 mg b.i.w. group had a greater than 1 $\log_{10}$ reduction in HCV RNA. Moreover, dose-dependent plasma cytokine elevations, including IFN-α and IFN-response proteins such as 2′-, 5′-oligoadenylate synthetase (OAS), were observed. However, dose-dependent mild-to-moderate flu-like symptoms and injection site reactions were frequent, as was the incidence of transient neutropenia and GI disorders [50]. Moreover, a clinical study in prior relapsers and nonresponders to peginterferon and ribavirin

yielded disappointing results. Further development of CpG 10101 as an anti-HCV therapeutic was subsequently suspended.

ANA245 (Isatoribine, Anadys Pharmaceuticals) is a small-molecule guanosine analogue (7-thia-8-oxoguanosine) capable of activating TLR7 [51], which, similarly to TLR9, is expressed on B cells and DCs. Its safety and efficacy were examined in a phase 1 proof of concept trial. Patients with chronic HCV were administered ANA245 800 mg i.v. daily (q.d.) for 7 days. The average HCV RNA reduction was −0.76 $\log_{10}$. Although no serious adverse events (SAEs) occurred, frequent mild-to-moderate AEs of insomnia, joint pain, headache, and asthenia were noted. An overall reduction in white blood cells (WBC), neutrophils, and platelets also occurred [52]. Further clinical development of ANA245 was discontinued.

Current Treatment

Currently approved, anti-HCV immunomodulators consist entirely of IFN-α-based modalities. We refer the reader to Chap. 10, which describes the current clinical use of IFN-α, as well as emerging IFN-based immunotherapies.

Immunomodulators and Other Agents in Development

Nitazoxanide (Alinia, Romark, Phase 2)

Nitazoxanide (NTZ) is a thiazolide that has been FDA approved in the United States for the treatment of diarrhea caused by the intestinal parasites *Cryptosporidium parvum* and *Giardia lamblia*. Its antiviral activity was discerned when HCV- and HBV-infected patients with HIV were treated for cryptosporidiosis and were noted to have decreased ALT levels. Subsequent in vitro studies showed NTZ to inhibit HCV and HBV replication [53]. The mechanism of action of NTZ is unclear, but it has been hypothesized that NTZ or its metabolite, tizoxanide, targets host cell signaling and enhances the intracellular activity of IFN-α. In support of this, NTZ has been shown to induce PKR phosphorylation, leading to increased phosphorylated eIF2α, a mediator of host cell defenses against viral infection [54]. Studies in HCV replicon systems show that NTZ resistance can be engendered by serial passages in increasing concentrations of NTZ. Interestingly, resistance to RBV or to the nucleoside analogue, 2′ CMA, was not noted, but there appeared to be increased susceptibility to IFN-α-2b. This effect appeared to be due to host changes, rather than virus mutations, as the replicons did not exhibit this increased sensitivity when transferred to treatment-naïve cells [55]. This is in contrast to subtherapeutic treatment with direct-acting antiviral agents, which leads to rapid emergence of resistant viral mutants with specific genomic mutations. Perhaps overoptimistically, it has been argued that the targeting of a host, rather than viral, target imposes a higher genetic barrier for resistance compared to direct antivirals [54]. However, this argument ignores the fact that viruses can quite easily mutate to evade host-mediated pressure. This has been illustrated in the field of HIV therapeutics, where targeting of the cellular HIV co-receptor, CCR5, can lead to outgrowth of HIV that can still use CCR5 for entry [56] or use CXCR4 as a co-receptor instead [57].

In an early randomized, double-blind, placebo (PBO)-controlled phase 2 trial of 47 Egyptian GT 4 CHC patients, 5 of whom had previously received peg-IFN-α/RBV therapy, 7/23 patients receiving NTZ 500 mg b.i.d. monotherapy for 24 weeks achieved undetectable HCV RNA, compared to 0/24 in the placebo (PBO) arm. Of note, four of these patients who had a baseline HCV RNA <400,000 IU/mL reportedly developed SVR [58]. NTZ has also been studied early on as lead-in therapy before combined therapy with peg-IFN-α. This strategy has been based on replicon model data supporting this pretreatment effect, as well as upon an initial pilot experience showing greater efficacy if NTZ was administered prior to, rather than simultaneously with, peg-IFN-α [53].

In the STEALTH (Studies to Evaluate Alinia (NTZ) for Treatment of Hepatitis C) C-1 trial, 120 Egyptian treatment-naïve patients with GT 4

CHC were randomized to one of three arms: (1) NTZ 500 mg b.i.d. × 12 weeks followed by NTZ 500 mg b.i.d. in combination with peg-IFN-α-2a 180 μg q.w. × 36 weeks; (2) NTZ 500 mg b.i.d. × 12 weeks followed by NTZ 500 mg b.i.d./peg-IFN-α-2a 180 μg q.w. with weight-based RBV × 36 weeks (triple therapy); or (3) peg-IFN 180 μg q.w. with weight-based RBV × 48 weeks (SOC). SVR occurred in 79, 61, and 50%, respectively, ($p = 0.023$ for arm 1 vs. arm 3). AE profiles were similar in the three arms, except for higher rates of anemia in the patients given peg-IFN-α/RBV [59]. A subsequent open-label study of 44 patients with mostly GT 4 CHC showed that a 4 week lead in period with NTZ 500 mg b.i.d. followed by NTZ 500 mg b.i.d. plus peg-IFN 180 μg q.w. for an additional 36 weeks yielded an SVR of 80%, similar to the SVR rates with a 12 week lead-in therapy followed by triple therapy [60]. This suggested that a 4 week lead-in period is adequate, although further research is warranted to determine if RBV can be eliminated from NTZ-containing regimens.

In the STEALTH C-2 trial conducted in the United States, 64 treatment-experienced (60% null, 20% partial) GT 1 CHC patients were randomly assigned to NTZ 500 mg b.i.d. for 4 week lead-in, followed by NTZ or PBO with peg-IFN-α-2a 180 μg q.w. and weight-based RBV × 48 weeks. HCV RNA did not significantly change during the lead-in period. Patients receiving NTZ had higher response rates than those taking PBO, but overall rates were low and did not reach statistical significance; NTZ vs. PBO RVR: 5% vs. 0%; complete EVR: 7% vs. 0%; partial EVR: 38% vs. 29%; SVR: 7% vs. 0%. The three patients with SVR were white, had high HCV RNA (>800,000 IU/mL), two of three were partial responders to prior treatment, and one had advanced fibrosis. The only NTZ-related AE was mild–moderate diarrhea [61].

In the STEALTH C-3 trial conducted in the United States, 112 treatment-naïve GT 1 CHC patients were randomized to receive either NTZ 500 mg b.i.d. ($n = 75$) or PBO ($n = 37$) for 4 week lead-in followed by continued NTZ or PBO with peg-IFN-α 180 μg q.w. and weight-based RBV for 48 weeks. One-third of patients had stage F3 or F4 liver disease. NTZ vs. PBO RVR: 12% vs. 19%; EVR: 62% vs. 49%; ETR: 63% vs. 46%; SVR: 44% vs. 32%. NTZ performed best in patients with high baseline HCV RNA: SVR rates in patients with HCV RNA >800,000 IU/mL for NTZ ($n = 62$) vs. PBO ($n = 31$) were 42 and 29%, respectively. There were no significant differences in SAEs between the two treatment groups, and notable AEs included mild to moderate diarrhea and urine discoloration [62].

Many questions regarding NTZ remain, including its mechanism of action, the resistance mutations it may theoretically engender, and whether it can be administered with DAA agents, with or without peg-IFN-α and/or RBV.

Vitamin D3

There has been widespread interest in the effects of vitamin D supplementation on the immune system. Vitamin D has been identified as a regulator of innate immunity in humans (reviewed in [63]), and low levels have been associated with active *Mycobacterium tuberculosis* [64], but its mechanism of action as an immune stimulator remains unknown. Low levels of the liver-hydroxylated form of vitamin D3, 25(OH)D, have been reported in patients with varied etiologies of chronic liver disease [65, 66], and a retrospective study has found that vitamin D receptor polymorphisms are associated with SVR [67].

Data regarding the therapeutic use of vitamin D in CHC derive primarily from retrospective studies that will need to be confirmed prospectively in randomized, double-blind trials among larger, more ethnically diverse cohorts. In Sicily, plasma 25(OH)D levels were significantly lower in 197 GT 1 CHC patients compared to 49 age- and sex-matched controls (25.1 vs. 43.1 μg/L, $p < 0.001$). Multivariate analysis showed an SVR odds ratio of 1.039 (1.002–1.077, $p = 0.03$) for each microgram per liter of 25(OH)D. Moreover, there was a correlation between liver CYP27A1 expression and serum 25(OH)D levels, and an inverse correlation of CYP27A1 and necroinflammatory activity. The authors speculated that HCV-induced necroinflammatory activity leads

to reduced expression of enzymes involved in liver hydroxylation of D3 [68].

Another retrospective study examined 42 patients who underwent liver transplant for HCV-related liver disease, and who were subsequently treated for recurrent HCV. Patients were treated with either standard IFN-α-2b, leukocyte IFN-α, or peg-IFN-α-2b with RBV 600 or 800 mg/day. In 15 patients, cholecalciferol 800 IU/day was given to avoid further bone loss in the presence of known pretransplant osteopenia or osteoporosis. Overall, 13 of 42 patients (31.0%) had SVR, and a low baseline 25-OH vitamin D level was associated with an unfavorable response to peg-IFN-α/RBV. SVR rates in severely deficient (≤10 ng/mL), deficient (>10 and ≤20 ng/mL), and near normal (>20 ng/mL) were: 1/10, 6/20, and 6/12, respectively. SVR rates in cholecalciferol-supplemented and unsupplemented groups were 8/15 and 5/27, respectively [69].

The effect of vitamin D supplementation on SVR was prospectively studied in one randomized, nonblinded study of GT 1 CHC patients conducted in Israel. Subjects received either vitamin D3 (1,000–4,000 IU q.d.) plus pIFN-a2b 1.5 μg/kg q.w. and RBV (1,000/1,200 mg q.d.) ($n=27$) or pIFN-a2b 1.5 μg/kg q.w. and RBV (1,000/1,200 mg q.d.) ($n=31$). The treatment group had a higher BMI, baseline HCV RNA, and liver fibrosis compared to controls. At 12 weeks, 26/27 (96%) of subjects who received vitamin D had HCV RNA <50 IU/mL, whereas 15/31 (48%) ($p<0.0001$) controls did. At 24 weeks post-treatment, 13/15 (86%) of vitamin D-treated vs. 5/12 (41%) ($p<0.001$) of controls reached SVR [70]. However, these extraordinary findings must be tempered by the extreme limitations of this nonblinded study. Moreover, it is unclear that the groups were matched according to prior treatment history. Moreover, the distribution of vitamin D doses among subjects is unclear, as is the rate of SVR as a function of vitamin D dose and underlying vitamin D deficiency. Future large randomized controlled trials in well-defined study populations, with adequately defined baseline and post-treatment vitamin D levels are warranted.

Cyclophilin Inhibitors

Cyclosporine A (CsA) is an immunosuppressive medication that binds cyclophilin (Cyp) of T cells and blocks calcineurin, a phosphatase that induces transcription of IL-2. CsA also suppresses HCV replication [71, 72]. Cyclophilins, part of a family of cellular peptidyl-prolyl isomerases, are known to be essential cofactors for HCV replication [73], but the mechanism of the antiviral effect of CsA is controversial. Cyp B has been shown to bind NS5B, and it increases subgenomic HCV replication via binding of HCV polymerase to RNA [74]. It has also been shown that CypA is essential for virus replication, that CypA binds NS5A [75], and that treatment of HCV replicons with CsA analogues elicits mutations in NS5A [76]. Others have shown that enhanced sensitivity of HCV replication to CsA is mediated through cyclophilin A, but analysis of full-length genome replication suggests that this is dependent upon the presence of HCV NS2 [76, 77]. It has been hypothesized that cyclophilin A may be required for peptidyl-prolyl isomerization of NS2–3 protease, as the NS2 crystal structure reveals an unusual *cis*-proline in position 164 of NS2 [78]; interestingly, this proline is conserved in all HCV isolates.

CsA derivatives that lack anticalcineurin activity, and thus, do not have significant immunosuppressive activity, are in human clinical trials as HCV inhibitors. Debio-025 (alisporivir, Debiopharm/Novartis), is the first-in-class Cyp inhibitor (reviewed in [79]). It has been studied in a randomized, double-blind, PBO-controlled phase 2 dose-escalation study of treatment-naïve CHC patients. Doses of 200, 600 and 1,000 mg q.d. in combination with peg-IFN-α-2a 180 μg q.w. × 4 weeks (without RBV, to simplify study design) were compared to monotherapy with either 1,000 mg q.d. Debio-025 or peg-IFN-α-2a 180 μg q.w. × 4 weeks. Because of the drug's long half-life, during the first week of treatment, a loading regimen of twice the allocated dose daily was administered, in order to bring patients rapidly to pharmacokinetic steady state. The peg-IFN-α-2a monotherapy, the Debio-025 600

and 1,000 mg combination treatments, and the Debio-025 1,000 mg combination treatment resulted in −2.49 (±1.95), −4.61 (±1.88), −4.75 (±2.19), and −2.220 (±2.40), respectively, mean $\log_{10}$ IU/mL decline at week 4 in treatment-naïve GT 1, 4 patients, and −5.69 (±1.58), −5.91 (±1.11), −5.89 (±0.43), and −4.22 (±1.33), respectively, in GT 2 and 3 patients. Individual viral load data did not indicate viral breakthrough, which suggests that Debio-025 may have a high resistance barrier. Of concern, 13 of 36 patients receiving 1,000 mg q.d. had isolated increases in conjugated and direct bilirubin. Hyperbilirubinemia was most pronounced during the first week, and was completely normalized in all subjects 3 weeks after the end of treatment. [80] Preclinical in vitro studies have shown that Debio-025 inhibits the biliary canalicular transporter multidrug resistance-associated protein 2 (MRP2), thus clinical hyperbilirubinemia may be due to isolated transporter inhibition in susceptible patients. Debio-025 is currently being evaluated in a phase 2b, 72-week trial comparing SOC (peg-IFN-α-2a 180 μg q.w. with weight-based RBV) vs. SOC in addition to Debio-025 600 mg q.d.

SCY-635 (Synexis) is a CsA derivative without calcinuerin-binding activity in Phase 1 development. It may be a weaker inhibitor than Debio-025 of the MRP2-conjugated bilirubin transporter [81].

HMG CoA Reductase Inhibitors

Statins, 3-hydroxyl-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitors, have pleiotropic effects and are widely used for the treatment for hypercholesterolemia. In vitro, statins have been shown to inhibit HCV replication. Anti-HCV activity is variable among different statins, with pravastatin and rosuvastatin having little anti-HCV activity. Their antiviral mechanism of action is unclear. Paradoxically, statins increase the hepatocyte LDL receptor, which is a known co-receptor for HCV. However, statins have other anti-inflammatory and immunomodulatory effects, such as activation of peroxisome proliferator-activated receptor α (PPAR-α) (reviewed in [82]), which among other things, downregulates NF-κB. Statins may also work in vitro by inhibiting geranylgeranylation of cellular proteins [83], which may make fewer lipid rafts available for HCV replication.

An analysis of the IDEAL (Individualized Dosing Efficacy Versus Flat Dosing to Assess Optimal Pegylated Interferon Therapy), in which 3,070 treatment-naïve, GT 1 CHC patients were treated, showed that SVR rates were higher in patients with pretreatment LDL ≥130 mg/dL or HDL ≤40 mg/dL, compared to patients with normal LDL and HDL (44.9% vs. 34.0%, respectively, $p<0.001$). Multivariate logistic regression showed that baseline high LDL level (OR 1.6, 95% CI: 1.4–1.8, $p<0.001$), low HDL (OR 0.5, 95% CI 0.3–0.8, $p=0.004$), and statin use (OR 2.0, 95% CI: 1.1–3.7, $p=0.02$) were independently associated with SVR [84].

Prospective clinical trials of statins have had mixed results so far, possibly because conventional statin doses yield a plasma concentration of 0.8–3 nM, whereas the in vitro inhibitory effect of statins is much higher, in the range of 1–10 μM. One study reported that fluvastatin 80 mg q.d. increased HCV RNA levels after 4 weeks of treatment in HCV and HIV co-infected persons [85], and another showed that high-dose fluvastatin yielded a modest decrease in HCV RNA in 50% of patients [86]. In another study, atorvastatin 20 mg q.d. (a low dose) prospectively given as monotherapy to ten CHC patients (eight of whom had GT 1 infection) did not significantly affect week 4 and 12 HCV RNA [87].

Anti-HCV miRNA

HCV recruits the liver-specific microRNA, miR-122, to increase replication. Evidence suggests that miR-122 binds to two complementary "seed" sequences upstream of the HCV IRES in the 5′ UTR and increases replication in part by stimulating IRES-mediated translation [88, 89]. Pretreatment miR-122 levels are decreased in CHC patients responding poorly to IFN [90]; miR-122 may be bound to HCV RNA in these patients,

and hence, of apparently lower concentration. Another known function of miR-122 is to affect expression of hepatocyte proteins whose genes also contain miR-122 seed sites; these proteins may act to enhance HCV replication. A concern of inhibiting miR-122 is the potential for toxicity by alterations in normal hepatocyte physiology. SPC3649 *(Santaris Pharma)* is a 15-base locked nucleic acid (LNA) antisense molecule that is complementary to miR-122 and is thought to sequester it. The phosphorothioate backbone of LNAs confers nuclease resistance as well as "locking" the oligonucleotide into a configuration that promotes target binding. IV administration of SPC3649 has been performed in chimpanzees with chronic HCV infection. 2/2chimpanzees receiving SPC3649 5 mg/kg i.v. q.w. for 12 weeks had a drop of 2.6 $\log_{10}$ at the end of 2 weeks of follow-up. However, the chimpanzees had significant AST and CPK spikes during treatment [91]. This echoes the toxicity of an earlier antisense therapy, ISIS-14803, a 20-unit antisense oligonucleotide with a phosphorothioate backbone, which binds to the HCV IRES. ISIS-14803 administered as i.v. or s.c. t.i.w.×4 weeks caused significant ALT flares in CHC patients, only 3/28 of whom had reductions in HCV RNA [92]. Further preclinical toxicity analyses will be necessary before this treatment, which is likely to be expensive to manufacture and clinically approve, is tested in humans.

TLR Agonists

The TLR9 agonist, IMO-2125 (*Idera Pharmaceuticals*), has been shown to induce IFN-α and ISGs in vitro. It has been tested in a randomized, PBO-controlled, double-blind phase 1 dose-escalation study in CHC (40 of 41 with GT 1 infection) nonresponders. IMO-2125 monotherapy ($n=8$ per dose cohort) or PBO ($n=2$ per dose cohort) was delivered s.c. q.w.×4 weeks. There were no SAEs, but 53% had transient (<1 day) of flu-like illness. Six of eight subjects receiving 0.32 mg/kg q.w.×4 weeks had >1 $\log_{10}$ decrease in HCV RNA, and this decrease correlated with IFN-α induction [93]. IMO-2125 is currently being studied in conjunction with RBV in a phase 1 dose-escalation study of treatment-naïve CHC GT 1 patients.

ANA773 (Anadys)

ANA773 is an oral prodrug of an active TLR7 agonist. It has been evaluated in a phase 1 double-blind, PBO-controlled, dose-escalation (800, 1,200, 2,000 mg q.o.d.×4 weeks) study of GT 1–4 CHC patients. Two of six patients in the 1,600 mg group and five of eight in 2,000 mg group had >1 $\log_{10}$ decline. The 2,000 mg group had an average 0.6 $\log_{10}$ HCV RNA decline, whereas the PBO group had a 0.1 $\log_{10}$ HCV RNA drop. There were no SAEs reported, and dose-dependent mild/moderate AEs, most notably, flu-like symptoms, occurred [94]. Anadys is pursuing partnerships to further this drug's development.

SD-101 (Dynavax)

SD-101 is a TLR9 agonist. It has been evaluated in a phase 1b single dose-escalation study of 34 treatment-naïve GT 1 CHC patients. SD-101 ($n=28$) or PBO ($n=6$) administered as single doses s.c. were well tolerated. Forty-eight hours after dosing, the average HCV RNA decrease was −1.33, −1.38, and −1.52 $\log_{10}$ for the 1, 3, and 5 mg dose groups, respectively [95].

Immunomodulatory Peptides (SCV-07, SciClone)

SCV-07, γ-d-glutamyl-l-tryptophan, is an immunomodulatory dipeptide that is thought to inhibit STAT3-driven gene expression and increase T-cell differentiation and function. In a single-blind dose-escalation study (seven daily injections, 0.01, 0.1, 1 mg/kg) among GT 1 CHC

relapsers, 2 of 9 patients in the 0.1 mg/kg group had >0.5 $\log_{10}$ HCV RNA drop at 2 week follow-up, and 1 of 11 patients in the 1 mg/kg group had >0.5 $\log_{10}$ drop at 30 day follow-up [96]. SCV-07 is currently being studied in phase 2 as 4-week lead-in followed by 4 weeks of combination therapy with RBV in 20 GT 1 CHC relapsers.

PD-L1 Blockade

As mentioned above, PD-1 is upregulated on peripheral and intrahepatic HCV-specific T cells in CHC patients [43] and on peripheral $CD8^+$ T cells in acute HCV-infected patients who fail to spontaneously clear virus [97]. Although PD-1/PD-L1 blockade enhances proliferative capacity [43], intrahepatic PD-1^+ $CD8^+$ T cells may be refractory to PD-1/PD-L blockade [98]. Moreover, it has recently been shown that PD-1 inhibits Treg proliferation via prevention of IL-2-mediated phosphorylation of STAT5 [99]. Despite these concerns, therapeutic PD-L1 blockade is an attractive anti-HCV immunotherapeutic approach. The anti-PD-1 mAb, CT-011 *(CureTech Ltd, Teva Pharmaceuticals)*, is a humanized monoclonal antibody that binds to PD-1, blocking its function. It is currently being tested in a nonrandomized, open-label phase 1 trial in GT 1 CHC patients.

IL-7 (Cytheris)

IL-7 is a cytokine produced primarily by the stromal cells of the thymus and is essential for lymphopoiesis. Animal models suggest that IL-7 administration improves thymic output and drives antigen-independent homeostatic proliferation in the periphery (reviewed in [100]). It is currently being evaluated in "ECLIPSE" trials: multicenter, phase 1/2a dose-escalation studies: recombinant human IL-7 administered q.w. in conjunction with peg-IFN-α/RBV × 4 weeks in GT 1 CHC nonresponders.

Anti-Phosphatidylserine mAb (Bavituximab, Peregrine Pharmaceuticals)

Bavituximab is a mAb targeting phosphatidylserine, which is located at the surface of virus-infected cells and enveloped viruses. Two phase 1 studies have been performed, the first showing that single i.v. doses of 6 mg/kg were tolerated in CHC nonresponders [101]. The other study was a dose-escalation trial in 24 HCV patients (15 of whom had GT 1 infection, 11 of whom were nonresponders) showing that 2 weeks of twice-weekly 90-min i.v. infusions were well-tolerated. Five of six subjects receiving a dose of 3 mg/kg achieved >0.5 $\log_{10}$ HCV RNA decline at 2 weeks [102]. Bavituximab is currently being studied in a phase 1 study of HCV and HIV co-infected subjects.

Antifibrotics

Targeting of mediators of hepatic fibrogenesis, such as hepatic stellate cells and TGF-β with agents such as IFN-γ and caspase inhibitors, have not been successful to date. Clinical trials of fibrosis inhibitors have been difficult to perform, in part due to suboptimal noninvasive biomarkers of fibrosis. Matrix metalloproteinases (MMPs) are proteolytic enzymes that have been implicated in maintenance of the integrity of the extracellular matrix. MMPs have been implicated in the pathogenesis of fibrosis, and hepatic MMP 9 is correlated with fibrosis stage in CHC patients [103]. CTS-1027 *(Conatus Pharmaceuticals)* is an oral small drug MMP inhibitor that has been studied as monotherapy in a phase 1 dose-escalation trial of CHC, primarily GT 1, prior treatment failures. None of the 87 patients studied had >1 $\log_{10}$ decrease in HCV RNA after 12 weeks of therapy, nor were there clear decreases in markers of inflammation. CTS-1027 is now being tested as combination therapy with peg-IFN-α-2a/RBV × 48 weeks in a phase 1, open-label

trial of 60 CHC null responders to prior peg-IFN-α-2a/RBV therapy. The pancaspase inhibitor, PF-03491390 *(formerly IDN-6556, Pfizer Pharmaceuticals)* has been studied in CHC patients in a dose-ranging phase 1 trial. Although HCV RNA was not affected, ALT levels were significantly lowered after 14 days of monotherapy [104]. Longer studies are being conducted to evaluate the effects of the drug on liver inflammation and fibrosis.

Therapeutic Vaccines

For reasons described earlier in this chapter, there are numerous scientific obstacles to the development of an HCV vaccine. Nevertheless, there have been clinical trials of several therapeutic vaccines that aim to ameliorate HCV infection. GI-5005 *(GlobeImmune)* is a heat-killed *Saccharomyces cerevisiae* that has been genetically modified to express HCV core and NS3. It has been hypothesized that GI-5005 can elicit HCV-specific T-cell responses and improve the rate of immune-mediated clearance of HCV-infected hepatocytes. Phase 1 trials suggested antiviral activity, with the intriguing observation of enhanced T-cell responses to a broader spectrum of epitopes than expressed in the product itself [105]. GI-5005 has been evaluated in a randomized phase 2 trial of treatment-naïve and nonresponder GT 1 CHC patients. Lead-in therapy with GI-5005 q.w. for 5 weeks, then q. mo. for 2 months, followed by GI-5005 q. mo. with peg-IFN-α-2a/RBV × 48 weeks (naïve patients) or ×72 weeks (nonresponders) has been compared to peg-IFN/RBV (×48 weeks for naïve patients, ×72 weeks for non-responders). Triple therapy significantly improved ETR (63% vs. 45%, $p = 0.037$) and ALT normalization (61% vs. 36%, $p = 0.018$) compared to peg-IFN-α/RBV alone. There was no difference in SVR in treatment-naïve patients, relapsers, or on-treatment breakthroughs. Interestingly, SVR in treatment-naïve, IL-28 genotype TT patients was greater for triple therapy (60%) compared to peg-IFN-α-2a/RBV (0%), but there were only five such patients in each group. [106] This intriguing observation warrants further study.

IC41 (Intercell)

IC41 is an HCV peptide vaccine containing CD4 and CD8 epitopes that uses poly-l-arginine as a $CD4^+$ T-cell adjuvant. It has been shown to induce functional anti-HCV $CD4^+$ and $CD8^+$ T cells in HCV nonresponder patients [107]. It has also been examined in combination with the topical TLR7/8 agonist, imiquimod, in a phase 2 study of 50 treatment-naïve GT 1 CHC patients. IC41 administered as biweekly intradermal injections × 8 weeks with imiquimod was compared to IC41 administered as weekly s.c. injections × 16 weeks. Administration of IC41 alone had no effect on HCV RNA. The combination group had an average HCV RNA decline of 0.21 $\log_{10}$ ($p = 0.0013$) at week 16. At week 38 (24 weeks after last vaccination), HCV RNA decreased by 0.47 $\log_{10}$ ($p < 0.0001$) [108]. Although HCV-specific T-cell responses were detected, their presence did not correlate with HCV RNA decline.

TG4040 (Transgene)

TG4040 is a modified vaccinia ankara-based vaccine encoding NS3, NS4, and NS5B. It has been evaluated in a phase 1 study of 15 treatment-naïve CHC patients, 6 of whom received three weekly doses, 9 of whom received a fourth injection at month 6. Seven of fifteen patients had an HCV RNA decrease ranging from 0.5 to 1.4 $\log_{10}$, and the two patients with the largest HCV RNA drop had the most robust responses to NS3 and NS4. The HCV RNA changes for the other eight patients were not reported [109]. Encouragingly, 5 of 15 patients had increased NS3- and NS5B-specific cellular immune responses [110]. TG4040 is currently being studied in phase 2, administered in conjunction with peg-IFN-α-2a/RBV in treatment-naïve GT 1 CHC patients.

ChronVac-C (ChronTech Pharma AB)

This is a codon-optimized NS3/4A DNA vaccine delivered intramuscularly by electroporation. It is in phase 1/2 trials.

Prophylactic Vaccines

As HCV rarely gives rise to protective immunity during the course of natural infection, the development of an effective vaccine capable of preventing HCV infection in uninfected individuals remains a daunting task. In short, much more research will be necessary to determine optimum immunogens, route of administration, and dosing schedule before a prophylactic vaccine becomes a reality. A vaccine containing HCV E1E2 GT 1a peptides *(Novartis)* has been adjuvanted in an oil-in-water emulsion, and a phase 1 dose-escalation trial at weeks 0, 4, 24, and 48 has been conducted among HCV-negative healthy volunteers. The vaccine appeared to be safe and well-tolerated. Anti-E1E2 antibodies were detected in vaccine recipients, but no dose–response effect was seen [111, 112].

Passive Immunotherapy

Although an individual's antibodies usually fail to prevent reinfection with HCV, there is evidence that HCV hyperimmune globulin may offer some degree of protection. In an epidemic of acute HCV occurring in the 1990s from a lot of intravenous γ globulin, HCV was transmitted to patients by plasma from which units testing positive for anti-HCV antibodies were not used. In contrast, recipients were not infected by plasma that contained units derived from HCV-infected patients who had not yet seroconverted. Subsequent analyses suggested that the exclusion of plasma testing positive for HCV antibodies (by EIA-2) may have removed neutralizing antibodies from the preparation [113].

Moreover, broadly reactive neutralizing antibodies present in IVIG preparations from HCV-positive donors can prevent HCV infection in chimpanzees. [114] These observations have led to speculation that HCV hyperimmune globulin, while not suitable for postexposure prophylaxis in the general population, may be efficacious in specialized settings, such as graft reinfection prophylaxis in liver transplant patients.

Summary and Conclusions

The field of HCV therapeutics is rapidly advancing, and it is expected that efficacious and safe directly acting anti-HCV agents will revolutionize the HCV treatment landscape. The initial use of such agents will involve their combination with peginterferon and ribavirin [115, 116]. A question of profound importance awaiting further evaluation is whether IFN-free combinations of directly acting antiviral drugs will have the capacity to eradicate HCV without the immunomodulatory effects of IFN. Preliminary data suggest that such combinations can effectively suppress HCV replication and longer-term studies to provide proof of concept that SVR is attainable with this approach have been initiated at the time of writing. Should this prove to be more difficult or attainable in only a proportion of patients, we may ultimately conclude, as some already believe, that an immunomodulatory component of therapy will always be needed, at least for some patients. In that event, the desire to improve upon the tolerability profile of IFN will fuel ongoing investigation of alternative immunomodulatory approaches such as those discussed in this chapter. In addition, the cherished goal of an effective HCV vaccine, though elusive thus far as it has been for HIV, will depend upon an enhanced understanding and application of HCV immunology.

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Measuring HCV RNA and Assessing Virologic Response

12

Jean-Michel Pawlotsky

Keywords
Real-time PCR • HCV RNA • Viral kinetics • Antiviral therapy

Introduction

Soon after the discovery of hepatitis C virus (HCV) in the early 1990s, in-house methods were developed by research laboratories to quantify HCV RNA levels. These techniques were lacking sensitivity, specificity, accuracy and reproducibility, emphasizing the need for standardized assays that could be widely used in the clinic. The first available standardized assay for HCV RNA quantification was the first-generation "branched DNA" (bDNA) assay, soon followed by standardized polymerase chain reaction (PCR)-based tests. These assays were used to improve our knowledge of HCV infection and related liver diseases. It soon became apparent that the HCV RNA level at baseline was an independent predictor of the outcome of therapies based on the use of interferon (IFN)-α. In addition, mathematical modeling of viral decay during antiviral therapy has substantially improved our understanding of the biology of HCV infection. Furthermore, monitoring of HCV RNA level kinetics during IFN-α-based therapy was shown to predict the likelihood of a favorable outcome and was subsequently used to tailor treatment duration in order to optimize the results of treatment. New assays based on "real-time" PCR were subsequently developed, with dynamic ranges of quantification better suited to clinical management. Recent data from Phase III trials with direct acting antiviral (DAA) drugs used in combination with pegylated IFN-α and ribavirin suggested that monitoring of viral kinetics will also be useful to tailor treatment duration when these combinations are available. The future utility of HCV RNA level monitoring in IFN-free treatment regimens remains to be established.

J.-M. Pawlotsky, MD, PhD (✉)
Department of Virology, National Reference Center for Viral Hepatitis B, C and Delta, Hôpital Henri Mondor, Université Paris-Est, 51 Avenue du Marechal de Lattre de Tassigny, Créteil 94010, France

INSERM U955, Créteil, France
e-mail: jean-michel.pawlotsky@hmn.aphp.fr

Measuring HCV RNA Levels: What Have We Learnt Over the Past Two Decades?

Assays for HCV RNA Level Measurement

Viral genomes are generally present in relatively small amounts in body fluids of infected patients, hindering their detection by simple molecular

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Table 12.1 Commercial HCV RNA quantification assays

Assay	Manufacturer	Method	Lower limit of detection	Dynamic range of quantification
Versant™ HCV RNA 3.0 assay	Siemens Medical Solutions Diagnostics, Tarrytown, New York	Semi-automated branched DNA signal amplification	615 IU/mL (2.8 Log_{10} IU/mL)	615–7,700,000 IU/mL (2.8–6.9 Log_{10} IU/mL)
Amplicor™ HCV Monitor v2.0	Roche Molecular Systems, Pleasanton, California	Manual RT-PCR	600 IU/mL (2.8 Log_{10} IU/mL)	600–500,000 IU/mL (2.8–5.7 Log_{10} IU/mL)
Cobas® Amplicor HCV Monitor™ v2.0	Roche Molecular Systems, Pleasanton, California	Semi-automated RT-PCR	600 IU/mL (2.8 Log_{10} IU/mL)	600–500,000 IU/mL (2.8–5.7 Log_{10} IU/mL)
Cobas® Taqman® HCV Test v2.0 for use with the High Pure System	Roche Molecular Systems, Pleasanton, California	Semi-automated real-time PCR	25 IU/mL (1.4 Log_{10} IU/mL)	25–300,000,000 IU/mL (1.4–8.5 Log_{10} IU/mL)
Cobas Amplirep-Cobas Taqman (CAP-CTM)	Roche Molecular Systems, Pleasanton, California	Semi-automated or fully automated real-time PCR	15 IU/mL (1.2 Log_{10} IU/mL)	43–69,000,000 IU/mL (1.6–7.8 Log_{10} IU/mL)
Abbott RealTi*me* HCV	Abbott Molecular, Des Plaines, Illinois	Semi-automated real-time PCR	12 IU/mL (0.5 mL input) (1.1 Log_{10} IU/mL) 30 IU/mL (0.2 mL input) (1.5 Log_{10} IU/mL)	12–100,000,000 IU/mL (1.1–8.0 Log_{10} IU/mL)
Artus HCV RT-PCR Kit	Qiagen, Germantown, Maryland	Semi-automated real-time PCR	34 IU/mL (1.5 Log_{10} IU/mL)	65–1,000,000 IU/mL (1.8–6.0 Log_{10} IU/mL)

RT-PCR: reverse transcriptase polymerase chain reaction

hybridization-based techniques. Thus, their detection and quantification requires a preliminary "amplification" step. This can be achieved by using two categories of molecular biology-based techniques: signal amplification techniques, such as bDNA, and target amplification techniques based on PCR.

Signal Amplification Techniques (bDNA)

In signal amplification techniques, the viral genomes are first hybridized to a holder, by means of specific "capture" oligonucleotide probes. Then, the signal emitted by the hybrids is amplified for detection and measurement.

In the bDNA assay [1], viral genomes are specifically captured on microwells by hybridization with oligonucleotide probes. Synthetic bDNA amplifier molecules are hybridized to immobilized target hybrids in the microwells and a pre-amplifier molecule is added to the bDNA complex to augment the signal amplification (i.e., assay sensitivity). Signal amplification is achieved through the multiple repeat sequences within each bDNA amplifier molecule that serve as sites for hybridization with alkaline phosphatase-conjugated oligonucleotide probes. Detection is based on alkaline phosphatase-catalyzed chemiluminescence emission from a substrate. Quantification is based on a standard curve generated simultaneously with known standards. A commercial bDNA assay was developed in the early 1990s, the third-generation of which is available (Versant™ HCV RNA 3.0 Assay, Siemens Medical Solutions Diagnostics, Tarrytown, New Jersey). This assay is robust, accurate and specific, but it lacks analytical sensitivity (dynamic range of quantification: 615–7,700,000 international units (IU)/mL, i.e., 2.8–6.9 Log_{10} IU/mL) (Table 12.1).

Target Amplification Techniques (PCR)

The principle of target amplification techniques is to synthesize a large number of copies of the viral genome (amplicons) in a cyclic enzymatic reaction. The amplicons can then be detected by various methods, and the amount of viral genomes in the clinical sample can be quantified.

The PCR method uses several temperatures and one enzyme, a thermostable DNA polymerase [2]. The amplicons are double-stranded DNA molecules. A reverse transcription step is required for HCV RNA, in order to synthesize a complementary DNA (cDNA) for use as template in the PCR reaction. Each complete PCR cycle doubles the number of DNA copies; after n cycles, 2^n copies of each DNA molecule present at the beginning of the reaction are theoretically synthesized. In fact, the reaction generally reaches a saturation plateau after 35–45 cycles.

Detection of PCR amplicons is classically based on specific hybridization to immobilized oligonucleotide probes. Amplicon-probe hybrids are revealed in an enzymatic reaction, followed by detection of a colored or luminescent signal. Quantification is based on competitive amplification of the viral template with a known amount of synthetic standard added to each reaction tube. The relative amounts of viral template and standard amplicons are measured at the end of the procedure and the results are read from a standard curve established in parallel. A commercial assay based on these principles has been widely used in clinical practice worldwide: Amplicor® HCV Monitor® v2.0 and its semi-automated version Cobas Amplicor® HCV Monitor® v2.0 (Roche Molecular Systems, Pleasanton, California). The dynamic range of quantification of this assay is claimed to be 600–500,000 IU/mL (2.8–5.7 Log_{10} IU/mL) (Table 12.1). We have shown that the upper limit of quantification is in fact of the order of 200,000 IU/mL (5.3 Log_{10} IU/mL) [3].

More recently, "real-time" PCR techniques have been developed. They will be discussed below.

Clinical Significance of HCV RNA Levels

The availability of methods measuring HCV RNA levels in the blood of HCV-infected patients led to the generation of a large number of studies that have considerably improved our knowledge of HCV infection.

Studies of acute HCV infections showed that HCV RNA becomes detectable in peripheral blood during the first or the second week after infection in the vast majority of cases. Acute HCV infection is characterized by a peak of HCV RNA. HCV RNA level subsequently decreases, generally concomitantly to elevation of alanine aminotransferase (ALT) levels. HCV RNA may persist at steady levels, disappear and subsequently reappear after a few days to weeks and then persist, or disappear and never reappear (cure of acute infection in 20–50% of cases) [4]. Therefore, in the context of acute hepatitis C, HCV RNA clearance must be checked on at least two occasions at an interval of several months before considering that infection is cured.

The vast majority of patients with chronic hepatitis C have HCV RNA levels between 5×10^4 and 5×10^6 IU/mL [5]. HCV RNA levels remain stable, or tend to slightly increase over time in chronically infected patients [6]. The HCV RNA level has no prognostic value: more severe liver disease is not associated with higher HCV RNA levels, and a high HCV RNA level does not predict a more severe outcome in the long term. In patients with end-stage liver disease, HCV RNA levels are generally substantially lower than in patients without cirrhosis or patients with compensated disease [7]. One possible explanation could be a lower number of infectable hepatocytes, resulting in an important reduction of virus production; however, this has never been proven.

The main clinical interest of the HCV RNA level is its prognostic value on the outcome of antiviral therapy. Indeed, baseline HCV RNA level has been shown to be an independent predictor of the sustained virological response (SVR) to IFN-α-based therapy, including therapy with standard or pegylated IFN-α, alone or in combination with ribavirin [8–12]. Nevertheless, the predictive value of HCV RNA levels is weaker than that of the HCV genotype and of the recently discovered genetic marker "*IL28B* genotype" in all studies [8–14].

Mathematical Modeling of Viral Kinetics

Mathematical modeling of HCV RNA level declines during antiviral therapy has been used to unravel a number of mechanisms related to the pathophysiology of HCV infection and HCV therapy.

Pathophysiology of HCV Infection

A seminal work by Neumann et al. showed that the half-life of free HCV virions in peripheral blood is on average 2.7 h, and approximately 10^{12} infectious virions are synthesized and cleared every day in a chronically infected patient [15], with slight differences according to the HCV genotype [16] or the race [17]. It was also shown that, during acute infection, endogenous type I interferon production slows virus generation, whilst infected cells appear to have a shorter half-life than noninfected cells [18].

HCV Therapy

Mathematical modeling of HCV RNA level decay during therapy has been used to better understand the mechanisms underlying treatment efficacy and failure. In patients receiving IFN-α-based therapy, HCV RNA generally declines in a biphasic manner [15, 19]. The rapid, first-phase decline results from direct inhibition of virus production by IFN-α antiviral effectors. It is dose-dependent, but saturable. The slower second-phase decline reflects progressive clearance of infected cells, which are more likely cured than eliminated [15, 19].

The mode of action of ribavirin in the treatment of chronic hepatitis C remains unknown. Ribavirin monotherapy reduces HCV RNA levels by no more than half a log on average, and this effect is transient during the first days of administration [20]. When combined with IFN-α, ribavirin significantly improves SVR rates by accelerating the second-slope of viral decline, thus preventing post-treatment relapses [21, 22]. Mathematical modeling of HCV RNA declines in patients treated with IFN-α with or without ribavirin suggesting that ribavirin decreases HCV virion infectivity in a dose-dependent manner in the context of potent inhibition of virus production by IFN-α [23]. Nevertheless, this model did not explain how ribavirin exerts this effect; importantly no experimental system has confirmed this hypothesis and it remains to be determined whether ribavirin makes produced virions less infectious, or noninfected cells less infectable by these viruses.

Liver Transplantation

Mathematical modeling of HCV RNA level kinetics was also used to better understand the mechanisms underlying reinfection of liver grafts after liver transplantation for HCV-related liver disease [24]. The half-life of HCV virions after removal of the old liver was calculated to be on average 0.8 h. The rise in HCV RNA level started on average 15 h after implantation of the new liver and subsequently reached a plateau. Most of virus production was found to originate in the liver, whilst approximately 20% of hepatocytes on average were estimated to be infected at the plateau phase [24].

Better Assays to Measure HCV RNA Levels

Within the past few years, classical HCV RNA quantification methods have been progressively replaced by methods based on real-time PCR, thanks to the development of commercial, standardized assays. Real-time PCR is far more sensitive than classical PCR or bDNA and is not prone to carryover contamination. The dynamic range of quantification is consistently wider, making real-time PCR particularly useful for quantifying the full range of viral levels observed in untreated and treated HCV-infected patients.

In the TaqMan™ technology [25], a probe labeled with a "reporter" fluorochrome and a "quencher" fluorochrome is designed to anneal to the target sequence between the sense and antisense PCR primers. As long as both fluorochromes are on the probe, the quencher molecule stops all fluorescence emission by the reporter. During each PCR reaction, as the DNA polymerase extends the primer, its intrinsic nuclease activity degrades the probe, releasing the reporter fluorochrome. Thus, the amount of fluorescence released during the amplification cycle and detected by the system is proportional to the amount of amplicons generated in each PCR cycle. Software is used to calculate the threshold cycle in each reaction with which there is a linear relationship with the initial amount of DNA. In each run, parallel processing

of a panel of quantified standards is used to establish a standard curve for quantification.

Assays based on real-time PCR are now used in clinical virology laboratories for HCV RNA detection and quantification. Three real-time PCR platforms are currently available (Table 12.1): the Cobas Taqman® platform, which can be used together with automated sample preparation with the Cobas AmpliPrep® system (CAP-CTM, Roche Molecular Systems); the Abbott RealTi*me* HCV platform (Abbott Molecular, Des Plaines, Illinois), which uses the $m2000_{RT}$ amplification platform together with the $m2000_{SP}$ device for sample preparation; and the Artus HCV RT-PCR kit (Qiagen, Germantown, Maryland). A fourth assay, developed by Siemens Medical Solutions Diagnostics, should be available soon. The intrinsic performance of these available tests differs. Indeed, approximately 15% of HCV genotype 2 and 30% of HCV genotype 4 samples are substantially underestimated in the first-generation CAP-CTM assay, most likely because of nucleotide mismatches [26, 27], whereas this problem has not been found with the Abbott assay [28]. A new version of the CAP-CTM assay will be released soon that will hopefully resolve this issue.

Measuring HCV RNA Levels and Assessing Viral Kinetics: The Pegylated IFN-α and Ribavirin Therapy Era

Prognostic Value of Baseline HCV RNA Level on Treatment Outcomes

Baseline HCV RNA level is an independent predictor of the SVR to pegylated IFN-α and ribavirin [10–12]. The threshold between "high" and "low" HCV RNA levels varies from one study to another between 400,000 and 800,000 IU/mL (5.6–5.9 Log_{10} IU/mL) [29, 30]. Patients with a "high" baseline HCV RNA level respond significantly less well than those with a "low" HCV RNA level. Nevertheless, the baseline HCV RNA level has a poor predictive value on the likelihood of an SVR at the individual level, because the range of HCV RNA levels observed at baseline is relatively narrow and other parameters (such as the HCV genotype and host genetics) play a stronger role.

Viral Kinetics on Pegylated IFN-α and Ribavirin Therapy

In patients receiving pegylated IFN-α and ribavirin, the HCV RNA decline is typically biphasic [15, 19]. A triphasic decline, characterized by a "shoulder phase" of 4–28 days between the first and second phases, has been described in a subset of patients. Mathematical modeling predicts that this triphasic decline occurs only in patients in whom a majority of hepatocytes are infected before therapy [31]. Different viral kinetics have been observed with pegylated IFN-α2a and -α2b, due to their different pharmacokinetic properties [32, 33]. Ribavirin addition has been shown to accelerate the second slope of viral decline, resulting in less relapses and a higher rate of SVR [21, 22].

Tailoring Treatment Duration to HCV Viral Kinetics

Decision to Treat and Indication of Treatment

The decision to treat chronic hepatitis C depends on multiple parameters including a precise assessment of the severity of liver disease (with a liver biopsy or noninvasive markers), the presence of absolute or relative contra-indications to therapy, and the patient's willingness to be treated. HCV genotype determination should be systematically performed before treatment, as it determines the duration of treatment, the dose of ribavirin, and the virological monitoring procedure [12].

Patients infected with HCV genotype 2 or 3 require 24 weeks of treatment and a fixed, low dose of ribavirin (800 mg daily) [12]. However, patients with a low baseline HCV RNA level (<400,000–600,000 IU/mL, i.e., 5.6–5.8 Log_{10} IU/mL) who achieve a rapid virological response (RVR, defined by an undetectable HCV RNA at week 4) may not need more than 16 weeks of therapy [34]. In contrast, 48 weeks of therapy may be needed in some patients infected with HCV genotypes 2 or 3 who do not achieve an RVR.

Patients infected with HCV genotypes 1 and 4 (and probably also 5 and 6) require a high, body-weight based dose of ribavirin (1,000–1,400 mg daily), and treatment duration must be tailored to the on-treatment virological response (see below).

Treatment Monitoring

Monitoring of HCV RNA levels is recommended to tailor pegylated IFN-α and ribavirin treatment to the actual virological response. A sensitive assay with a broad dynamic range of quantification, ideally a real-time PCR assay, should be used. In HCV genotype 1-infected patients (and probably also patients infected with genotypes 4, 5 and 6), the HCV RNA level should be measured before therapy, and 4 and 12 weeks after its initiation. Patients with a low baseline HCV RNA level (<400,000–800,000 IU/mL, i.e., 5.6–5.9 Log_{10} IU/mL) who achieve an RVR can be treated for 24 weeks [35]. The lack of a 12-week virological response (i.e., no change or an HCV RNA decrease of less than 2 Log_{10} at week 12) indicates that the patient has virtually no chance to achieve an SVR and should stop treatment [36, 37]. In contrast, when a 2-Log_{10} drop in HCV RNA level has been observed at week 12, treatment must be continued until week 48 if HCV RNA is undetectable at week 12, or until week 72 if HCV RNA is still detectable at week 12 [38–40].

The SVR corresponds to a cure of infection in more than 99% of cases [41].

Measuring HCV RNA Level and Assessing Viral Kinetics: The Direct Acting Antiviral Drug Era

Utility of HCV RNA Level Measurement in Therapy with Pegylated IFN-α, Ribavirin and a Protease Inhibitor

The results of Phase III trials with two HCV protease inhibitors, telaprevir and boceprevir, in combination with pegylated IFN-α and ribavirin in both treatment-naïve patients and nonresponders to a first course of pegylated IFN-α and ribavirin, were recently reported [42–45].

Telaprevir will be used at 750 mg 3 times per day in combination with pegylated IFN-α2a, 180 μg/week, and ribavirin, 1.0–1.2 g/day according to body weight. In treatment-naïve patients, telaprevir will be administered for the first 12 weeks of therapy, and response-guided therapy will be used to tailor the duration of additional pegylated IFN-α2a and ribavirin administration: 12 weeks in patients who achieve an extended rapid virological response (eRVR, undetectable HCV RNA at weeks 4 and 12), i.e., a total treatment duration of 24 weeks; and 36 weeks in those who do not, i.e., a total treatment duration of 48 weeks [43, 45]. In treatment-experienced patients, telaprevir will most likely be administered for 12 weeks in combination with pegylated IFN-α2a and ribavirin, and treatment with pegylated IFN-α2a and ribavirin will be continued for an additional 36 weeks, i.e., until week 48, without using response-guided therapy (pending presentation of the final results of the Phase III trial).

Boceprevir will be used at 800 mg 3 times per day in combination with pegylated IFN-α2b, 1.5 μg/kg/week, and ribavirin, 0.8–1.4 g/day according to body weight. In treatment-naïve patients, boceprevir will be administered for 24 weeks, after a lead-in phase of 4 weeks with pegylated IFN-α2b and ribavirin alone. Thus the total treatment duration will be 28 weeks in patients who achieve an RVR (undetectable HCV RNA at week 4 of boceprevir administration, i.e., at week 8 of therapy), while patients who do not achieve an RVR will receive pegylated IFN-α2b and ribavirin for an additional 20 weeks, i.e., until week 48 [44]. In treatment-experienced patients, the triple combination of boceprevir, pegylated IFN-α2b and ribavirin will be administered for 32 weeks after a lead-in phase of 4 weeks with pegylated IFN-α2b and ribavirin in patients who achieve an RVR (undetectable HCV RNA at week 4 of boceprevir administration, i.e., at week 8 of therapy) for a total treatment duration of 36 weeks, and 44 weeks in those who do not for a total treatment duration of 48 weeks [42]. Importantly, everyday practice may be difficult, especially for nonexpert practitioners, as response-guided therapy will be based on different definitions of "RVRs" assessed at different time points of therapy for telaprevir and boceprevir.

Utility of HCV RNA Level Measurement with IFN-Free Regimens

DAAs generally induce a biphasic HCV RNA level decline. In the absence of an early selection of resistant HCV variants (drugs with a high barrier to resistance), the second-phase decline is steady. In contrast, when the barrier to resistance is low, the initial decline is followed after a few days to weeks by a reincrease of HCV RNA levels due to the outgrowth of resistant viral variants.

Prevention of resistance is typically based on the combination of several antiviral drugs that are potent and have no cross-resistance. Only short-term administration of DAAs without IFN-α (up to 2 weeks) has been reported thus far. Recent data has suggested that combining a protease inhibitor with either a nonnucleoside inhibitor of HCV RNA polymerase or an NS5A inhibitor does not increase the barrier to resistance and leads to early selection of variants that are resistant to both classes of drugs [46, 47]. In contrast, the combination of a nucleoside analogue with a protease inhibitor over 2 weeks has been associated with a biphasic decline of viral replication without any rebound due to selection of resistant variants [48]. Longer-term administrations are now needed.

Mathematical modeling has suggested that an IFN-free regimen should include a combination of drugs with a genetic barrier to resistance of at least three amino acid substitutions [49], meaning that resistance to this combination can occur only if variants preexist with more than three substitutions that confer resistance to the different drugs. Nevertheless, this remains to be demonstrated clinically. In addition, ribavirin was recently shown to delay the emergence of resistant variants when combined with two DAAs with a low barrier to resistance in the absence of IFN-α, probably by accelerating the second slope of viral decline, a mechanism similar to that involved in combination with IFN-α [47]. Further studies are now needed to identify the best IFN-free regimen (combination of drugs, role of ribavirin, duration) and the role of HCV RNA level monitoring in treatment optimization.

Conclusions

Measurement of HCV RNA levels has come a long way since it was first made available in the early 1990s. It is now widely used to tailor pegylated IFN-α and ribavirin treatment duration, and will still be useful for that purpose when telaprevir and boceprecir are prescribed in combination with these two drugs in patients infected with HCV genotype 1. It is likely that virological response-guided therapy will remain essential in the future, especially in an era of IFN-free regimens. Nevertheless, a number of studies need to be performed with highly sensitive and accurate assays based on real-time PCR in order to establish the best use of viral kinetics monitoring.

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Response-Guided Therapy

13

Mitchell L. Shiffman

Keywords
Chronic HCV • Interferon treatment • IL28B • Sustained virologic response

Introduction

The treatment of chronic hepatitis C virus (HCV) has historically been for a fixed duration with a fixed dose of peginterferon alfa-2a or alfa-2b and ribavirin [1–4]. According to this algorithm patients with HCV genotypes 1 and 4 are treated for 48 weeks and those with genotypes 2 and 3 for only 24 weeks. Stopping rules prevent continuing treatment in those patients with chronic HCV genotype 1 who have very little likelihood of achieving a virologic response and sustained virologic response (SVR). These included not having a 2 $\log_{10}$ reduction in HCV RNA from the pre-treatment baseline by treatment week 12 and remaining HCV RNA positive at treatment week 24. In contrast, patients with genotypes 2 and 3 are simply treated for 24 weeks. This approach to the treatment of chronic HCV yields SVR rates of approximately 40–45% for patients with genotype 1, 75–80% for patients with genotypes 2 and 3, and 70–75% for patients with HCV genotype 4 [1–5].

It is now known that patients with the same genotype do not all respond to treatment in the same way. Rather, there is a spectrum of virologic response where patients become HCV RNA undetectable at variable times after treatment has been initiated [6–9]. The rate at which patients become HCV RNA undetectable is affected by several virologic and host factors. Viral factors which affect time to virologic response include the genotype and baseline serum level of the virus [1, 2, 4, 5]. Host factors which affect the rate at which a patient becomes HCV RNA undetectable includes the degree of hepatic fibrosis, race, sex, body weight, and insulin resistance [1–4, 10–14]. More recently the genetics of the host, in particular the specific polymorphism of the IL28B gene, has been shown to be one of the most important factors affecting time to response [15]. Differences in IL28B polymorphism explain most, but not all, of the racial differences observed in virologic response and SVR.

The spectrum of virologic response observed in patients with chronic HCV receiving peginterferon and ribavirin therapy provides the opportunity to specifically tailor treatment in each patient. This approach is referred to as response-guided

M.L. Shiffman, MD (✉)
Liver Institute of Virginia, Bon Secours Health System,
12720 McManus Boulevard, Suite 313,
Richmond and Newport News, VA 23602, USA
e-mail: mitchell_shiffman@bshsi.org

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therapy (RGT), and allows the duration of peginterferon and ribavirin to be adjusted based upon the time at which the patient became HCV RNA undetectable. This concept has already been incorporated into treatment algorithms utilizing direct acting anti-viral agents, peginterferon, and ribavirin triple therapy. The concepts of RGT can also be very helpful regarding peginterferon and ribavirin dose modifications in patients experiencing significant adverse events from treatment. RGT treatment decisions can only be implemented when HCV RNA is monitored at frequent, regular intervals during treatment so that the time at which the patient has become HCV RNA undetectable is well defined. This chapter will review data which enabled the concept of RGT to be developed and describe how this approach can be utilized to optimize the treatment of patients with chronic HCV now and in the future.

Patterns of Virologic Response

The patterns of virologic response are depicted in Fig. 13.1. Although patients can become HCV RNA undetectable at any time after treatment is initiated it is convenient to divide virologic response into three major time points, weeks 4, 12, and 24. Patients who become HCV RNA undetectable within the first 4 weeks after initiating treatment are referred to as having a rapid virologic response (RVR). Patients who become HCV RNA undetectable after week 4 but by week 12 have a complete early virologic response (cEVR). Finally, patients who become HCV RNA undetectable after week 12 but by week 24 are referred to as being "slow to respond" (STR). Two patterns of virologic non-response are also recognized. Patients with a partial virologic response (PVR) are at least partially sensitive to peginterferon. These patients have an early virologic response (EVR) characterized by a 2 log decline in serum HCV RNA within the first 12 weeks of treatment. However, after week 12 serum HCV RNA does not continue to decline and at week 24 serum HCV RNA remains detectable. In contrast, patients with null response (NR) are relatively insensitive to interferon and have less than a 2 log decline in HCV RNA from the pre-treatment baseline by week 12. The vast majority of patients with NR have viral and/or host factors associated with non-response including an IL28B genotype associated with insensitivity to interferon. Patients with null or partial response will not have further declines in HCV RNA with continued therapy. Thus, treatment should be discontinued as soon as the NR or PVR patterns are recognized. No patient should remain on peginterferon and/or ribavirin treatment beyond 24 weeks if they are not already HCV RNA undetectable in serum.

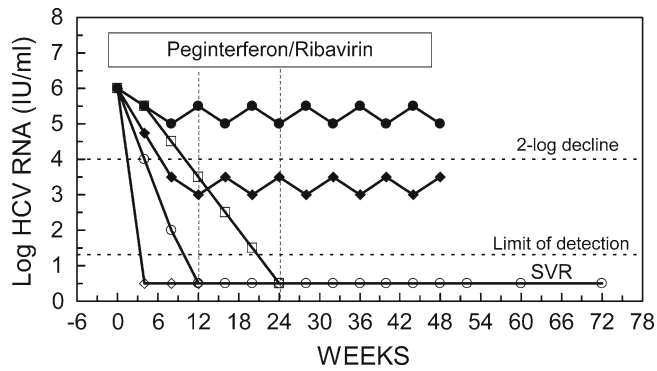

Fig. 13.1 The spectrum of virologic responses patterns which can be observed when patients with chronic HCV are treated with peginterferon and ribavirin. Rapid virologic response (*open diamonds*), complete early virologic response (*open circles*), slow to respond (*open squares*), partial virologic response (*solid diamonds*), and null response (*solid circles*) (with kind permission from Springer Science + Business Media: Shiffman [9])

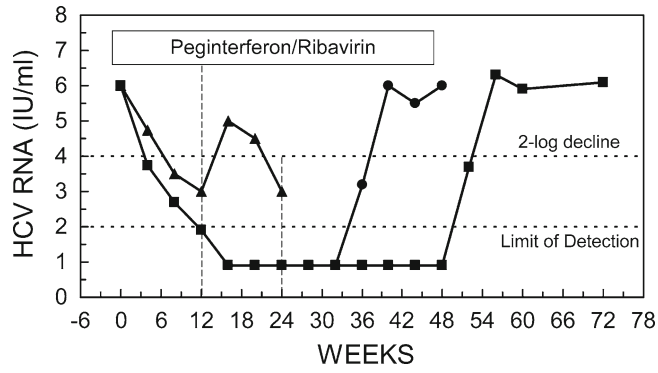

Fig. 13.2 Loss of the virologic response patterns. Loss of response occurs when a patient with a decline in serum HCV RNA then has a rise in serum HCV RNA back toward the pre-treatment baseline before ever becoming HCV RNA undetectable (*solid triangles*). Breakthrough occurs when serum HCV RNA becomes detectable during treatment after the patient had already become HCV RNA undetectable (*solid circles*). Relapse occurs when HCV RNA becomes detectable after completing a full course of treatment (*solid squares*) (with kind permission from Springer Science + Business Media: Shiffman [9])

Documenting when patients become HCV RNA undetectable is one of the most important aspects of treating chronic HCV and the cornerstone of RGT. Unfortunately, many physicians who treat chronic HCV do not measure the serum level of HCV RNA at enough time points; they fail to document that HCV RNA is indeed declining during treatment and that the patient has become HCV RNA undetectable. As a result, many patients who are not responding to peginterferon and ribavirin therapy are treated longer than necessary. In addition, some patients with a virologic response may be missed when HCV RNA is assessed at only limited time points. This is especially common when patients who achieve a virologic response interrupt or discontinue therapy because of adverse events or for non-medical reasons and the virologic response is lost. These events produce virologic response patterns referred to as "Loss of Virologic Response" (Fig. 13.2). Three such patterns exist. Loss of response is characterized by an initial decline in serum HCV RNA which rebounds back toward the pre-treatment baseline before the patient has become HCV RNA undetectable. Breakthrough occurs when a patient who was previously HCV RNA undetectable in serum suddenly develops recurrent viremia. Loss of response and breakthrough are rarely observed in patients who are compliant with their treatment regimen. The vast majority of patients who lose their response missed at least 1–2 doses of peginterferon and/or several consecutive days of ribavirin [13, 14]. The most common reason why patients miss doses is to lesson the adverse events of these medications. The final "Loss of Virologic Response" pattern is relapse. This is characterized by recurrence of HCV RNA in serum after the patient completes a full course of treatment. The frequency of relapse is related to how quickly patients become HCV RNA undetectable. Thus, patients with RVR have the lowest and patients with the STR virologic pattern have the highest relapse rates. Relapse is also more common in patients who miss doses or prematurely discontinue ribavirin [13, 15].

The concepts of RGT and appropriate treatment decisions can only be applied when the precise virologic pattern of response, non-response, or loss of response has been accurately identified. To accomplish this, serum HCV RNA should be assessed every 4 weeks from the onset of therapy until either the patient has become HCV RNA undetectable or one of the non-response patterns, NR, PVR, or loss of response, has been defined and treatment is discontinued. Once a patient has responded to treatment and has become HCV RNA undetectable, HCV RNA should continue

to be assessed at 3-month intervals (i.e. weeks 12, 24, 36, 48, 60, and 72) to ensure that breakthrough has not occurred and to document that the patient has achieved SVR or developed relapse.

The Basic Concepts of Response-Guided Therapy

There are three basic concepts to understanding RGT; (1) the faster after initiating treatment a patient becomes HCV RNA undetectable the higher is the SVR rate; (2) the longer it takes for a patient to become HCV RNA undetectable the longer the patient will need to be treated with peginterferon and ribavirin to maximize SVR; and (3) patients who become HCV RNA undetectable at the same time and are treated for the same duration have similar SVR rates regardless of genotype and other baseline clinical characteristics.

Table 13.1 provides data which support these principles for patients with genotypes 1, 2, and 3. Insufficient data are available for patients with genotype 4 to be included in this analysis. These data were derived from several large prospective clinical trials where the duration of treatment varied; and retrospective analyses from these large databases [3, 6, 9, 16–30]. Studies which did not provide SVR rates based upon when during treatment the patient first became HCV RNA undetectable were not included in these analyses. For simplicity, the table lists only mean values. The calculations account for variations in the sample size among the various studies analyzed. It should be kept in mind that each of these HCV clinical trials yielded slightly different overall SVR rates. This was likely affected by the size of these trials and variations in patient demographics, entry criteria, study design, and drop-out rates. As a result, the SVR rates listed in Table 13.1 should be considered a reasonable approximation and utilized to compare the impact of "time to virologic response" and "duration of therapy" both within and across genotypes.

Table 13.1 Mean sustained virologic response rates for patients with various virologic response patterns treated for various periods of time with peginterferon and ribavirin

Duration of treatment (weeks)	Virologic response pattern				
	RVR		cEVR		STR
	GT 1	GT 2 or 3	GT 1	GT 2 or 3	GT 1
16		83%		42%	
24	81%	89%		57%	
48	83%	90%	60%	72%	34%
72			63%		52%

Data represent mean values which were calculated based upon data obtained from refs. [16–33]
RVR rapid virologic response. Serum HCV RNA becomes undetectable before treatment week 4; *cEVR* complete early virologic response. Serum HCV RNA becomes undetectable after treatment week 4 and by treatment week 12; *STR* slow to respond. Serum HCV RNA becomes undetectable after treatment week 12 and by treatment week 24

Patients with RVR (HCV RNA undetectable by treatment week 4) who were treated for 24 weeks had SVR rates that ranged from 77 to 88% for patients with HCV genotype 1 and 85–100% for patients with genotypes 2 or 3. Although the mean SVR observed for patients with genotypes 2 or 3 when treated for 24 weeks was about 6% higher than for patients with genotype 1 treated for the same duration these values overlapped and are unlikely to be significantly different. Prolonging the duration of therapy from 24 to 48 weeks in patients with genotype 1 resulted in only a 2% mean increase in SVR (range: 75–91%). Only a single retrospective analysis contained data for patients with genotypes 2 or 3 who achieved RVR and were treated for 48 weeks [30]. Several studies have evaluated a treatment duration of less than 24 weeks for patients with HCV genotypes 2 or 3 achieving RVR. Although the duration of therapy varied from 12 to 16 weeks in these studies all have been combined and analyzed collectively for simplicity. Reducing treatment duration to only 12–16 weeks in patients with genotypes 2 or 3 who achieved RVR reduced SVR to 71–98%. In several prospective, randomized controlled trials the SVR in patients where the duration of therapy was less than 24 weeks was significantly lower than in patients who received treatment for 24 weeks [27–29]. Only a single prospective, randomized, controlled, trial has compared 24 vs. 48 weeks of peginterferon and ribavirin treatment in patients with HCV genotype 1 who achieved a RVR [31]. In this study SVR rates of 84% were achieved in both the

24- and 48-week treatment groups. Thus, for patients with RVR the optimal duration of therapy appears to be 24 weeks and increasing treatment duration further appears to offer little incremental benefit even in patients with HCV genotype 1. No study has specifically evaluated whether patients with HCV genotype 1 and a high baseline viral load, or those with cirrhosis, who achieve a RVR have significantly lower SVR rates when treated for 24 as opposed to 48 weeks.

Patients with cEVR (HCV RNA undetectable between treatment weeks 4 and 12) who were treated for 48 weeks had SVR rates that ranged from 38 to 73% in patients with genotype 1 [3, 6, 9, 16–30]. Only a single retrospective study has evaluated reducing the duration of therapy to 24 weeks in these patients [21]. This led to a marked reduction in SVR. Extending the duration of therapy to 72 weeks in patients with genotype 1 and cEVR has also been evaluated in a single study [18]. The SVR reported in that study was very similar to that observed for 48 weeks of treatment. In patients with genotypes 2 or 3 and cEVR treatment for 24 weeks yielded an SVR of 36–77%. Reducing the duration of therapy in patients with genotypes 2 or 3 and cEVR to 12–16 weeks reduced SVR to only 26–57%. Only a single retrospective analysis has examined the impact of extending treatment duration in patients with genotypes 2 or 3 and cEVR to 48 weeks [30]. This yielded an SVR rate of 72%. Thus, for patients with cEVR the optimal duration of therapy appears to be 48 weeks even in patients with genotypes 2 or 3. A randomized, prospective, controlled clinical trial to test the impact of extending treatment to 48 weeks in patients with HCV genotypes 2 or 3 and cEVR is nearing completion.

Data for patients with the STR (HCV RNA undetectable between weeks 12 and 24) virologic pattern is only available for patients with genotype 1. Nearly all patients with genotypes 2 and 3 become HCV RNA undetectable by treatment week 12 [1, 2, 27]. SVR rates of 18–52% were observed in genotype 1 patients with the STR virologic pattern following 48 weeks of treatment. Extending the duration of therapy to 72 weeks increased SVR rates in these patients to 44–69% [7, 9, 17–20]. Thus, for patients who are STR the optimal duration of therapy appears to be 72 weeks.

The Impact of Baseline Response Factors

Several demographic, histologic, biochemical, and virologic characteristics have been associated with a lower SVR rate. These include patients with African American race, Hispanic ethnicity, obesity, cirrhosis, insulin resistance, and high levels of serum HCV RNA. In contrast, Asian or Caucasian patients, or those without obesity, mild fibrosis, absence of insulin resistance, and low levels of HCV RNA have higher SVR rates [1–4, 10–14]. However, according to the concepts of RGT patients with the same virologic response pattern will achieve similar rates of SVR regardless of these demographic, host, and viral factors [9]. Data supporting this is presented in Table 13.2 [32]. This table summarizes data obtained from a retrospective analysis of several large clinical trials.

Table 13.2 Sustained virologic response rates in patients with HCV genotype 1 and various patterns of virologic response during treatment with peginterferon and ribavirin

	Virologic response pattern		
	RVR	cEVR	STR
All in group	75%	63%	33%
BMI > 27	77%	59%	33%
Caucasian	76%	67%	34%
African American	67%	73%	28%
HCV RNA (IU/mL)			
<400,000	78%	72%	41%
>400,000	71%	62%	32%
Cirrhosis			
Yes	75%	55%	34%
No	75%	65%	33%

Based on data from ref. [23]
RVR rapid virologic response. Serum HCV RNA becomes undetectable before treatment week 4; *cEVR* complete early virologic response. Serum HCV RNA becomes undetectable after treatment week 4 and by treatment week 12; *STR* slow to respond. Serum HCV RNA becomes undetectable after treatment week 12 and by treatment week 24

Patients with a RVR had similar rates of SVR, in the 67–77% range regardless of race, body weight, serum HCV RNA level, or degree of fibrosis at baseline. Patients with cEVR also had similar rates of SVR, in the 59–73% range, regardless of these baseline characteristics. Finally, patients with the STR pattern had SVR rates in the 28–41% range. Patients with RVR had higher SVR rates than patients with cEVR regardless of these baseline host and virologic factors. Patients with the STR virologic pattern had the lowest SVR rates regardless of these baseline factors. It is therefore apparent that the reason patients with poor baseline response factors have lower SVR rates is because they have less RVR, and cEVR, and a higher likelihood of the STR and non-response patterns. However, any patient who achieves an RVR or cEVR will have SVR rates that are as good as any other patient with these virologic response patterns regardless of their baseline host or virologic factors.

The Genetics of Interferon Response and Response-Guided Therapy

Recent studies have demonstrated that host genetics play a major factor in determining SVR during interferon-based therapy [15, 33]. This appears to be modulated by a single nucleotide polymorphism within the IL28B gene. Patients who have the CC haplotype for this gene have a twofold higher likelihood of achieving a SVR compared to patients with either the CT or TT haplotype. This is because patients with IL28B-CC have much greater interferon sensitivity and are much more likely of achieving RVR and cEVR than patients with either the CT or TT haplotype. Despite the strong association between IL28B genotype and SVR the concepts of RGT still apply and time to response is a more important determinant of SVR than IL28B genotype. Although patients with the IL28B-CC genotype have a much higher likelihood of achieving an RVR than patients with other haplotypes of the IL28B gene, all patients with RVR have similar, very high rates of SVR regardless of their IL28B genotype [33]. How IL28B status will be utilized in the management of patients with chronic HCV in the future remains to be defined. However, it is readily apparent that a favorable IL28B genetic pattern, the CC-haplotype, can explain why a patient with multiple poor prognostic factors at baseline (i.e. cirrhosis, high viral load, obesity, and insulin resistance) can achieve a RVR. There is therefore little doubt that measuring IL28B will provide useful information to assist in the management of patients with chronic HCV.

Applying the Concepts of Response-Guided Therapy

Based upon the data in Tables 13.1 and 13.2, and the general principles of RGT it is readily apparent that the duration of peginterferon and ribavirin should be based upon the time to response; not for an arbitrary period based upon genotype and other baseline factors. Patients with RVR should be treated for 24 weeks. Patients with cEVR should be treated for 48 weeks; and patients who are STR should ideally be treated for 72 weeks.

Despite optimizing the duration of therapy some patients will still relapse following treatment. The rate of relapse increases with the time required to become HCV RNA undetectable and according to the concepts of RGT prolonging treatment further is unlikely to significantly reduce relapse and impact SVR. Thus, retreatment of a patient with prior relapse for a longer duration is unlikely to yield an SVR if the patient had already received treatment for the appropriate duration based upon the concepts of RGT outlined in Table 13.1. Retreating patients with HCV genotypes 2 or 3 for 48 weeks will remain an important management strategy if these patients achieved cEVR but relapsed after receiving only 24 weeks of peginterferon and ribavirin. In contrast, retreating patients with HCV genotype 1 who had a STR pattern and relapsed following 48 weeks for 72 weeks will not be necessary in the future. Several clinical trials have now demonstrated that retreatment of HCV genotype 1 patients who relapsed with either telaprevir or boceprevir in combination with peginterferon and ribavirin yields a high rate of RVR and treatment of these patients for only 6 months is associated with an

SVR rate of over 90% [34–36]. Awaiting the availability of these protease inhibitors is therefore an alternative for the genotype 1 patient with prior relapse.

Some patients with PVR can become HCV RNA undetectable if the interferon dose is intensified. This can be accomplished by utilizing a higher dose of peginterferon or daily doses of high-dose interferon alfa-con1 [37, 38]. This approach is best utilized after treatment week 12 and before week 24. The goal of interferon intensification is to enable the patient to become HCV RNA undetectable by treatment week 24 and if successful, treatment should be extended to 72 weeks to maximize the chance of achieving SVR. In the future, these patients will have a high likelihood of responding to peginterferon, ribavirin, and a protease inhibitor [34–36]; and there will likely be no need to intensify interferon or treat patients for up to 72 weeks.

Utilizing Response-Guided Therapy to Manage Adverse Events

Managing the adverse events of peginterferon and ribavirin is one of the most challenging and controversial areas of HCV treatment [39]. Although some retrospective analyses have suggested that reducing the doses of peginterferon and/or ribavirin may lead to a decline in SVR both prospective studies and retrospective analyses have failed to confirm that the use of growth factors improves SVR compared to patients managed by judicious dose reduction especially if the adverse events which necessitated dose reduction occurred after the first 8 weeks or after patients were already HCV RNA undetectable [40–43]. The only published prospective, randomized, controlled trial to evaluate epoetin-alfa vs. dose reduction failed to demonstrate that the use of this hematologic growth factor increased SVR [44].

Several studies have now demonstrated that the time at which the reduction in the ribavirin dose occurred during treatment is an important factor in achieving an SVR [32, 42]. One of these studies was a retrospective evaluation of several large databases [32]. This study evaluated the impact of dose reducing either peginterferon or ribavirin based upon whether this dose modification was performed before or after the patient became HCV RNA undetectable. In general, reducing the dose of peginterferon or ribavirin prior to becoming HCV RNA undetectable increased relapse and reduced SVR. In contrast, reducing the dose of peginterferon and ribavirin after the patient had become HCV RNA undetectable appeared to have minimal impact on SVR, especially if the patient had achieved a RVR.

Another retrospective analysis evaluated the impact of utilizing epoetin-alfa to correct ribavirin-induced anemia as opposed to dose reduction [42]. Overall, patients treated with epoetin-alfa had an SVR rate of 50% compared to 48% for patients treated with dose reduction. However, patients who developed rapid and profound anemia within the first 8 weeks after initiating treatment did have a significantly higher SVR when epoetin-alpha was utilized in lieu of dose reduction, 45 vs. 27%. The vast majority of patients managed by ribavirin dose modification within the first 8 weeks who went onto achieve an SVR were already HCV RNA undetectable by treatment week 4 and had achieved a RVR. It is apparent from this data that patients who develop anemia rapidly after the initiation of treatment have lower rates of SVR even when epoetin-alpha is utilized compared to patients who develop anemia later during the course of treatment. This is because the vast majority of these patients require ongoing dose modifications, must deal with adverse events for a longer period of time, and have a higher likelihood of being unable to complete the proper duration of therapy. As a result, confirming when the patient becomes HCV RNA undetectable and treating according to the concepts of RGT may allow many patients to continue treatment as opposed to prematurely discontinuing treatment.

Modifying the doses of peginterferon and ribavirin should best be performed in small decrements. This maintains maximal exposure to these drugs while at the same time improving adverse events. Ribavirin should be reduced in 200 mg decrements, from 1,200 mg/day to 1,000 and then 800 mg/day at 1-week intervals. In the vast majority

of patients, the dose does not need to be reduced by more than 2 "steps" before the decline in hemoglobin either stabilizes or improves. Reducing the ribavirin dose by this method actually has minimal impact in total cumulative ribavirin exposure and in most cases prevents this from falling below 80%. In contrast, interrupting ribavirin dosing has a profound impact on total cumulative ribavirin dosing [39]. A retrospective analysis has demonstrated that reducing ribavirin dose had minimal impact on SVR but interrupting ribavirin dosing led to a significant decline in SVR [43]. The negative impact of discontinuing ribavirin prematurely on breakthrough and relapse was also demonstrated in a prospective study [45]. Peginterferon can also be reduced by marginal amounts without impacting SVR [43]. Peginterferon alfa-2a should be reduced from 180 μg/week to 135 mg/week. A randomized, prospective study has demonstrated that no significant difference in SVR exists between patients treated with either 1.0 or 1.5 μg/kg/week [3]. Finally, although dose modification may be associated with lower SVR rates in some patient groups, it is better to manage adverse effects with dose reduction rather than have frustrated patients suffering adverse events discontinue therapy prematurely. Reducing the doses of peginterferon and/or ribavirin will significantly improve adverse events, alleviate the need to interrupt or discontinue treatment, and allow these patients to remain on treatment for the proper duration (24, 48, or 72 weeks) based upon their time to virologic response (4, 12, or 24 weeks).

The Future of Response-Guided Therapy with Anti-Viral Agents

Within the near future two highly potent protease inhibitors, telaprevir and boceprevir, which directly inhibit HCV, will be approved for use by various regulatory bodies in many countries and be widely available and utilized along with peginterferon and ribavirin for treatment of patients with chronic HCV genotype 1 [34–36, 46–48]. In treatment naïve patients the use of the triple combination therapy will enhance SVR from approximately 45% to nearly 69–75% [46–48]. Retreatment of patients with HCV genotype 1 who previously failed to achieve a SVR with peginterferon and ribavirin will also enhance SVR. However, the magnitude of the retreatment effect with the triple combination will be dependent upon the previous response to peginterferon and ribavirin [34–36]. In patients with previous relapse SVR rates of over 90% have been observed following retreatment with the triple combination. Patients with previous PVR appear to have SVR rates of approximately 55–65% when retreated with an HCV protease inhibitor, peginterferon, and ribavirin. However, patients with prior NR and poor interferon responsiveness have low rates of SVR, only about 30–35%, when retreated with triple combination therapy.

The concepts of RGT have already been applied to triple combination therapy and utilized in phase 3 clinical trials of telaprevir and boceprevir in both the treatment naïve and retreatment populations [35, 46–48]. In these studies the term extended RVR (eRVR) was utilized to describe patients who achieved a RVR and then remained HCV RNA undetectable through treatment weeks 20–24. It will be necessary to monitor HCV RNA even after patients have become HCV RNA undetectable at week 4 in patients receiving protease inhibitors because of the possibility that virus resistant to the direct acting anti-viral agent has emerged during treatment and to identify recurrent viremia if it occurs. Recurrent viremia is most likely to occur in patients who develop significant adverse events from peginterferon and/or ribavirin and require that these medications be interrupted or in patients who are genetically insensitive to interferon based upon their IL28B genotype.

According to the basic principles of RGT patients with HCV genotype 1 who achieve an eRVR with a protease inhibitor, peginterferon, and ribavirin could be treated for 24 weeks. In contrast, patients who do not achieve an eRVR will require 48 weeks of therapy but will still have SVR rates that are less than those observed in patients with eRVR. The data available to date with both boceprevir and telaprvir have confirmed these basic tenants in both the naïve and

retreatment populations [35, 46–48]. When patients with an eRVR were treated for a shorter duration, only 24–28 weeks, SVR rates of approximately 85–95% were observed. In contrast, patients who initially achieved an RVR but developed breakthrough viremia or patients who failed to achieve an RVR with a protease inhibitor, peginterferon, and ribavirin had SVR rates of only about 45–55% even when treated for 48 weeks. Since many patients treated with a protease inhibitor become HCV RNA undetectable within 1–2 weeks it is entirely possible, given the basic tenants of RGT, that the duration of therapy could be reduced to only 12 weeks in some patients who are treated with triple combination therapy. This is most likely to be successful in patients with the IL28B-CC genotype. Randomized controlled trials to explore this possibility will likely be conducted in the future. It remains unclear if the concepts of RGT will also apply when multiple anti-viral agents are available to treat chronic HCV without peginterferon and/or ribavirin.

Conclusions

Our understanding of how to successfully treat chronic HCV with peginterferon and ribavirin continues to expand. Based upon all of the data that has accumulated to date, there appears to be a clear association between "time to virologic response," "duration of therapy," and SVR. These data form the basic principles of RGT. According to these concepts the duration of therapy should be based upon when the patient becomes HCV RNA undetectable; 24 weeks for patients with RVR, 48 weeks for patients with cEVR, and 72 weeks for patients with the STR virologic pattern. These patterns can only be defined by assessing HCV RNA at frequent intervals. Once patients have become HCV RNA undetectable, they need remain HCV RNA undetectable on treatment for the proper duration of treatment. In some cases this may require aggressive management of adverse events and reduction in the dose of peginterferon and/or ribavirin as opposed to prematurely discontinuing treatment. The principles of RGT appear to apply equally well when HCV is treated with triple combination therapy, either boceprevir or telaprevir, peginterferon, and ribavirin. It remains unclear if treatment duration could be reduced to less than 24 weeks in patients who become HCV RNA undetectable within 1–2 weeks of initiating triple combination therapy. Whether the concepts of RGT will apply equally well when HCV is treated with multiple anti-viral agents without peginterferon and/or ribavirin remains to be defined.

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Managing the Side Effects of Therapy

14

Curtis Cooper

Keywords
Pegylated interferon • Ribavirin • Side effects • Adverse events • Cytopenias • Depression • Mental health • Rash • Neurocognitive

Historical Perspective

The cornerstone of hepatitis C antiviral therapy for the last 20 years has been subcutaneously injected interferon α [1, 2]. Interferon is well noted for multiple side effects that negatively influence physical well-being and mental health. Side effects are typically less severe than with the larger doses used for some malignancies including melanoma [3]. Almost all patients experience fatigue and anorexia but most are able to continue with their usual daily activities including maintaining employment. However, as many as a quarter of patients need to reduce their work hours, employment responsibilities, or take a leave of absence. Most patients report mood volatility and some degree of depressive symptoms. Approximately 5–10% develop clinical depression requiring mental health support and/or pharmaceutical intervention. Patients with pre-existing psychiatric disease are at greater risk for relapse or exacerbation. The risk of this is generally predicted by mental health stability 6–12 months preceding the initiation of interferon.

Standard interferon was originally dosed at least three times per week by subcutaneous injection. Most patients experienced flu-like symptoms immediately after injection that lasted for several days. Side effects impaired patient quality of life, adherence to treatment regimens, and ultimately sustained virological response (SVR) rates.

Pegylated interferon (PegIFN) essentially replaced standard interferon in 2001 [4, 5]. The addition of polyethylene glycol (PEG) molecules prolonged interferon's half-life allowing the number of weekly doses for treatment to be reduced to one. Fewer doses reduced the discomfort associated with multiple weekly injections. Many patients inject at the end of the work week allowing for a day or two of rest post-injection when flu-like symptoms are most severe. This has resulted in improved quality of life while on therapy, increased productivity, and facilitated adherence which benefits SVR rates [6–8].

Other formulations of interferon including albuminated-interferon which is injected every other week or once monthly have been assessed [9, 10]. Although allowing for the same above

C. Cooper, MD, FRCPC (✉)
Department of Medicine, Division of Infectious Diseases, University of Ottawa, The Ottawa Hospital, G12-501 Smyth Road, Ottawa, ON, Canada K1H 8L6
e-mail: ccooper@ottawahospital.on.ca

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described benefits, the SVR rate achieved with albuminated-interferon was essentially similar to that observed with pegylated-interferons. However, a concern regarding a potential for pulmonary toxicity led to a decision not to pursue further development of this product.

Ribavirin (RBV), co-administered with interferon, has been considered standard of care since the mid- to late 1990s based on clinical trial evidence demonstrating improved SVR compared with interferon mono-therapy [1, 2]. Although multiple mechanisms of action have been purposed, the precise anti-hepatitis C antiviral and immunological activities of RBV remains to be fully resolved. RBV contributes additional side effects including fatigue, rash, cough, and hemolytic anemia (see Current Treatment). Newer formulations of RBV have had a minimal effect on reducing these symptoms. Anemia was diminished with Taribavirin, a prodrug of RBV [11]. However, the combination of peginterferon and tarabavirin was associated with a slightly lower rate of sustained virologic response and a higher rate of diarrhea compared to peginterferon and RBV. As a result, it is unlikely that the development of taribavirin will continue.

Current Treatment

Hepatitis C virus (HCV) antiviral therapy frequently produces side effects that negatively impact quality of life, necessitate medication dose reduction, and in some cases mandate premature treatment interruption. Life-threatening and fatal treatment-related toxicities are well recognized but rare. Aggressive management of potentially treatment-limiting side effects may improve patient well-being, optimize medication adherence, and maximize the likelihood of achieving a SVR. Health care providers should evaluate on-treatment patients frequently in an effort to identify and manage side effects before they progress. Typically, on-treatment patients are assessed every 2 weeks for the initial 2 months of therapy and then monthly thereafter for the duration of treatment. Most side effects resolve within 2–4 weeks after interruption of therapy.

The side effects associated with PegIFN-RBV-based HCV antiviral therapy are diverse. The following is a description of commonly experienced toxicities as well as rare but serious adverse events grouped primarily by system.

Constitutional

Post-Injection Symptoms

Many PegIFN-RBV recipients will experience "flu-like" symptoms occurring several hours after injections. Symptoms typically include fever (20–30%), headache (40–50%), and myalgias (20–30%). These symptoms can persist for several days and can prove to be incapacitating for some. In general, the severity diminishes after two to three injections. The use of acetaminophen or non-steroidal anti-inflammatory drugs for prophylaxis or treatment can minimize the severity of these symptoms. Headaches occurring after interferon injection can last hours to days. If acetaminophen and non-steroidal anti-inflammatory drugs are insufficient, amitriptyline, used on a prn basis, is often very effective for more severe headaches.

Fatigue

Treatment-related fatigue is very common (incidence of 60–90%). Fatigue significantly reduces quality of life and is a contributing factor in those discontinuing treatment. The etiology is multifactorial and reflects the systemic effects of both medications including the neuropsychiatric effects of interferon (e.g., fatigue, depression) and RBV-related anemia. Hemoglobin levels should be assessed at each visit. Thyroid stimulating hormone (TSH) should be evaluated every 12 weeks while on treatment as rarely, interferon-related hypothyroidism can contribute to patient fatigue.

A collection of neuropsychiatric side effects termed "neurovegetative" symptoms usually emerge during the initial 12 weeks of treatment and generally last until completion of therapy. Psychomotor slowing, altered sleep, and fatigue are included within this category of symptoms. The pathophysiology is not well understood but

is at least partially related to alterations in basal ganglia neurochemistry and dopamine signaling [12]. Antidepressant agents which exert noradrenergic/dopaminergic activity have been proposed for management (bupropion, venlafaxine, and duloxetine), although efficacy has not been clearly demonstrated in the literature. Neurovegetative symptoms typically respond poorly to selective serotonin re-uptake inhibitors (SSRI).

Psychostimulants including methylphenidate or modafinil are of uncertain benefit. The role of exercise regimens has not been well-evaluated for fatigue related to HCV treatment but is generally thought to be a safe recommendation for most patients [13].

Gastrointestinal

Gastrointestinal side effects are common with HCV therapy but rarely necessitate dose reduction or treatment interruption. Nausea (30–40%), anorexia (20–30%), diarrhea (15–25%), emesis (10–15%), abdominal pain (10–15%), dyspepsia (5–10%), and constipation (5%) are typically mild to moderate in severity and usually responsive to symptom-directed interventions.

Nausea is often temporally related to RBV dosing and may be diminished by administration with food and/or the use of dimenhydrinate. Emesis is less common and should be managed with oral rehydration and antiemetics (dopamine antagonists such as promethazine, prochlorperazine, and metoclopramide). For more severe nausea and vomiting, a 5-hydroxytryptamine-3 receptor ($5\text{-}HT_3$) antagonist (e.g., odansetron) can be considered. Oral tetrahydrocannabinoids (dronabinol) can be very effective in patients with nausea, anorexia, and weight loss, as they act both as antiemetics and appetite stimulants [14]. Although not medically recommended, many patients obtain and smoke marijuana to alleviate nausea and combat anorexia. Diarrhea occurs in 5–10% of treatment recipients and is managed with oral rehydration and/or antimotility agents including loperamide. Dyspepsia is managed with gastric acid suppressing agents including H_2 or proton pump inhibitors.

Most gastrointestinal symptoms can be managed symptomatically. However, uncommon and rare adverse events may occur, necessitating medical work-up including endoscopy, aggressive intervention, and in some cases treatment discontinuation. Gastrointestinal bleeding should lead to an evaluation for esophageal varices; especially in those known to be cirrhotic. Although a rare complication of treatment, hemorrhagic/ischemic colitis has been reported. Signs and symptoms include abdominal pain, bloody diarrhea, and fever. Resolution generally occurs within several weeks of interferon interruption. Interferon usually has a neutral effect on pre-existing inflammatory bowel disease (Crohn's Disease, ulcerative colitis) and these disorders should not be considered contraindications to interferon-RBV therapy [15, 16].

Hematologic

Anemia

As a result of RBV-induced hemolysis and interferon-induced bone marrow suppression, anemia is common. Patients experience a median decline in hemoglobin concentration during treatment of 3–4 g/dL. However, in some cases the decline can be much greater. Anemia exacerbates treatment-associated fatigue, contributes to mood disorders, and is a major clinical issue contributing to treatment interruption. There are several interventions to consider in the management of anemia.

Ribavirin Dose Reduction

In general, RBV dosing is reduced by 200 mg/day and reevaluated 1 week thereafter. Product monograms recommend RBV discontinuation if hemoglobin levels fall below 8.5 g/dL. If RBV dose reduction occurs prior to achieving full virological clearance, the likelihood of achieving SVR is reduced. Therefore, many clinicians will attempt to maintain at least some portion of the originally prescribed RBV dose while pursing other measures to increase the hemoglobin level.

Erythropoietin

The use of erythropoietin in HCV patients receiving interferon and RBV has been demonstrated to increase hemoglobin and to improve quality of life in at least one prospective, randomized, placebo-controlled trial [17]. Study participants were genotype 1-infected with hemoglobin levels falling below 10 g/dL [18]. Unfortunately, no data on SVR was collected as part of this study. In a retrospective analysis, erythropoietin use was associated with higher SVR and lower treatment discontinuation rates only in patients who developed anemia during the first 8 weeks of therapy but not among those who developed anemia after treatment week 8. This suggests that preservation of RBV doses by erythropoietin use may be most critical before full HCV RNA clearance has been achieved. Another key finding was that the SVR rate was higher in those who developed on-treatment anemia than those who did not. This was independent of erythropoietin use and RBV dosing levels. This implies that anemia is a marker of achieving optimal RBV drug levels during therapy. Despite the compelling results of this study there remains considerable debated pertaining to erythropoietin use during HCV antiviral therapy. In another prospective study, the use of erythropoietin was not associated with an increased SVR rate compared to patients managed by RBV dose reduction [19].

In several patient populations including those with end-stage renal disease and cancer patients, erythropoietin has been associated with increased thrombosis risk, cardiovascular events, malignancy progression, and pure red cell aplasia (PRCA) [20]. In contrast, the use of erythropoietin in the context of HCV is thought to be safe. The incidence of these complications in a population of HCV treatment recipients receiving erythropoietin was not increased while on treatment or for a mean 13 months following completion of therapy compared to those who did not receive erythropoietin while on interferon-RBV [21]. Of 449 patients treated with erythropoietin and PegIFN and RBV in the IDEAL study, adverse events were similar to those observed in anemic patients not prescribed erythropoietin [18].

Red Blood Cell Transfusion

Red blood cell transfusion is used to support patients until erythropoietin has an opportunity to exert its effect or until the effect of RBV dose reduction is realized. Transfusion is suggested in situations where patient fatigue threatens their ability to remain on treatment.

Autoimmune hemolytic anemia in a rare complication observed in those on interferon-based treatment. It is often challenging to distinguish this complication from RBV-induced hemolysis. However, a rapid and profound decline in hemoglobin levels can help to distinguish between the two diagnoses.

Neutropenia

PegIFN-induced bone marrow suppression often leads to neutropenia. In the IDEAL study, the absolute neutrophil count (ANC) fell below <750 cells/mm^3 in approximately one quarter of treatment recipients and below <500 cells/mm^3 in 5% of patients [18]. The risk of infections to those with neutropenia is unclear but clinical experience suggests that the medical significance is minimal. A retrospective study did not identify a link between treatment-emergent neutropenia and the incidence of severe or serious infection [22].

Product monographs advise PegIFN dose reduction for those with an ANC <750 cells/mm^3, and discontinuation at ANC levels below 500 cells /mm^3. In practice, many clinicians elect to observe patients with increased frequency and avoid dose reductions unless the neutrophil count falls below 500 cells/mm^3. The use of neutrophil stimulating factors (G-CSF) has not been thoroughly evaluated and is generally not recommended.

Thrombocytopenia

The incidence of severe thrombocytopenia (platelet counts <25,000/mm^3) in patients on treatment is low (≈1%). Furthermore, significant bleeding in those with thrombocytopenia is rare. Product monograms advise PegIFN dose reduction for platelet counts <50,000/mm^3 and discontinuation at levels below 25,000 platelets/mm^3. In practice, experienced clinicians will often elect to observe patients with increased frequency and avoid dose

Table 14.1 Common neuropsychiatric complications of interferon-based HCV therapy

Side effect	Incidence (%)	Contributing factors	Syndrome	Treatment
Depression	20–60	• Current depressive and/or anxiety symptoms • Depression during previous treatment • RBV-induced anemia • Longer duration of treatment • Lack of social support • Thyroid dysfunction • Organic brain impairment	Depression-specific • Low mood • Anhedonia • Anxiety • Cognitive complaints • Suicidal ideation and/or completion (if severe)	Selective serotonin re-uptake inhibitors (SSRIs)
			Neurovegetative • Fatigue • Anorexia • Pain • Psychomotor slowing	Combined serotonin-noradrenaline (norepinephrine) antidepressants (SNRIs)
Anxiety	10–15	• Current anxiety disorder • Anxiety during previous treatment	• Palpitation • Agitation • Restlessness	• Venlafaxine • Benzodiazepines
Mania or hypomania	3–5	• Family history of bipolar disorder • Past manic or hypomanic episodes • Bizarre, agitated, or aggressive behavior	• Extreme irritability/ agitation • Euphoria • Increased energy • Rate of speech • Hypersexuality	• Immediate psychiatric consultation • Mood stabilizer and neuroleptics • Stop IFNα and SSRIs, which may exacerbate mania

reductions unless platelet levels decline to the 20,000/mm^3 range. The thrombopoetin receptor agonist eltrombopag increases platelet counts in those with HCV-related cirrhosis [23]. However, no clear benefit in terms of reduced bleeding risk was identified and an increased risk for portal vein thrombosis in cirrhotic patients was noted. As a consequence, the use of this product is not recommended in most circumstances.

Idiopathic thrombocytopenia (ITP) may rarely complicate interferon-based HCV therapy [24]. This is usually identified by a precipitous and profound decline in platelet count (<25,000/mm^3) following the initiation of therapy. ITP mandates treatment interruption and urgent hematology referral.

Mental Health and Neurocognitive Side Effects

A number of neuropsychiatric adverse effects occur spontaneously or are exacerbated by interferon. Although the pathophysiology is not fully understood, neurobiological mechanisms play a central role. Depression is common (severe in up to 15% of patients) and can present within weeks to months following the initiation of therapy and cognitive dysfunction (Table 14.1) [25–27]. Anxiety and mania are less frequently encountered but warrant a rapid and intensive clinical response.

Patients with pre-existing psychiatric disorders should not be excluded for interferon-based treatment. However, these patients should be carefully assessed for mental health stability prior to therapy [28]. Patients with pre-existing mental illness can achieve SVR rates that are comparable to patients without mental health co-morbidity. A multidisciplinary approach including experienced physicians, mental health specialists, addiction assistance, and social workers increases the likelihood of success [29, 30].

Depression is the most common psychiatric side effect complicating therapy [31, 32]. Patients should be evaluated at least every 4 weeks while on therapy. Self-assessment tests may be useful for depression screening and during therapy to

provide an initial measure of depression severity. Examples include the Beck's Depression Index (BDI) and Centers for Epidemiologic Study – Depression (CES-D). The emergence of mild depressive symptoms during treatment is common and does not mandate interferon dose reduction or discontinuation. Management often includes the use of antidepressants and increased frequency of clinical monitoring (i.e., 2–4 times monthly). Moderate or severe depression often mandates mental health care support (e.g., psychologist, psychiatrist) and interferon dose reduction or discontinuation. Active suicidal ideation should result in treatment discontinuation and possible hospitalization.

Antidepressants started during HCV therapy should be continued at least 2–3 months following treatment cessation. Depressive symptoms may rebound if antidepressants are prematurely discontinued. Gradual tapering of the dose is recommended. Antidepressant medications may be required for longer periods of time in those with severe depression [33]. The value of pretreatment, prophylactic antidepressants remains uncertain.

Anxiety disorders are often exacerbated by interferon. Anxiolytics including venlafaxine and benzodiazepines are usually effective in stabilizing patients so that they can complete HCV treatment. Venlafaxine is also beneficial for those with treatment induced-irritability.

Insomnia is a very common side effect that diminishes patient quality of life and contributes to a decline in mood stability. Attention to improved sleep hygiene practice (i.e., cool dark room, use of bed for sleep only, set sleeping schedule) as well as avoidance of caffeine, alcohol, and food in the evenings can be beneficial. Trazodone is often used as first-line therapy. Benzodiazepines may be useful in the short term but issues related to tachiphylaxis limit the long-term efficacy of this class of drugs.

Sexual dysfunction is common while receiving HCV treatment and is multifactorial in etiology [34]. Diminished sexual activities characterized by a loss of sexual desire, erectile and ejaculatory dysfunction is reported by half of males during treatment. Fear of pregnancy and HCV medication teratogenicity may also negatively affect sexual activity while on treatment. There is little data on the efficacy or safety of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 (PDE5) inhibitors (e.g., sildenafil citrate) [35].

Infectious Diseases

Interferon therapy suppresses immune function. Serious or severe infections develop in 3–5% of patients treated with PegIFN and RBV over a 48-week course of therapy. Infectious complications during PegIFN therapy are not directly related to neutropenia, suggesting that the mechanisms underlying infection risk are more complex [22].

Oral Cavity Infections

Infections occur most commonly in the oral cavity, respiratory tract, and skin [22]. Thrush, dental abscesses, and oral herpes simplex virus ulcers are the most common oral cavity infections occurring during HCV therapy. Nystatin swish and swallow or oral fluconazole are very effective for thrush. Dental abscesses should be treated with penicillin, or if allergic, clindamycin followed by prompt dental evaluation and definitive management to prevent systemic dissemination. The severity and duration of oral herpes simplex virus can be attenuated if oral antiviral medication (e.g., acyclovir) is initiated within the first 24 h of onset.

Respiratory Infections

Respiratory infections are typically caused by community-acquired viruses or bacteria including *Streptococcus pneumonia* and *Hemophilus influenza*. Acute bacterial bronchitis is more common than pneumonia. The majority of acute bronchitis is viral in etiology. The decision to initiate antibiotic therapy is dictated by clinical judgment, the severity of illness, and culture results. Influenza immunization is recommended in those living with HCV infection, irrespective of HCV antiviral therapy. Vaccine efficacy may be

Table 14.2 Dermatology side effects of HCV antiviral therapy

Very common (≥1/10)	Common (≥1/100 to <1/10)	Uncommon (≥1/1,000 to <1/100)	Very rare (<1/10,000)	Case reports
• Dermatitis • Pruritis • Dry skin • Alopecia	• Erythematous rash • Maculopapular rash • Eczema • Acne • Furunculosis • Face or peripheral edema • Abnormal hair texture • Nail disorder • Sweating/night sweats • Urticaria • Photosensitivity	• Herpes simplex • Cellulitis	• Toxic epidermal necrolysis • Stevens-Johnson syndrome • Angioedema • Erythema multiforme • Porphyria cutanea tarda	• Cutaneous sarcoidosis • Linear IgA bullous dermatosis • Xerostomia • Lichenoid reaction • Sweet's syndrome

diminished while on interferon but some degree of vaccine-produced immunity is better than none.

Cutaneous Infections

Bacterial cellulitis is usually caused by Group A *Streptococcus* or *Staphylococcus aureus*. Empiric antibiotic therapy should be guided by local resistance profiles and patient allergic profile. Injection site abscesses resulting from poor interferon injection technique or injection drug use misadventure occur on occasion. Management includes antibiotics and surgical drainage if abscesses are greater than 3–4 cm. Herpes simplex virus or varicella zoster virus (shingles) may produce painful, burning, vesicular lesions on an erythematous base. Systemic dissemination is much less common but requires intravenous antiviral therapy and HCV treatment interruption. Cutaneous fungal infections are occasionally observed in the skin folds. Topical antifungal medication as well as moisture control is usually effective.

Dermatologic Disorders (Table 14.2)

HCV infection itself is associated with skin disorders including porphyria cutanea tarda (PCT) and lichen planus. Rarely, cryoglobulinemia can manifest as vasculitis. These disorders are not absolute contraindications to interferon-based therapy and may actually improve with successful clearance of HCV viremia [36].

Local maculopapular skin reactions and dry skin frequently develop on HCV therapy and are managed with topical hydrating lotions, antipruritics, and topical corticosteroids. Mild to moderate alopecia may be seen in approximately 20% of interferon recipients but usually does not result in obvious balding. It is usually reversible with treatment discontinuation. Gentle hair products are often advised. Some experts recommend topical minoxidil but supportive data is limited.

Interferon-based therapy can produce psoriatic exacerbations [37]. Treatment interruption is not mandatory but increased monitoring and assessment by dermatology is required. The development of psoriatic arthritis mandates immediate discontinuation of therapy.

Several rare but severe dermatological complications require immediate interferon interruption. These include toxic epidermal necrolysis, Stevens-Johnson syndrome, angioedema, extensive erythema multiforme, and cutaneous sarcoidosis.

Injections are not typically painful. Interferon often produces non-palpable erythematous lesions at the injection site. These fade within weeks to 2 months. Rarely (<1:10,000), cutaneous and subcutaneous necrosis reactions can occur at the site of injection.

Autoimmune Including Thyroid

Endocrine Conditions

Hypothyroidism

Hypothyroidism is the most common autoimmune complication of interferon-based HCV therapy. Both immune modulatory mechanisms (Hashimoto's thyroiditis) and direct thyrotoxic effects (destructive thyroiditis) can produce hypothyroidism [38]. The incidence is 3–4%, more common in women and often associated with a family history of thyroid disease. HCV itself may contribute to the likelihood of developing autoimmune disease following interferon exposure [39, 40]. The risk of thyroid disease is four times greater in those with pre-existing thyroid peroxidase antibodies [41]. The appearance of thyroid autoimmunity antibodies while on interferon therapy may precede the onset of clinical thyroid disease [42]. However, routine monitoring of thyroid antibodies is not normally pursued due to poor positive and negative predictive values [43, 44]. Thyroid function should be monitored by TSH measurement prior to treatment and every 3 months thereafter until treatment completion. TSH elevation requires subsequent free thyroxine (T4) levels and thyroid antibody measurement. Symptoms of hypothyroidism may overlap with typical interferon side effects.

If symptomatic hypothyroidism is identified, there are several actions to consider. One option is immediate interruption of HCV therapy. This may reduce the likelihood of permanent thyroid dysfunction. However, there is minimal data to support this approach. Furthermore, premature treatment interruption will reduce the likelihood of SVR. An alternate course of action is to continue IFN therapy and initiate thyroid replacement treatment (e.g., levothyroxine).

Hyperthyroidism

Hyperthyroidism is less frequently encountered. Typically, this manifests as non-autoimmune thyroiditis which is followed thereafter by hypothyroidism and eventual resolution. In rare cases Graves' disease is identified by complete TSH suppression in conjunction with detectable thyroid-stimulating antibodies. Hyperthyroidism typically mandates discontinuation of HCV therapy and consultation with endocrinology.

Diabetes

Type I diabetes mellitus develops in less than 1% of interferon recipients and is often irreversible [45]. Prior to treatment, half will display markers of pancreatic autoimmunity, suggesting that interferon may accelerate a pre-existing diabetogenic process [46]. Some Type II diabetics will note greater difficulty in maintaining blood glucose within therapeutic range following the initiation of HCV antiviral therapy.

Rheumatologic Conditions

Although rare (<1% incidence), interferon may precipitate the clinical onset of autoimmune-mediated rheumatology conditions including rheumatoid arthritis and systemic lupus erythematosus [47]. This mandates the immediate and permanent discontinuation of interferon therapy and consultation with rheumatology. A key risk factor is a personal or family history of autoimmune or rheumatological disease. Pre-treatment screening should include a laboratory evaluation of antinuclear antibody test and rheumatoid factor. However, increased levels of these measures in the context of HCV infection are non-specific.

For patients with pre-existing autoimmune or rheumatological disease, the benefits and risks of interferon exposure require careful consideration. Active, suboptimally treated rheumatologic disease represents an absolute contraindication to interferon exposure, whereas those with well-controlled autoimmune disease may be considered. Interferon-based HCV treatment should be reserved for individuals at high risk for progression to end-stage liver disease. Of note, HCV-mediated autoimmune conditions (e.g., mixed cryoglobulinemia) often improve with HCV therapy. This may be permanent if a SVR is achieved.

Ophthalmologic

Discontinuation of interferon should be considered in those who develop new or deteriorating

ophthalmologic disorders. These rarely occurring conditions include optic neuritis, significant cotton wool spots, retinal hemorrhage, papillary edema, macular edema, retinal vascular obstruction, corneal ulcer, or loss of visual acuity, visual fields, and/or complete loss of vision. Subclinical retinal abnormalities are more common (30%). These are reversible following completion of interferon therapy. Patients with established or suspected ophthalmologic disorders and those at risk due to co-morbidities (e.g., diabetes mellitus and hypertension) should have re-treatment and periodic on-treatment fundoscopic retinal evaluation. Patients with on-treatment ocular symptoms including diminished visual acuity and/or visual field abnormalities should be assessed promptly by ophthalmology.

Pulmonary

Cough and/or dyspnea are observed in at least 20% of patients on PegIFN and RBV. Although the mechanism is not entirely clear, mucosal dessication by RBV is a primary contributing factor [1]. The pathophysiology of dyspnea is multifactorial and includes anemia, the fatiguing effect of interferon, and rarely as a consequence of treatment-precipitated cardiac or pulmonary disease. HCV treatment may be associated with a reversible decline in gas transfer as measured by the diffusion lung capacity for carbon monoxide (DLCO) which may contribute to this symptom.

Several pulmonary complications are rarely observed with interferon therapy. Interferon may produce flares in pre-existing sarcoidosis or trigger new onset of this disorder. The incidence is approximately 0.5%. Treatment discontinuation results in improvement in most cases [48]. Interstitial pneumonitis is a potentially fatal complication [49]. Symptoms include fever, dyspnea, and cough. Diagnostic findings include a restrictive pattern on spirometry and ground glass infiltrates on high-resolution chest computerized tomography scans. This diagnosis mandates interruption of HCV therapy, initiation of steroids, and consultation with a lung disease specialist.

Cardiovascular

Interferon and RBV-induced anemia may precipitate myocardial ischemia in those with pre-existing coronary artery disease [50]. An evaluation of coronary artery disease risk factors prior to initiating treatment is recommended. Persons at risk for or with known coronary artery disease should undergo additional evaluation by cardiology prior to initiation of HCV treatment. Interferon-induced cardiomyopathy and pericarditis are rare but potentially serious complications which mandate treatment suspension [51, 52].

Future Treatment

Currently, HCV antiviral therapy is characterized by long duration, numerous side effects, difficult administration, and suboptimal success. Alternative therapeutic options are required. Fortunately, there are multiple orally administered HCV antivirals in clinical development that hold great promise for improved efficacy and tolerability. These medications include polymerase inhibitors (nucleoside and non-nucleoside analogs), protease inhibitors and other orally administered compounds (non-structural protein 5a inhibitor) which suppress viral replication by selectively inhibiting synthesis of virus structural proteins. As a group, these molecules achieve rapid viral suppression, very high rapid virologic response (RVR) rates, and much higher SVR rates when combined with peginterferon and RBV. This has been demonstrated in both interferon-RBV naïve and treatment-experienced patients (e.g., virologic relapsers and non-responders). As achieving a RVR may allow for shorter duration therapy, this represents a major advance in reducing the cumulative side effects of interferon and RBV. The attrition rate of agents has been high due to various toxicities. However, several reasonably well-tolerated compounds with demonstrated efficacy are poised to become the standard of care for HCV treatment by 2011.

Optimism must be tempered by concerns related to the rapid development of drug resistance with resulting HCV viremia rebound.

Intensive emphasis on adherence to HCV antiviral therapy will be critical to the success of these new HCV therapies. The first two protease inhibitors likely to be used in clinical practice (boceprevir and telapravir) require thrice daily dosing which will challenge adherence. Other molecules in early stages of development can be dosed once or twice daily and will help to overcome this barrier to treatment success.

It has been proposed that RBV, and the associated toxicities of this medication, may be eliminated by replacement with one of these new molecules in combination with interferon. However, several key studies (SPRINT-1, PROVE 2, PROVE 3, STEALTH-C) demonstrate that RBV is important in maximizing the success of the HCV protease inhibitor-interferon-RBV combination treatment, as response rates were lower and relapse rates higher in the RBV-sparing arms of these trials [53–55].

There is accumulating evidence that combinations of these molecules (e.g., protease inhibitor plus nucleoside or non-nucleoside reverse transcriptase inhibitor) may eventually lead to interferon and RBV-sparing HCV treatment regimens. In the INFORM study, combination R7128 (nucleoside analog) plus R7227 (protease inhibitor) dosed over a maximum 14-day period in treatment naïve recipients achieved greater than additive antiviral activity [mean −3.9 log 10 IU/mL change from baseline (range: −5.0 to −2.9)], with no viral rebound and no adverse events requiring dose modification or discontinuation [56]. In vitro data, including a study that found a lack of cross-resistance between protease inhibitors, nucleoside inhibitors, and non-nucleoside inhibitors, support the potential use of combination therapy [57]. These data support the plausibility of effective interferon and RBV-sparing regimens which would substantially improve the side effect profile of HCV therapy.

Conclusions

Current standard of care HCV therapy is characterized by multiple side effects which challenge most patients' ability to complete all medications for the entire duration of recommended treatment. Careful and frequent monitoring during treatment is essential to identify issues before they progress in severity, threaten patient well-being, and force treatment interruption. It seems likely that with the arrival of orally doses protease inhibitors and polymerase inhibitors, HCV treatment regimens will be reduced in duration and improved in overall tolerability.

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The Genetics of Virologic Response

15

Alison B. Jazwinski and Andrew J. Muir

Keywords
Hepatitis C • Genome • Human/genetics • Genome-wide association study • Genotype • Interleukins/genetics* • Pharmacogenetics

Introduction

The likelihood of response for an individual patient treated with standard pegylated interferon alpha and ribavirin for hepatitis C is impacted by a combination of viral factors and patient factors. Infection with viral genotypes 2 or 3 is favorable for response to treatment compared to infection with other viral genotypes [1]. Patient factors known to improve treatment response include female sex, non-African-American ethnicity, and age. Other clinical predictors of treatment response include baseline viral load, degree of fibrosis, insulin resistance, and steatosis on liver biopsy [1–4]. Recent discoveries of genetic variation within the HCV virus and within the human population have provided new insights into differences in treatment response among various populations. The field of medicine is changing and it appears that we will soon enter an era where determination of the genetic makeup of pathogens and patients will be standard practice for clinical decision making. The developments that have occurred within the field of HCV treatment are a step in this direction.

A.B. Jazwinski, MD
Departments of Gastroenterology and Hepatology, Duke University School of Medicine and Duke Clinical Research Institute, Durham, NC, USA

A.J. Muir, MD, MHS (✉)
Department of Medicine, Duke University Medical Center, DSMC, Box 3913, Durham, NC 27710, USA
e-mail: muir0002@mc.duke.edu

Viral Genetic Factors

Variation Within the HCV Sequence Predicts Treatment Response

Significant variation exists within the HCV genome due to the poor proofreading ability of the viral polymerase and frequent mutations. HCV has six viral genotypes with less than 72% homology at the nucleotide level. Subtypes within the genotypes have nucleotide identities between 75 and 86%, and individual isolates of a given subtype have even more variation. Furthermore, HCV replicates as quasispecies and often multiple variants of the virus exist within the infected individual [5–8]. The clinical implications of quasispecies are not entirely clear. One study evaluated quasispecies in 59 patients with particular focus on the E2 hypervariable region 1.

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Increased quasispecies heterogeneity correlated with the estimated duration of HCV infection, risk factor for infection (transfusion), HCV RNA levels ($P < 0.05$), and genotype 1 infection. In the 29 patients receiving treatment with interferon alpha, patients who achieved SVR had lower pre-treatment quasispecies heterogeneity compared to patients with relapse or no response. Quasispecies heterogeneity was not a predictor in the multivariate analysis as viral load was the stronger predictor of response [9].

It has been long known that treatment response varies depending on the viral genotype that has infected the individual. Patients infected with genotypes 2 and 3 have much higher response rates to standard treatment than patients infected with other genotypes [3]. The effect of the other layers of variation on treatment response has been historically less understood.

As part of the Viral Resistance to Antiviral Therapy of Chronic Hepatitis-C (VIRAHEP-C) clinical study conducted by the National Institutes of Health to compare treatment outcomes of African-Americans and whites, the virus of 94 patients was sequenced prior to treatment. Patients were divided into groups based on their virologic response at day 28 of treatment with pegylated interferon alpha and ribavirin. Individuals that had >3.5 $\log_{10}$ reduction in HCV viral load at day 28 were classified as "marked" responders, those with 1.4–3.5 $\log_{10}$ viral load reductions were classified as "intermediate" responders, and those with <1.4 $\log_{10}$ viral load reductions were classified as "poor" responders. Statistically significant ($P < 0.01$) differences were noted in the amount of sequence variation between patients with "marked" and "poor" response, with higher sequence variation noted in genotype 1a, E2, NS3, and NS5A and genotype 1b core, E2, NS2, and NS3 [10, 11]. This work identified the effect of individual amino acid changes in the viral proteins. However, amino acids interact with other residues within the same protein and in other proteins. Therefore, the same group performed a global analysis of the viral genome in the context of treatment response by analyzing the complete protein coding region of pretherapy HCV sequences [12]. They used the principle of covariance, which analyzes pairs of amino acid positions that vary among independent viral isolates. In doing this genome-wide analysis, they found novel genetic interactions that were interwoven through the HCV genome and that differed between responders and nonresponders to IFN-based therapy and may permit prediction of outcome of therapy. Although this work requires validation it may provide the basis for a sequence-based test that could predict the susceptibility of individual HCV isolates to interferon-based therapies.

Host Genetic Factors

Genetic Predictors of Sustained Virologic Response in HCV genotype 1

Interleukin-6

The VIRAHEP-C study also evaluated genetic variation in interleukin-6, which is involved in the immune response to infections. In vitro studies had suggested genetic diversity leading to varied levels of interleukin-6 expression. The interleukin-6 T-T-G-G-G-G-C-A-G-A haplotype was associated with lower rate of achieving SVR among Caucasian Americans with a relative risk 0.80, 95% CI 0.66–0.98. After adjusting for confounders, the rs1800797-(G)-rs1800796-(G)-rs1800795-(G) haplotype was independently associated with a reduced rate of SVR (RR 0.78, 95% CI 0.62–1.0). These findings were limited to the Caucasian group in this study with no clear variation associated with treatment response in African-Americans [13].

IL28B

A major advance in this field occurred in 2009 when several genome-wide association studies (GWAS) independently identified SNPs near the *IL28B* gene to be associated with SVR in patients with genotype 1 hepatitis C. Table 15.1 summarizes the findings from the original three GWAS of hepatitis C treatment response. Subsequent reports have validated this finding in cohorts from the United States [14, 15], Switzerland [16], France [17], and Austria [18].

Table 15.1 Characteristics of the original three GWAS and the most significant SNPs identified

Study	N	Platform	Phenotype	Ethnicity	SNPs	*P* values
Ge et al. [20]	1137	Ilumina 610 Quad BeadChip (565,759 of 610,000)	SVR (adherent)	European-American, African-American, Hispanic	rs12979860	1.37×10^{-28}
					rs12980275	2.54×10^{-27}
					rs8099917	3.70×10^{-26}
					rs12972991	1.72×10^{-21}
					rs8109886	1.54×10^{-16}
					rs4803223	7.85×10^{-16}
					rs12980602	6.10×10^{-9}
					rs8103142	N/A
					rs28416813	N/A
Tanaka et al. [23]	142/172	Affymetrix SNP 6.0 (621,220 of 900,000)	NVR* (adherent)	Japanese	rs8099917	2.68×10^{-32}
					rs11881222	2.84×10^{-31}
					rs8105790	1.98×10^{-31}
					rs7248668	1.84×10^{-30}
					rs4803219	2.45×10^{-29}
					rs8103142	1.40×10^{-29}
					rs28416813	5.52×10^{-28}
					rs12980275	2.84×10^{-27}
					rs10853728	1.23×10^{-16}
Suppiah et al. [22]	293/555	Illumina Infinium HumanHap 300/ CNV370 – Quad genotyping beadchip (311,159)	SVR (did not control for adherence)	European	rs12980275	7.74×10^{-10}
					rs8099917	9.25×10^{-9}
					rs8103142	3.83×10^{-4}
					rs8105790	3.70×10^{-4}
					rs8109886	1.27×10^{-4}
					rs12980602	1.02×10^{-3}
					rs4803224	5.87×10^{-3}
					rs10853728	7.42×10^{-2}

The initial discovery came from the IDEAL trial, which compared the efficacy of peginterferon alpha-2a to peginterferon alpha-2b. African-American patients from another HCV treatment study were added to enrich the African-American population, which resulted in a total of 1,137 samples for analysis [19]. A polymorphism located on chromosome 19 at a position 3 kilobases (kb) upstream of the *IL28B* gene, rs12979860 (Fig. 15.1), was found to be strongly associated with SVR in adherent European-American, African-American, and Hispanic patient groups infected with HCV genotype 1 (combined $P=1.37\times10^{-28}$) [20]. When individuals were genotyped according to *IL28B* status, those individuals that carried two copies of the C allele for the polymorphism were found to have higher rates of SVR than individuals that have two copies of the T allele (Fig. 15.2). Patients of European-American and Hispanic descents with genotype CC had a twofold increase in SVR compared to patients with a TT genotype. Patients of African-American descent with the CC genotype had threefold higher rates of SVR compared to patients with a TT genotype [20]. When compared to other common predictors of response to treatment, the *IL28B* genotype was the strongest baseline predictor of SVR with odds ratio (OR) of 7.3 in European-American patients, 6.1 in African-American patients, and 5.6 in Hispanic patients. In the multivariate analysis, low viral load and less fibrosis remained predictors of SVR. Despite the finding of the higher response rate in African-Americans with the CC genotype, race remained a predictor of treatment response. The C allele for *IL28B* is more prevalent in European-American patients than in African-American patients, and this explains approximately half of the difference in treatment response rates between African-American and European-American

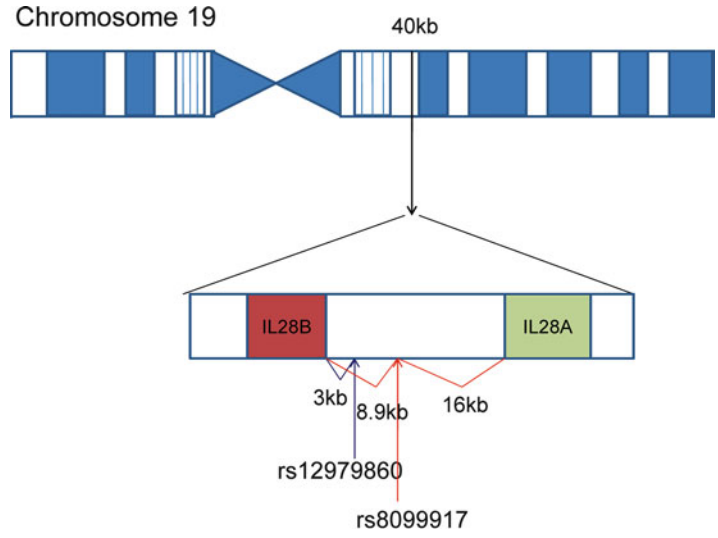

Fig. 15.1 Ideogram of chromosome 19. The rs12979860 and rs8099917 SNPs are located near the gene for *IL28B*

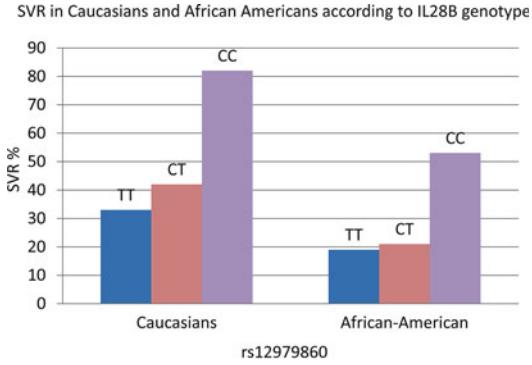

Fig. 15.2 Results from the Ge et al. study describing *IL28B* genotype and achievement of SVR

Table 15.2 C allele frequencies for various populations throughout the world

Ethnicity	Rs12979860 C allele frequency
Cambodian	97.9
Korean	93.5
Chinese	93.6
Japanese	91
Irish	73.9
European-American	67.4
Indian	65.5
Russian	64.1
Mexican	55.5
Ethiopian Jews	54.8
Masai	40.0

patients [20]. Additionally, the frequency of the C allele varies throughout the world and in general, Asian countries have the highest frequency followed in sequence by European countries, North American countries, and African countries (Table 15.2) [21].

In another GWAS that included patients of European ancestry, the SNP rs8099917 in the intergenic region between *IL28A* and *IL28B* (Fig. 15.1) had the strongest signal associated with SVR. This SNP was located 8.9 kb downstream from *IL28B* and 16 kb upstream of *IL28A*. Individuals with a G allele for this polymorphism have higher rates of non-response to treatment than those with two copies for the T allele. In multivariate analysis, rs8099917 was an independent predictor of response to treatment without evidence of confounding from the other parameters [22]. This study did not take non-adherence to treatment into account.

A third GWAS of adherent Japanese individuals found strong associations between non-response to treatment and two SNPs located on chromosome 19 – rs12980275 and rs8099917. In patients that did not achieve a virologic response, the nonresponder allele for SNP rs12980275 was

present at much higher frequency than in patients that did achieve a virologic response (74.3 vs. 12%). Similarly, the frequency of the nonresponder allele for SNP rs8099917 (G allele) was 75.5% in patients that did not achieve virologic response vs. 9.4% in those that did achieve virologic response. In a logistic regression model to predict non-response at week 12, rs8099917 had the most significant effect with an OR 37.68 [23].

Each of these studies identified SNPs that were associated with treatment outcome at the genome-wide level, and each study identified SNPs in close proximity to the *IL28B* gene. The report of different SNPs from these studies is likely explained by a number of factors. The rs12979860 SNP was not present on the chips used in all of the studies. In addition, only the IDEAL study analysis included African-American patients. The GWAS approach provides clues that an area of the genome may be relevant to the outcome under investigation but does not necessarily explain the mechanism. IL28B is a type III interferon and a member of the interferon lambda family that includes IL-28A and IL-29. Type III interferons have similar structure and function to the type I interferons, including interferon alpha. IFN-lambdas have been shown in vivo and in vitro to have antiviral activity against HCV genotype 1 and a phase 1 trial of IFN lambda in patients with HCV has shown anti-viral activity [24–26]. These studies support the relevance of this region of the genome to HCV treatment outcomes, but the mechanism behind the variation in treatment response is unknown at this time and is under investigation.

Genetic Factors and Patients Infected with HCV Genotypes 2 and 3

Patients infected with HCV genotypes 2 and 3 have higher rates of achieving SVR than patients infected with other HCV genotypes. Approximately 80% of patients infected with genotypes 2 and 3 will achieve an SVR with standard pegylated interferon alpha and ribavirin therapy, and can be treated with a shorter course of therapy (24 weeks vs. standard 48 weeks) [27].

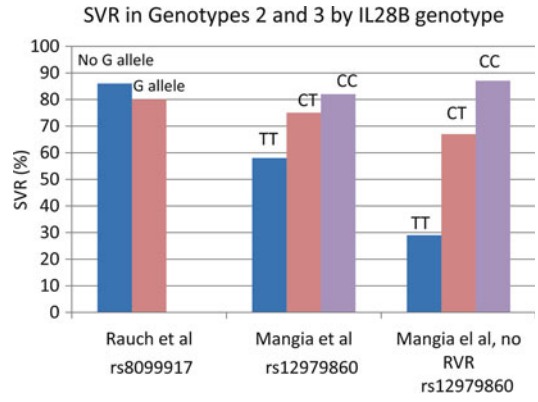

Fig. 15.3 SVR rates in patients with genotypes 2 and 3. The only significant difference is seen in the patients that do not achieve RVR. Patients with the CC genotype have the highest rates of subsequent SVR

A recent study evaluated the impact of *IL28B* on treatment response in patients infected with genotypes 2 and 3 (Fig. 15.3). The study was a retrospective evaluation of patients from a randomized controlled trial of 12 or 24 weeks of treatment. *IL28B* genotype was not associated with RVR or SVR in patients with genotypes 2 and 3 as it is in patients with genotype 1. However, in the subset of patients with genotypes 2 and 3 who did not achieve an RVR, *IL28B* genotype was strongly associated with the ability to achieve an SVR (SVR: 87 vs. 67% vs. 29% for CC vs. CT vs. TT; OR, 4.0; 95% CI, 1.9–8.5; $P = 0.0002$) [28].

Further studies are required to determine whether identification of *IL28B* genotype in patients that do not achieve an RVR can accurately guide the duration of therapy. Studies are needed to see if patients with the unfavorable genotype (TT) benefit from longer duration of treatment.

The Association of Genetics and Viral Kinetics

IL28B

Historically, lower baseline viral loads have been associated with improved response to treatment. Interestingly, the C allele for SNP rs12979860, which was related to better treatment response, was associated with higher baseline viral loads in

the IDEAL cohort. Using a value of 600,000 IU/mL as the cutoff point between determining high and low viral loads in the IDEAL cohort, there was not a relationship between the *IL28B* polymorphism and values above or below this level, suggesting that the association of the polymorphism with clearance and viral load may be independent [20].

The "responder" *IL28B* genotype was strongly associated with viral clearance after 4 weeks of treatment (RVR, rapid virologic response), after 12 weeks of treatment, and with lower rates of relapse post treatment in the IDEAL cohort. Homozygous CC genotype patients experienced enhanced viral clearance as early as 2 weeks into treatment with a median reduction in viral load of 2-$\log_{10}$IU/mL greater in than non-CC patients [29]. When on-treatment testing is considered, decline in HCV viral burden is the strongest predictor of eventual treatment success. Interestingly, combining pre- and on-treatment predictors revealed that the responder CC genotype predicts eventual SVR in that group of patients who failed to achieve RVR (in Caucasians, 66% SVR rate in non-RVR CC compared to 31% SVR (CT) and 24%(TT) non-RVR patients) [29].

The relationship between *IL28B* genotype and RVR was also determined in a cohort of 682 patients with HCV. This cohort contained 371 patients with genotype 1, 208 patients with genotypes 2/3, and 102 patients with genotype 4. The responder *IL28B* polymorphisms (either CC genotype rs12979860 or TT genotype rs8099917) were associated with reduction in HCV viremia as early as 24 h after the first dose of peginterferon alpha and ribavirin with 1.28 ± 0.49 vs. 0.77 ± 0.49 $\log_{10}$IU/mL reduction in patients infected with HCV genotype 1 and 1.60 ± 0.59 vs. 0.77 ± 0.55 log IU/mL in patients with genotype 4 (both with $P < 0.001$). Higher rates of RVR were seen in patients with the CC genotype at 38.3 vs. 11.6% for non-CC genotype in patients infected with HCV genotype 1 and 76.5 vs. 23.5% in patients with HCV genotype 4. Patients infected with genotypes 2 and 3 had more frequent RVR in carriers of rs12979860 CC genotype (75.3 vs. 52.6%) but SVR rates were similar between patients with CC genotype and patients with a T allele. The results were similar when rs8099917 was analyzed [18].

Human Major Histocompatibility Complex

In the VIRAHEP-C cohort, 373 patients were tested for major histocompatibility complex (MHC) allele carriage and their effects on viral decline at week 4 [30]. The rate of viral decline was higher for noncarriers of DQA1*04 than carriers and the magnitude of the rate of decline depended on race. African-Americans carriers had 1.19 $\log_{10}$IU/mL viral decline compared to 1.51 $\log_{10}$IU/mL viral decline for noncarriers. Caucasian American carriers had 0.98 $\log_{10}$IU/mL viral decline compared to 2.66 $\log_{10}$IU/mL decrease for noncarriers. A similar effect was seen for the DQB1*0402 and A*03 alleles, with Caucasian American noncarriers having the fasted decline in viral load. For DQB1*0402, Caucasian American noncarriers had 2.65 $\log_{10}$IU/mL and carriers 0.94 $\log_{10}$IU/mL viral decline and African-Americans noncarriers had 1.49 and carriers had 1.21 $\log_{10}$IU/mL viral load decline. For A *03, Caucasian American noncarriers had 2.75 $\log_{10}$IU/mL, carriers 2.10 $\log_{10}$IU/mL and African-American noncarriers had 1.39 $\log_{10}$IU/mL and carriers 1.65 $\log_{10}$IU/mL viral load declines at week 4. Caucasian American carriers of Cw*03 had higher rates of viral decline than noncarriers (2.99 vs. 1.29 $\log_{10}$IU/mL), but African-American noncarriers of Cw*03 had higher rates of viral decline than carriers (1.51 vs. 1.19 $\log_{10}$IU/mL).

Genetics and HCV Clearance After Acute Infection

IL28B

IL28B genotype has also been associated with the spontaneous clearance of HCV. In a large candidate gene study, the C allele for SNP rs12979860 was present in significantly more patients in the clearance group than in the persistence group (80.3 vs. 66.7% $P = 7 \times 10^{-8}$) in patients of European ancestry. In patients of African ancestry, the C allele was present in 56.2% of the patients

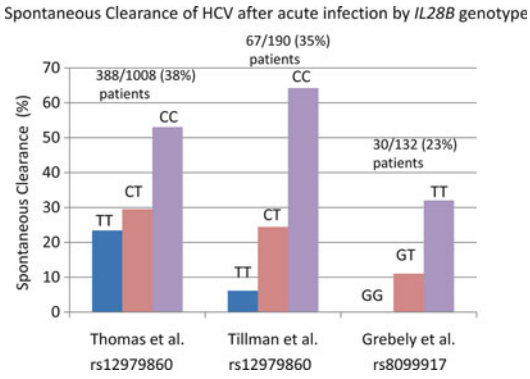

Fig. 15.4 Percentage of spontaneous clearance of HCV virus after acute infection according to *IL28B* genotype

that cleared the virus vs. 37% that did not clear the virus. Patients with the CC genotype were three times more likely to clear the HCV virus relative to patients with the CT and TT genotypes (Fig. 15.4) [21]. In this study, approximately 20% of the patients were co-infected with HIV.

A second large cohort of patients, including 914 HCV monoinfected patients and 448 HCV/HIV co-infected patients found the SNP rs8099917 to be associated with chronic hepatitis C infection with genome-wide significance ($P = 6.07 \times 10^{-9}$). The minor G allele, associated with poor treatment response, was associated with risk of chronicity. *IL28B* was the only region associated with clearance when employing a genome-wide approach [16].

In an analysis of patients from the German anti-D cohort, a group of female patients exposed to the same strain of HCV (genotype 1b) during the 1970s, the CC genotype for rs12979860 was again found to be associated with spontaneous clearance of HCV after acute infection. In this cohort, patients had 64, 24%, and approximately 6% chance of spontaneously clearing the virus with CC, TT, and CT genotypes, respectively (Fig. 15.4). Additionally, patients with a C allele were more likely to experience jaundice during acute HCV infection compared to the other genotypes suggesting that a more robust immune response may be present in patients with one or two C alleles [31].

Most recently, Grebely et al. assessed the role of host genotype in a cohort of patients of injecting drug users acutely infected with HCV [32]. Before inclusion of host genotype, jaundice was associated with clearance in this cohort. In 79 of the 132 patients with available genotyping data, *IL28B* good responder genotype (TT rs8099917) was associated with spontaneous clearance (Fig. 15.4). Patients with good responder genotypes were more likely to have a seroconversion-like illness with jaundice (TT vs. non-TT 32 vs. 5%, $P = 0.047$). Host *IL28B* genotype was the only factor significantly associated with time to spontaneous clearance in multivariable Cox proportional hazards analysis after adjustment for gender and acute seroconversion illness with jaundice (adjusted hazard ratio = 3.78, 95% CI, 1.04–13.76, $P = 0.044$). This study also included patients who were offered HCV treatment with pegylated interferon alpha ±ribavirin ($n = 111$). A higher percentage of patients with homozygous good responder *IL28B* variant had RVR than other *IL28B* variants (35 vs. 57%, $P = 0.16$). While no relationship with SVR was observed, overall numbers were small with poor rates of treatment adherence ($n = 54$).

Polymorphisms in Genes Associated with Steatosis and Insulin Resistance

Steatosis and insulin resistance are also known to have effects on HCV treatment response. In another study of 351 patients from the VIRAHEP-C study, a number of genes were tested for association with steatosis or insulin resistance in patients infected with HCV genotype-1 [33]. The genetic variants studied were in collagen type-1 alpha-1, cytochrome P450 2E1, interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-1 receptor type-1, leptin receptor (LEPR), chemokine (C-C motif) ligand 2, chemokine (C-C motif) ligand 8, tumor necrosis factor-alpha and transforming growth factor beta-1 (TGF-β1)

Differences in genetic associations with steatosis and insulin resistance have been reported in Caucasians and African-Americans. Caucasian Americans had higher odds of steatosis if they possessed the interleukin-6 *rs2069845* AG or GG genotype compared to the AA genotype (OR 2.5, 95% CI 1.1–6.0). African-American patients had 4.4 times higher odds of steatosis if they possessed

the *TGF-B1 rs2278422*-GG genotype vs. the CC or CG genotype. Also, in African-Americans with a 1 unit higher HOMA2-IR score there was a higher rate of steatosis in patients with the TGF-β1rs2241716-GG genotype than in patients with AA or AG genotype (OR 3.3, 95% CI 1.6–6.9).

In both Caucasian American and African-American patients, there was an association with IL-10 rs3024496-CT or TT genotypes and steatosis with 1 unit higher HOMA2-IR scores than patients with CC genotype. In Caucasian Americans the OR was 7.7, 95% CI 2.3–25.4 for the CT genotype and 9.3, 95% CI 1.5–59.2 for the TT genotype compared to OR of 1.5, 95% CI 0.5–4.2 for the CC genotype. In African-Americans the OR was 3.4, 95% CI 1.3–8.8 for the CT genotype compared to 0.3, 95% CI 0.1–1.3 for the CC genotype. There was also an association with LEPR rs1892534-AG genotype and steatosis in both African-American and Caucasian American patients, the African-American group had triple the odds of having steatosis and Caucasian American patients had 29 times the odds of having steatosis compared to patients with AA genotype.

Racial differences in the likelihood of insulin resistance also exist. African-American patients with the IL-10 rs3024496-CT genotype or the rs1800890-TT genotype had lower odds of insulin resistance compared to rs3024496-CC genotype and rs1800890-AA or AT genotype, respectively. African-American patients with TGF-β1 rs2278422 GG genotype had lower odds of insulin resistance than those with CC or CG genotype. Caucasian Americans with the IL-6 rs1880242 TT genotype had 0.3 times lower odds of insulin resistance compared to GG or GT genotype.

Clinical Applications of Genetic Testing

Currently clinicians weigh a variety of factors when determining which patients with hepatitis C should be treated and when during the course of their infection this should occur. At this point, it is clear that *IL28B* genotype status is related to treatment response in patients infected with HCV genotype 1. There is also an increasing body of evidence to define the relationship between *IL28B* genotype in viral kinetics in all genotypes of HCV infection. A clinical diagnostic is currently available for determining IL28B genotype and may be used to help informed clinicians determine when to initiate treatment. Testing for *IL28B* genotype can help determine those patients that are most likely to achieve an SVR and thus should be considered favorable candidates for treatment. Similarly, patients that have an IL28B genotype that is not favorable for treatment response combined with known poor predictors for treatment response (African-American race, high viral loads, increased degrees of fibrosis) may be counseled of their poor chance of viral clearance and in the absence of any urgent indication for therapy may opt to wait for more effective treatments in development. At the time of the release of the commercially available *IL28B* test in 2010, the impact of the test on clinical practice was unclear. Many patients were already deferring treatment due to the anticipated approval of the HCV protease inhibitors boceprevir and telaprevir in 2011 [34, 35]. The influence of the *IL28B* genotype on treatment outcomes with combination therapy and protease inhibitors will be important to understand and is under investigation.

In patients with acute HCV infection, there remains conjecture regarding the timing and nature of treatment for HCV infection [27]. For acute infection, early treatment after a short period of observation (e.g., 12 weeks) is generally accepted [27, 31]. The knowledge that certain patients are less likely to clear acute HCV infection prompts the question of whether these patients (i.e., with unfavorable *IL28B* genotype) might benefit from early initiation of therapy in acute infection to optimize clearance. In contrast, patients with the "good responder" genotype might be observed for clearance, in the knowledge that the minority who fail to clear should respond well to salvage pegylated interferon alpha and ribavirin therapy.

Ultimately, determination of IL28B genotype in combination with on-treatment factors such as achievement of RVR may help to determine an appropriate length of therapy. Further work is

ongoing to determine how to best incorporate these factors with other known predictors of response to individualize treatment regimens and durations for patients.

Conclusions

HCV treatment is time-consuming, difficult to tolerate, and only successful in approximately 50% of patients. Novel work has been summarized in this chapter and includes both determinations of viral genome factors and patient genetic factors to assess the likelihood that a patient will respond to treatment. In the near future we may be able to sequence the infecting virus as well as the patient's genome to determine the likelihood of treatment response and to assess treatment duration.

More research is necessary to incorporate all these findings together to understand their use in clinical medicine. Further work is also ongoing to identify patients that are more likely to develop treatment-limiting adverse events and side effects which will also have important implications in clinical care [36]. Continued study of the biologic pathways and viral–cell interactions is also crucial for better understanding of the disease and to develop new treatment regimens.

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Maintenance Therapy with Peginterferon

16

Raza Malik and Nezam H. Afdhal

Keywords
Cirrhosis • Hepatocellular carcinoma • Liver failure • Varices

Introduction

After the discovery of the HCV virus in 1989, epidemiological studies indicated that 4.1 million Americans are infected with the virus [1]. A chronic hepatitis occurs in 80% of individuals infected, with progression to cirrhosis in up to 30%, giving rise to a large population of patients with advanced liver disease. Individuals who develop cirrhosis are at high risk of decompensation with liver failure and liver cancer making this an important condition that requires appropriate treatment [2]. The primary goal of therapy in patients with chronic hepatitis C infection should be aimed at achieving a sustained virological response, rendering the patient HCV RNA negative to prevent the future development of cirrhosis and liver cancer [3]. In patients that present with well-compensated cirrhosis, successful anti-viral therapy can prevent future decompensation and liver failure. There have been significant advances in the treatment of chronic hepatitis C infection in the last decade, with a threefold increase in SVR. Initial SVR rates with interferon monotherapy were 6–12%, increasing to 42% with interferon and ribavirin and finally to 54–56% with PEG-IFN and ribavirin [4]. However, despite better response rates with modern anti-viral therapies there are still a significant number of patients failing treatment (approx 50%). A number of viral and host factors have been associated with treatment failure, with the viral genotype being the most important. Genotypes 2 and 3 have excellent SVR rates of 76–83%, whilst genotype 1 patients are between 42–46%. Low baseline HCV RNA levels (<800,000 IU/mL) and female sex also predict a favorable SVR rate amongst those with chronic HCV. Advanced fibrosis/cirrhosis, African American race, and obesity predict a poor response to PEG-IFN and ribavarin. In the USA, genotype 1 is the predominant hepatitis C genotype, which has resulted in a large cohort of patients who have failed PEG-IFN and ribavirin treatment [5, 6]. Demographic factors, such as the growing obesity epidemic, are also playing an increasing role in HCV progression to cirrhosis.

R. Malik, MD, PhD • N.H. Afdhal, MD (✉)
Department of Medicine, Liver Center, Beth Israel Deaconess Medical Center, Harvard Medical School, 110 Francis Street, Boston, MA 02215, USA
e-mail: nafdhal@BIDMC.harvard.edu

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Evaluation of Treatment Failures

In patients who failed treated with interferon monotherapy or standard interferon and ribavirin, re-treatment with PEG-IFN and ribavarin is associated with SVR rates of 30 and 10%, respectively, therefore consideration should be given to re-treatment. Patients whose initial course of antiviral therapy with PEG-IFN and ribavirin was characterized by poor adherence or major dose reductions (<80% total interferon regimen) could be considered for another full treatment course [7].

What is Maintenance Therapy?

Maintenance therapy focuses upon alternate clinical endpoints such as improvement in liver inflammation and liver fibrosis, either prevention or delay in the development of cirrhosis, hepatic decompensation ,and hepatocellular carcinoma.

As part of maintenance therapy, good liver health is essential. All patients should be advised to abstain from alcohol and maintain a healthy body mass index to avoid the potent co-factors of alcohol and NASH. In addition, avoidance of hepatotoxic medications, including some alternative and or herbal medications should be encouraged [8, 9].

Current options for maintenance therapy includes [10–13]:

- Low-dose long-term PEG-IFN
- Ribavarin
- Anti-fibrotics
- Iron depletion

This chapter will focus on maintenance therapy with Peginterferon as this is the most established to date.

Candidates for Maintenance Therapy

Patients with significant fibrosis/compensated cirrhosis (Metavir Stage 3/4 or Ishak 4-6) who failed standard anti-viral therapies are felt to be the optimal candidates for maintenance therapies. The annual estimated risk of decompensation with established cirrhosis is 3% and the risk of hepatocellular carcinoma is approximately 3–5% [14, 15]. In view of this significant risk of complications, the option to just observe is not appealing to either the physician or the patient alike.

Maintenance Therapy with Interferon

Interferon has a wide variety of anti-viral and immune-modulating functions making it an obvious candidate for maintenance therapy. Originally, a small pilot study was conducted in 1999 in HCV nonresponders to determine if histological progression could be halted [16]. This randomized controlled trial was conducted in 53 patients who received 6 months of interferon alpha-2b therapy, in whom HCV RNA remained positive but a histological response was seen. Twenty-seven patients were assigned to continue standard interferon (3 MU three times a week) for 24 months and 26 patients discontinued treatment and were observed with the two groups well matched demographically. In patients receiving maintenance interferon there was a significant improvement in serum ALT level, log HCV-RNA titer, and hepatic inflammation. In fact, after 30 months of treatment, there was a mean reduction in fibrosis score (from 2.5 to 1.7) with 80% of patients showing histological improvement. Further evidence of benefit with maintenance interferon was seen in patients in whom therapy was withdrawn, where discontinuation of interferon was associated with an increase in fibrosis score and histological progression (30%).

These findings led to three major clinical trials investigating the use of maintenance PEG-IFN to prevent the progression in chronic hepatitis C infection (see Table 16.1).

HALT-C: Hepatitis C Anti-Viral Long-Term Treatment Against Cirrhosis

This study was an NIH (National Institutes of Health) sponsored study that recruited 1,145 patients from ten different sites into a lead in phase. The patients (standard interferon and ribavirin

Table 16.1 Maintenance therapy trials (Adapted from Di Bisceglie et al. [17], Lok et al. [18], Afdhal et al. [19])

	HALT-C	COPILOT	EPIC3
Patient selection	Ishak 4–6	Ishak 3–6	Metavir 2–4
Liver disease	CTP <6	CTP <7	CTP <6
Total number	1,400	555	1,700
Maintenance number	1,050	555	616
Maintenance therapy	PEG Alfa 2a	PEG Alfa 2b	PEG Alfa 2b
Dose	90 mcg	0.5 μg/kg	0.5 μg/kg
Arms	Placebo	Colchicine	Placebo
Run in period	Yes (24 weeks)	No	Yes (12 weeks)
Duration (years)	3.5	4	3–5 years
Clinical outcomes			
(PEG/CON %)	21 vs. 24%	17 vs. 24%	21 vs. 14%
Histology			
Fibrosis	No Difference	N/A	No difference
Inflammation	Improved	N/A	Improved
Portal HTN	No difference	Improved ($p<0.02$)	Improved ($p<0.02$)

non-responders) with chronic HCV and advanced fibrosis (Ishak stage 4–6) were randomized to a combination of pegylated interferon (Pegasys®) 180 μg/week and ribavirin (1,000–1,200 mg/day) for 24 weeks. There were 662 non-responders, and 151 relapsers from the lead in phase put forward into the maintenance study. In addition, an external express cohort with the same inclusion/exclusion criteria and persistent HCV infection were inserted to make a total of 1,050 patients (428 cirrhotic, 622 noncirrhotic) for the maintenance study. A total of 517 patients were randomized to pegylated interferon (Pegasys®) 90 μg/week and 533 patients to an untreated control observation group. The majority of the patients in the study were genotype 1 (>90%), with high viral load (>log 6) and were stratified equally for baseline characteristics. The patients were followed with clinical, serum, radiological assessment (at 3–6-month intervals) and liver biopsy at 1.5 and 3.5 years for histological assessment. The primary outcomes were important clinical (evidence of hepatic decompensation as shown by (1) increase in CPT score to 7 or higher, (2) variceal hemorrhage, (3) ascites, (4) spontaneous bacterial peritonitis, (5) hepatic encephalopathy, (6) HCC development, and (7) death) and histological parameters (development of cirrhosis on liver biopsy with progression of Ishak fibrosis score by 2 points or more). Secondary outcomes included quality of life, serious adverse events, events requiring dose reductions, and development of presumed hepatocellular carcinoma. The trial lasted 4 years including the 6-month lead in phase [17].

In this study maintenance therapy with PEG-IFN did not result in a reduction in clinical events or histological improvement. However, there was an improvement in viral load, serum ALT, and necroinflammatory scores in the group treated with PEG-IFN alpha-2a. More recently a report from Lok et al looked at the longer-term effects of PEG-IFN maintenance in HALT-C patients with respect to the late development of HCC [18]. Overall 88 patients developed HCC, 37 in the PEG-IFN arm and 51 of 533 controls. There was a significantly lower rate of HCC development in the PEG-IFN patients at 7 years in those patients with cirrhosis at baseline with a hazard ratio of 0.45 (95% CI 0.24–0.83), which was most evident in patients who had a 2-point reduction in inflammation. This study illustrates two of the shortfalls of all the maintenance trials: the arbitrary time to stopping treatment and the inability to define on treatment success either by inflammatory or viral endpoints.

Colchicine Vs. Peg-Intron Long-Term Trial

This study recruited 555 patients with chronic hepatitis C from 40 sites in the United States (supported by Schering Plough Corp.). It compared weight-based low-dose peginterferon alfa-2b (subcutaneous injection of 0.5 μg/kg/week, one-third the dose used in standard HCV combination therapy) vs. colchicine (0.6 mg orally, twice daily), a potential anti-fibrotic medication, in 555 chronic hepatitis C patients with advanced liver fibrosis (Ishak 3-6) who previously failed interferon-based therapies [19]. It was a 4-year study with similar baseline characteristics in the two study arms. Over the 4 years of the randomized study, investigators monitored the patients to determine how many reached a primary endpoint, defined as death, liver transplant, hepatocellular carcinoma, variceal bleeding, or liver failure (increase in Child-Pugh-Turcotte (CPT) by 2 points with ascites, jaundice, or encephalopathy). They analyzed their findings for all 555 patients, who received at least one dose of their assigned drug, in two ways: based on all events that occurred during the entire four years of the study, regardless of whether a patient was still taking their assigned drug or not (the "intent-to-treat" or ITT analysis), and based on only the events that occurred while patients were taking their assigned drug (the "on drug" analysis). Although the final data of this study have never been published in a peer reviewed manuscript, the investigators did present the final data at the European Association for the Study of Liver Diseases (EASL) meeting in Milan, Italy, 2008 [19]. A primary endpoint was reached by 17.8% (51/286) of patients in the peginterferon alfa-2b group vs. 20.4% (55/269) in the colchicine group in the ITT analysis, and by 12.2% (35/286) and 16.0% (43/269) patients, respectively, in the on-drug analysis (treatment differences were not statistically significant). Among patients who had portal hypertension (42.3 and 48.0% of patients in the peginterferon alfa-2b and colchicine groups, respectively), peginterferon alfa-2b therapy resulted in significantly improved event-free survival in both the ITT and on-drug analyses (Wilcoxon $p = 0.041$ and 0.028, respectively). Further, variceal bleeding, a complication of portal hypertension, was almost abolished with peginterferon alfa-2b in both the ITT (10 vs. 1 patients) and the on-drug (10 vs. 0 patients) analyses. In the ITT analysis, hepatocellular carcinoma occurred in 7.7 and 5.9% of patients in the peginterferon alfa-2b and colchicine groups, respectively, a non-significant difference. A total of 49% of patients discontinued their medication before the end of the 4-year study, with 36% due to failure to comply and 13% due to side effects.

This study indicates that maintenance PEG-IFN alfa-2b may be effective in HCV cirrhotic patients with portal hypertension as it improves the event-free survival in this sub-group of patients. Publication of the final study results are awaited.

EPIC3

This was the largest study conducted to date to evaluate the re-treatment of patients with chronic hepatitis C infection, with advanced fibrosis, who had failed at least 12 weeks of interferon-based therapy previously [20]. Initially, this prospective, multicenter, open-label study evaluated the efficacy and safety of peginterferon alfa-2b (1.5 μg/kg/week) plus weight-based ribavirin (800–1,400 mg/day) in 2,333 chronic HCV-infected patients with significant fibrosis/cirrhosis. A total of 497 patients (22%) achieved an SVR, with response rates better in patients previously treated with standard interferon/ribavirin compared to PEG-IFN/ribavirin (25 vs. 17%) and also in relapsers compared to non-responders (38 to 14%).

Patients who did not respond to re-treatment by week 12 were offered entry into the maintenance interferon study arm, which assessed the histological benefits of low-dose (0.5 μg/kg) and long-term (3–5years) peginterferon alfa-2b treatment in patients with F2/F3 metavir fibrosis score. The majority of patients had genotype 1 (92%) and high viral loads (70% viral load >600,000 IU/L) and were stratified equally for baseline characteristics.

The investigators presented the maintenance data (up to 3 years) at the EASL meeting in Vienna, Austria, 2008. In the primary analysis, the maintenance PEG-IFN alfa-2b group ($n=270$) showed no difference with the control observation group ($n=270$) in fibrosis response. However, in sub-group analysis in patients treated for >2.5 years there was a trend towards improvement (21 vs. 14%). A large group of patients (35% – 192/540) had no post-maintenance biopsy (PEG-IFN alfa-2b, $n=88$; observation, $n=104$) and were hence classed as no change making the interpretation of these results difficult.

There was a significant difference in inflammatory score (≥1 point improvement in metavir activity score) between the two groups, with the maintenance PEG-IFN alfa-2b group having a significant improvement in inflammatory activity compared to the control group (20 vs. 9%).

The mean duration of treatment was 2.3 years among patients receiving PEG-IFN alfa-2b and 2.4 years among the control group. In a similar fashion to COPILOT, there was evidence of a benefit in the subgroup with portal hypertension ($p<0.02$). In the 348 patients with pre-retreatment and end-of-treatment liver biopsies, the mean duration between the biopsies was 3.6 years in the PEG-IFN alfa-2b group and 3.9 years in the control group. There were major adverse events in 20% (53/270) of patients in the PEG-IFN alfa-2b group and 11% (31/270) of patients in the control observed group. The final publication of this and the 5-year data are awaited.

This study suggests that maintenance PEG-IFN alfa-2b improves inflammatory activity but does not improve fibrosis or prevent progression. However, as there were large numbers of patients that did not get a post maintenance liver biopsy, which likely affected the analysis; the results from the 5-year analysis will provide more definitive information on improvement in fibrosis.

In conclusion, when we evaluate the three large trials we are finally left with some controversy based on the study designs and endpoints. We can certainly conclude that maintenance peginterferon therapy had no overall effect on fibrosis progression or regression but there was a clear improvement in necro-inflammation. Whether with a longer duration this would effect fibrosis is unclear but we can conclude that this did not occur with 4 years of treatment. The overall results on clinical outcomes were also not favorable for either of the peginterferon studies, although peginterferon maintenance therapy showed a distinct benefit in the subgroup with portal hypertension in both COPILOT and EPIC. Since IFN has been shown to reduce portal pressure, there is both a mechanism for this effect and a potential rationale to use this relatively well-tolerated treatment in patients with cirrhosis and portal hypertension.

Discussion

Despite considerable progress in treatment strategies for patients with chronic HCV in the past decade, 50% of patients still fail to respond to IFN-based treatment. All non-responders need to be carefully assessed to ensure that the current gold standard of PEG-IFN and ribavarin at appropriate dose and duration have been administered. In patients who failed an adequate course of anti-viral therapy with PEG-IFN and ribavirin, an estimate of the underlying liver disease is required as this is the clinically relevant endpoint. Individuals with mild disease both in terms of inflammation and fibrosis can be managed conservatively as the risk of progression to cirrhosis and liver cancer in the medium to long term is low. These patients can be advised to follow a course of conservative management or opt for retreatment with a combination of a DAA, peginterferon and ribavirin. Non-responders to retreatment with DAA triple therapy can be conservatively managed until the next advance in this field emerges. Individuals with a moderate degree of liver fibrosis will require close monitoring with non-invasive surrogate markers of liver inflammation and fibrosis (serum ALT, serum biomarkers, Transient Elastography) and/or liver biopsy to establish their risk of disease progression and to determine if they fall into the high-risk category. Patients with severe liver fibrosis who failed an adequate course of anti-viral therapy with PEG-IFN and ribavirin are at

risk of disease progression to cirrhosis, hepatic decompensation, and hepatocellular carcinoma. These patients are good candidates for maintenance therapy. There is no unequivocal evidence to date that maintenance therapy with PEG-IFN improves liver fibrosis and prevents progression in liver disease in patients with chronic hepatitis C infection. However, both the HALT-C and EPIC studies showed an improvement in inflammation which may benefit a sub-set of patients. In addition, the Colchicine vs. Peg-Intron Long-Term Trial (CO-PILOT) and EPIC studies indicate that a sub group of patients with established portal hypertension may benefit, with an improvement in event-free survival related to improvement in portal hypertension. Maintenance therapy does make sense if patients can be maintained HCV RNA undetectable long term; although no significant reduction in outcomes was demonstrated in these patients in any of the maintenance therapy studies.

In terms of the management of chronic hepatitis C treatment failures, there is an additional variable that needs to be considered. This is the prospect of small molecule direct-acting anti-virals (e.g., protease inhibitors – telaprevir and boceprevir) available for use in cirrhotic patients and how they will affect management strategies. Despite the fact that these agents will be initially licensed for the treatment of genotype 1 treatment naïve patients, there will be a major debate on their future use in patients who are treatment failures and which combinations of regimens are to be used.

Side Effects

The treatment of cirrhosis with IFN and RBV is challenging due to the side effect profile seen in these patients. In the cirrhosis studies, both compensated and decompensated, the discontinuation rates can reach above 40%. All the expected side effects of therapy are seen in cirrhotic patients but in particular the hematological side effects can be much more severe in this population [21, 22].

Management of Thrombocytopenia

Thrombocytopenia has been reported in up to 70% of patients with cirrhosis [23]. In addition, thrombocytopenia is a common side effect of interferon alpha treatment and thrombocytopenia can lead to difficulty with maintenance of full-dose interferon [24]. Thrombocytopenia in cirrhosis is due to a combination of hypersplenism and underproduction of thrombopoeitin. Thrombopoeitin is a growth factor which is produced in the liver and is involved in proliferation of megakaryocytes and platelet production. Thrombocytopenia can prevent patients from starting IFN therapy and most trials exclude patients with patient counts <80,000/mL and so the SVR rates in these patients is unknown. In addition, discontinuation and dose reduction for thrombocytopenia is common in HCV cirrhosis trials.

There are a number of therapeutic approaches under development for the management of thrombocytopenia including: eltrombopag, a thrombopoeitin receptor agonist, recombinant human IL-11, and various thrombopoeitin mimetics. Eltrombopag is currently being tested in phase III studies in over 1,000 patients with cirrhosis (ENABLE TRIALS) to examine whether it will allow initiation and maintenance of treatment with interferon alpha in patients with cirrhosis and HCV. A recent randomized phase II study of eltrombopag in patients with HCV showed that 4 weeks of therapy led to platelet counts of >100,000/μL in up to 95% of patients vs. 0% in the placebo group ($p < 0.001$) [25] . In addition, continuing eltrombopag for 12 weeks allowed significantly more patients to complete 12 weeks of anti-viral treatment (up to 65%, compared to 6% in the placebo group).

Management of Neutropenia

Cirrhotic patients often have low total white cell counts secondary to hypersplenism and are very susceptible to PEG-IFN induced neutropenia. Antonini reported 73 infections in 23% of 319 subjects treated with PEG-IFN [26]. Infection was independent of type of IFN and neutrophil

count but was associated with age >60 years. However, cirrhotic patients already have increased risk of infections and in one trial in patients awaiting liver transplant there was an association of severe infections including SBP and bacteremia associated with poor liver function, low white cell count, and use of PEG-IFN. We currently recommend the use of filigrastin in patients with cirrhosis on IFN when the neutrophil count falls below 750 cells/mL.

Maintenance Therapy in the Era of Direct Acting Anti-Virals

The introduction of the first DAA treatments is imminent in combination with PEG-IFN and RBV. The goal of these treatments is to increase SVR and SVR still remains the pivotal endpoint of treatment. There is some speculation that long-term viral suppression may be possible with combinations of DAA but this is speculative. The proof of principle that even 24 weeks of viral suppression can lead to an SVR was recently demonstrated using 2 DAA's in interferon treatment failures. At EASL 2011 using a dual DAA regimen of an NS5A inhibitor and a protease inhibitor, Lok et al showed that 4 of 11 treatment failures achieved an SVR [27]. All the other patients that failed to achieve SVR had a virological breakthrough with resistant mutants to both agents. For maintenance therapy this has 2 critical points, firstly that you do not need IFN to cure HCV but equally as important is that failure to suppress is associated with breakthrough resistance. Therefore, if we can suppress effectively long term we should be able to cure HCV and there seems to be little role for an all DAA maintenance regimen since by its very nature it will either cure or fail.

In conclusion, maintenance therapy although full of promise has failed to yield the anticipated results with uniform prevention of disease progression. The only patients in whom I would recommend maintenance therapy are those with cirrhosis and portal hypertension in whom some clinical benefit with reduction in bleeding and also HCC has been shown. The future with DAA promises cure rather than maintenance and this should absolutely remain the goal of future drug development.

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Part III

Anti-Viral Therapy for Chronic HCV

Antiviral Targets in HCV

17

B. Kronenberger and S. Zeuzem

Keywords

HCV life cycle • Targets for direct antiviral therapy • Attachment inhibitors • Entry inhibitors • Translation inhibitors • Post-translational processing inhibitors • Replication and assembly inhibitors • NS3/4A inhibitors • NS5B polymerase inhibitors • NS5A inhibitors • Clinical development of direct antiviral therapy • Interferon-free combination therapy

Introduction

For almost one decade pegylated interferon alfa in combination with ribavirin has been the standard of care (SOC) in the treatment of patients with chronic hepatitis C [1, 2]. Despite numerous attempts to optimize peginterferon alfa/ribavirin therapy more than half of all patients with chronic HCV, genotype 1 cannot be cured with this treatment. The limitations of antiviral therapy with peginterferon alfa/ribavirin may be overcome by direct antiviral agents (DAA) which specifically target hepatitis C viral proteins.

Due to substantial progress in the development of DAA, treatment of chronic hepatitis C is about to enter a new era (Fig. 17.1). The most advanced DAA are directed against the NS3/4A protease or the NS5B polymerase. Inhibition of these targets blocks post-translational processing of the viral polyprotein or inhibition of viral replication, respectively. Many more targets for DAA targeting viral entry, initiation of translation, virus assembly and release have been identified and may be the targets of future agents.

B. Kronenberger, MD • S. Zeuzem, MD (✉)
Department of Internal Medicine 1, Hospital of the Johann Wolfgang Goethe-University, Theodor-Stern-Kai 7, 60590, Frankfurt am Main, Germany
e-mail: Zeuzem@em.uni-frankfurt.de

Development of DAA from a Historical Perspective

Discovery of the HCV Life Cycle

For many years, the understanding of the hepatitis C virus (HCV) life cycle has been hampered by the lack of reliable and efficient cell culture systems. Primary hepatocytes can be infected by serum-derived HCV, however, this system only supports low-level replication which is not sufficient to perform pharmacological studies [3]. In 1999, Lohmann et al. described a subgenomic HCV replicon that replicated at high levels upon transfection into a human hepatoma cell line [4]. The HCV replicon system provided the basis

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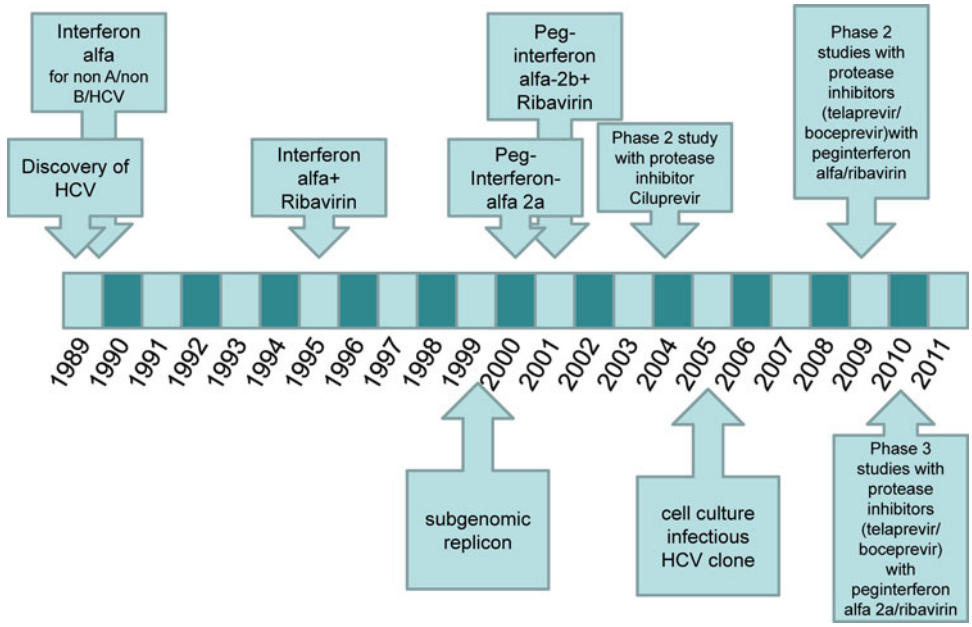

Fig. 17.1 Milestones in the development of HCV therapy

for detailed molecular studies of HCV and the development of antiviral drugs. The major limitation of the replicon system, however, is the lack of the structural proteins which are necessary for attachment, entry, and assembly.

To study attachment and cell entry, recombinant HCV-envelope glycoproteins, HCV-like particles produced by insect cells, and HCV pseudotypes particles consisting of HCV envelope glycoproteins assembled onto retro- or lentiviral core particles were developed [5]. In 2005, Wakita et al. described the isolation of a HCV genotype 2a JFH1 strain from a patient with fulminant hepatitis which replicated efficiently in Huh7 cells without cell culture adaptive mutations and resulted in secretion of viral particles that were infectious for cultured cells and a chimpanzee [6]. The cell culture infectious HCV clone enables the study of the whole HCV replication cycle. The subgenomic replicon and the cell culture infectious HCV are powerful tools for large-scale screening of HCV inhibitors against multiple viral targets.

Structure of the Hepatitis C Virus

Due to the development of in vitro models, tremendous progress has been made in the understanding of the HCV replication cycle since the description of the HCV in 1989. Nevertheless, the structure of HCV still is not completely elucidated. In analogy to other members of the flaviviridae family such as the dengue or tick-bone encephalitis virus, HCV is thought to adopt a classical icosahedral scaffold in which the two envelope proteins E1/E2 are anchored to the host cell-derived double layer lipid envelope. Underneath the membrane is the nucleocapsid composed of multiple copies of the core proteins in complex with the genomic RNA [7, 8].

Life Cycle of the Hepatitis C Virus

Infection starts with adsorption and entry of HCV to the target cell (Fig. 17.2) [8]. Adsorption and entry is the result of a complex interaction

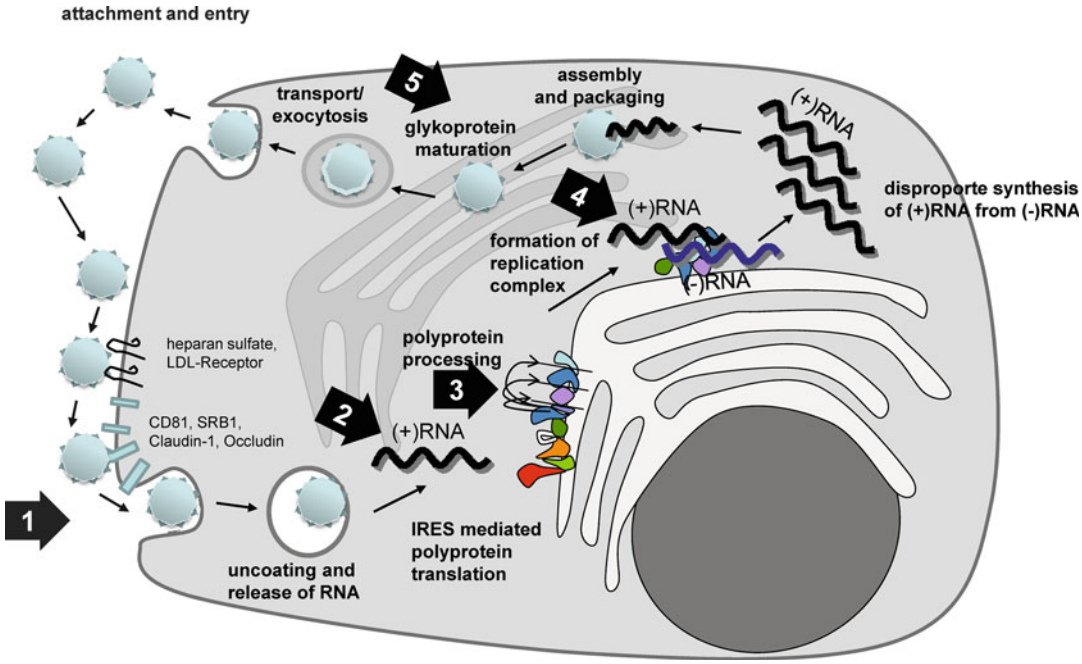

Fig. 17.2 HCV life cycle. The *arrows* indicate targets of antiviral therapy. (1) Entry inhibitor, (2) translation inhibitor, (3) post-translation processing inhibitors, (4) replication inhibitors, (5) assembly and release inhibitors (based on data from Moradpour et al. [8] and Pawlotsky et al. [82])

between the envelope proteins E1/E2 and a couple of cellular receptors [9]. After cell entry the envelope proteins fuse with the membrane of the endoplasmic reticulum and release the HCV RNA into the cytoplasm. The HCV genome, a positive-sense 9.6 kb RNA molecule, encodes for a polyprotein of approximately 3,100 amino acids. Translation of the HCV polyprotein is initiated by an internal ribosome entry site (IRES) located in the 5′ non-translated region (NTR) of the HCV genome. Host- and virally encoded proteases process the polyprotein into non-structural (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) proteins, which are required for HCV RNA translation and replication, and structural proteins (core, envelope E1, E2, and p7) (Fig. 17.3).

Attachment and Entry

Viral Determinants

The envelope proteins E1 and E2 are type I transmembrane glycoproteins, with N-terminal ectodomains and a short C-terminal transmembrane domain and assemble as non-covalent heterodimers. Experimental studies with pseudotypes lacking envelope proteins or bearing-mutated envelope proteins clearly demonstrated that envelope proteins are essential for infectivity [7].

HCV is the result of continuous de novo infection and elimination of infected cells. Due to the high de novo infection rate of this virus, blocking de novo infection could be a promising strategy to treat HCV. Antibodies directed against the hypervariable region (HVR1) within the envelope 2 protein or antibodies directed against the N-terminal E1 region have been shown to inhibit host cell recognition and attachment [10, 11]. Therefore, the envelope proteins are potential targets for antiviral therapy. However, the sequence of envelope proteins shows a high variability especially in the HVRs making it difficult to develop specific inhibitors.

Cellular Receptors

CD81 was the first cellular HCV receptor that was identified by expression cloning using a cDNA library derived from the human T cell

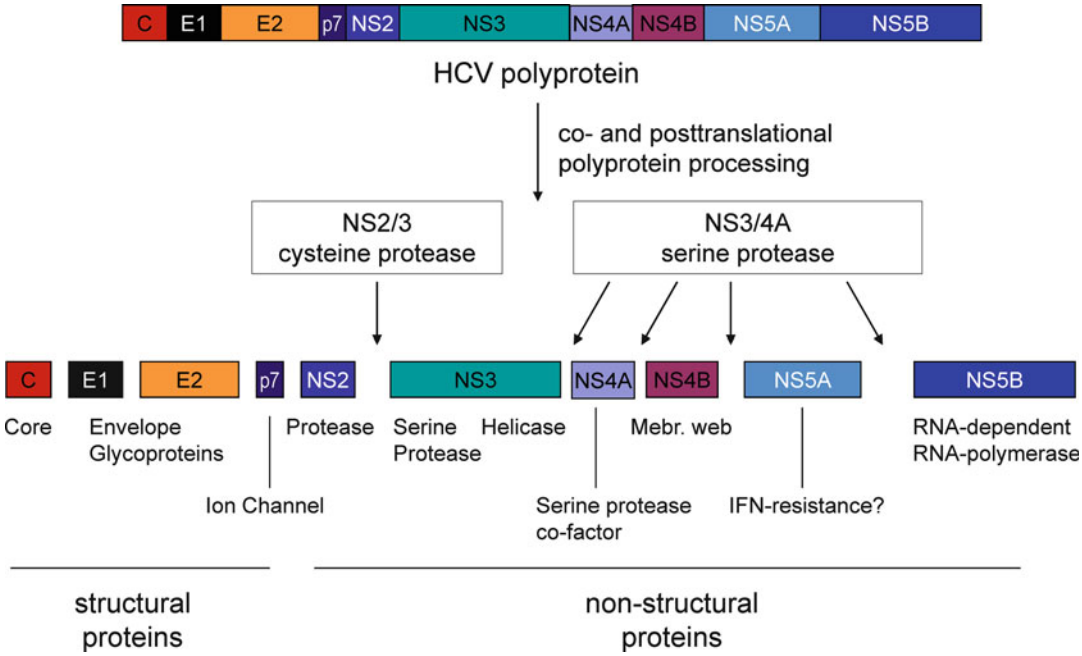

Fig. 17.3 Organization of the HCV polyprotein and co- and post-translational polyprotein processing

lymphoma cell line Molt-4 with the ability to bind recombinant E2 proteins [12]. The 25-kDa cell surface protein CD81 is widely expressed and is involved in pleitropic activities. CD81 is essential for HCV but not sufficient for HCV infection. Due to the conserved structure of CD81, it appears as a promising target to block HCV entry. Anti-CD81 antibodies inhibit HCV pseudotype and cell culture infectious HCV particles to enter Huh-7 hepatoma cell lines [5, 6]. Furthermore, silencing of CD81 by small interfering RNAs (siRNAs) renders hepatoma cell lines resistant to HCV pseudotype and cell culture infectious HCV infection [13].

The scavenger receptor class B type I (SR-BI) was the second essential HCV receptor which was detected by recombinant E2 binding to the hepatoma cell line HepG2 lacking expression of CD81 [14]. SR-BI is a 82-kDa protein which is highly expressed in liver and steroidogenic tissues as well as in human monocyte-derived dentritic cells. SR-BI has the ability to bind HDL, LDL, and is involved in bidirectional cholesterol transport at the cell membrane. Administration of antibodies against SR-BI as well as silencing of SR-BI is associated with loss of HCV pseudotype infectivity [15].

Other cell surface molecules such as heparan sulfate, the LDL-receptor, as well as the C-type lectins DC-SIGN and L-SIGN have been shown to bind the HCV E2 protein, however, all these factors were not sufficient to render HCV non-permissive cell line permissive to HCV infection [16]. Another important step in understanding the HCV entry mechanism was the identification of claudin-1, a tight junction component that is highly expressed in the liver [17]. Claudin-1 was shown to be required for HCV infection of human hepatoma cell lines and was the first factor to confer susceptibility to HCV when ectopically expressed in non-hepatic cells. Nevertheless, non-human cells were still not susceptible to HCV even when human CD81, SR-BI, and claudin-1 were expressed indicating that further essential receptors for HCV were required. Using a similar screen that has led to identification of claudin-1,

occludin was identified as a fourth host-cell protein essential for HCV entry [18]. Occludin renders mouse cells susceptible to HCV pseudoparticle infection. In addition to occludin, HCV pseudoparticle infection of murine cells required expression of CD81, SR-BI, and claudin-1.

Translation

The long open reading frame of the HCV genome is flanked at the 5′- and 3′-ends by short highly structured NTRs [7, 8]. The 5′-end contains an IRES which is required for the initiation of HCV polyprotein translation. The 5′-NTR consists of four highly ordered domains. Domains I and II are relevant for replication, domains II–IV together with the first 24–40 nucleotides of the core region constitute the IRES. HCV translation initiation occurs through the formation of a binary complex between the IRES and the 40S ribosomal subunit. Micro-RNA 122 was shown to bind to the 5′-NTR and enhance viral replication [19]. The 3′-NTR consists of a short variable poly U/UC region with a length of 80 nucleotides and an almost invariant RNA element of 98 nucleotides, the X-tail [7, 8]. The conserved elements are essential for HCV replication in cell culture. Detailed structural analyses have been performed of the 5′-NTR particularly with the IRES.

Post-translational Processing

Translation of the HCV open reading frame leads to the formation of a polyprotein precursor. The endoplasmic reticulum protease processes the structural proteins. The NS2/3 protease mediates a single cleavage at the NS2/NS3 junction, whereas the NS3/4A protease cleaves at four downstream sites in the polyprotein.

NS2

The crystal structure of the catalytic domain of the NS2 protease revealed a dimeric cysteine protease with two composite active sites [20]. NS2-deficient subgenomic replicons replicate efficiently in cell culture indicating that NS2 itself is not strictly required for genome replication.

New findings with the cell culture infectious HCV system indicate that NS2 is an essential cofactor for virus assembly [9, 21]. This function involves interaction of NS2 with the core and the envelope proteins as well as with p7 [9]. In addition, NS2 interacts with cellular factors such as cyclophilin A. Additional findings report that NS2 is able to modulate apoptosis and gene expression [9].

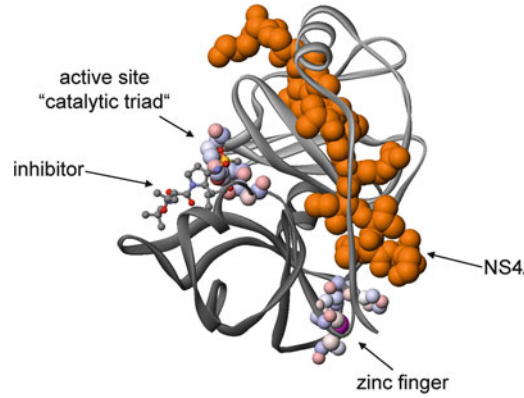

Fig. 17.4 Structure of the NS3/4A protease

NS3/4A

NS3 carries in the N-terminal part a serine-type protease [22]. The enzyme has a typical chymotrypsin-like fold and is composed of two beta barrel domains displaying on their interface the classical active site residues of serine-type proteases (Fig. 17.4). NS3 possesses intrinsic proteolytic activity. NS4A is a co-factor that increases NS3-associated polyprotein cleavage. NS4A anchors the protease to intracellular membranes, contributes to its complete folding, stabilizes the protease against degradation, and activates protease activity by changing the geometry of the catalytic triad. The NS3/4A cleaves at four downstream sites in the polyprotein to generate the N-termini of the NS4A, NS4B, NS5A, and NS5B proteins. The NS3/4A serine protease has also been shown to inactivate the host proteins Trif and Cardif which are involved in the interferon response mediated by toll-like receptor 3 (TLR3) and retinoic-acid inducible gene I (RIG-I), respectively [23]. Furthermore, it has been shown that NS3 is also an integral part of the viral RNA replication complex, functions as a RNA helicase and a nucleotide triphosphatase (NTPase) [24].

Replication

NS4B – Formation of the Replication Complex

NS4B is a highly hydrophobic molecule predicted to contain four transmembrane regions. NS4B induces the formation of an intracellular membrane structure, which represents the site of HCV replication, and is required to assemble the other NS proteins within these membrane-associated replication complexes [9]. An arginine-rich-like motif within NS4B that mediates binding to the 3′-terminus of the negative HCV strand is important for HCV RNA replication [9].

NS5A – Replication, Modulation of Cellular Processes

NS5A is a pleiotropic protein with key roles in both viral RNA replication and modulation of the physiology of the host cell. NS5A is composed of an N-terminal amphipathic alpha helix serving as a membrane anchor and three distinct domains that are separated by the low complexity sequences LC I and LC II [8, 9]. Domain I appears to be involved in RNA binding. Domain II may be involved in inhibition of interferon-induced dsRNA activated protein kinase (PKR). Domain III is poorly conserved and seems to be of minor importance for HCV replication. The X-ray crystal structure of domain I has been revealed [25].

NS5A is a phosphoprotein, basally phosphorylated and hyperphosphorylated forms have been identified [26]. The casein kinase CKII has been identified to be involved in NS5A hyperphosphorylation. The detailed role of NS5A phosphorylation is unclear. A model suggests that NS5A phosphorylation serves as switch between HCV replication and assembly [9]. NS5A is mostly known due to its potential effect on interferon-alfa signaling. Furthermore, NS5A has been shown to affect cell growth of target cells and apoptosis [9].

NS5B – RNA-Dependent RNA Polymerase

NS5B is the RNA-dependent RNA polymerase and the catalytic core of the replication complex. The polymerase activity appears to be modulated by interaction with the viral factors NS3 and NS5A and the host factor cyclophilin B. NS5B

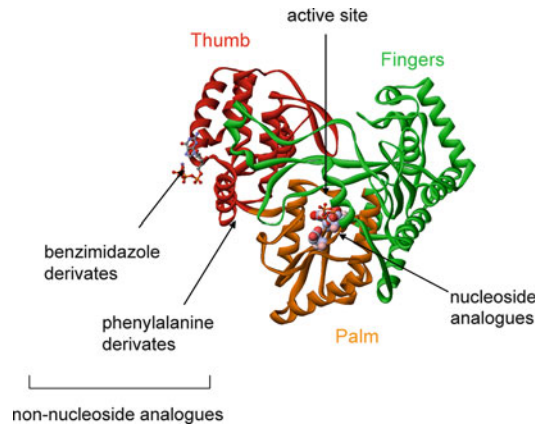

Fig. 17.5 Structure of the NS5B RNA-dependent RNA polymerase

reveals the typical polymerase structure, a classical "right hand" shape of thumb, palm, and finger (Fig. 17.5) [27]. Multiple interactions between the finger and thumb subdomains create a tunnel in which a single-stranded RNA molecule is directly guided to the active site.

Assembly and Release

HCV particles presumably form by budding into the endoplasmic reticulum or an endoplasmic reticulum-derived compartment and exit the cell through the secretory pathway [8]. Assembly and release of HCV particles involves a complex interplay between viral proteins that is not fully understood. Interactions between NS2, E1, and p7 as well as between NS2 and NS3 were shown to be essential for virus assembly [7]. The HCV core protein targets to early and late endosomes. Movement of core protein to the early and late endosomes and virus production were shown to require an endosome-based secretory pathway [28].

The small HCV protein p7 has the ability to form a membrane ion channel. Recently, Steinmann et al. showed that p7 has a central role in HCV assembly and release of cell culture infectious HCV particles [29]. p7 resembles viroporins, a class of proteins known from other viruses such as HIV-1 and influenza A virus. Members of this group of functionally related proteins form membrane pores that promote virus

Table 17.1 Entry inhibitors

Drug	Company	Target	Study phase (identifier)
Directed against viral structures			
MBL-HCV-1	University of Massachusetts	Anti-HCV antibody	Phase 1 (NCT01121185)
XTL-6865	XTL Biopharmaceuticals	Anti-HCV Antibody	Preclinical
HuMax-HepC Antibody	Genmab	Anti-HCV Antibody	Preclinical
Polyclonal Antibody	Civacir	Anti-HCV Antibody	Stopped (NCT00473824)
Directed against host structures			
ITX-5061	iTherX Pharmaceuticals	Downregulation of SR-B1 (Host target)	Phase 1 (NCT01165359)
ITX4520	iTherX	SR-B1	Preclinical
Anti-CD81	Non-commercial	CD81	Preclinical
Anti-Claudin	Non-commercial	Claudin-1	Preclinical
Unknown target			
PRO 206	Progenics	Entry inhibitor	Preclinical
SP-30	Samaritan Pharm.	Entry inhibitor	Preclinical
Therapeutic vaccination			
GI 5005	Globeimmune	Therapeutic vaccine	Phase 2 (NCT00606086)
IC41	Intercell	Therapeutic vaccine	Phase 2 (NCT00602784)
TG4040	Transgene	Therapeutic vaccine	Phase 2, planned (NCT01055821)
Hepavaxx C	ViRex Medical	Therapeutic vaccine	Preclinical

release and in some cases also virus entry. Conserved p7 residues crucial for functioning of this protein were identified that could be relevant for drug design.

Targets of DAA

Entry Inhibitors

Entry Inhibitors Against Viral Structures

As shown in Table 17.1, numerous compounds for entry inhibition have been developed. Approaches for entry inhibition include monoclonal or polyclonal antibodies against the envelope proteins or therapeutic vaccines. Proof of principle for in vivo inhibition of HCV infection by entry inhibitors was provided by the fully humanized monoclonal antibody MBL-HCV1 directed against a linear epitope of HCV E2 glycoprotein. Three chimpanzees received a single dose of the Anti-E2 antibody intravenously before challenge with HCV 1a strain H77. No HCV RNA was detected in the serum of the 250 mg/kg-dosed chimpanzees through week 20 while the 0 and 50 mg/kg-dosed chimpanzees both became infected by day 14 [30]. The antibody is being currently evaluated in a phase 1 trial (Table 17.1).

Another approach to prevent HCV infection is therapeutic vaccination. T- and B-cell epitopes are administered to elicit a humoral or cellular immune response. The therapeutic vaccine GI 5005 expressing NS3 and core antigens was administered in combination with peginterferon alfa-2a and ribavirin in treatment-naïve and non-responder genotype 1 patients. Triple therapy with GI-5005 showed superior end of treatment response rates when compared with standard therapy (63% vs. 45%) [31]. However, the sustained virologic response (SVR) rates were not improved. Other therapeutic vaccines are in development (Table 17.1).

Entry Inhibitors Against Host Structures

Promising targets are the HCV receptors CD81, SR-B1, occludin, and claudin-1. CD81 is highly conserved and essential for HCV infection. The problem of CD81 is the ubiquitious distribution

that may be associated with adverse effects. Monoclonal anti-CD81 antibodies have been shown to block HCV infection in vivo. Furthermore, potential small molecule inhibitors have been reported. However, clinical data on DAA targeting CD81 are not yet available.

The entry inhibitor ITX-5061 is directed against SR-B1. In preclinical trials the compound has shown a potency in the picomolar range, and was equally potent against both genotype 1 and genotype 2 viruses. The ability of ITX-5061 to reduce viral load in treatment-naïve and previously treated patients with HCV infection is currently being evaluated in a placebo-controlled, randomized trial (Table 17.1).

Occludin and claudin-1 are major components of bicellular tight junctions that are located in the apical part of lateral membranes and comprise an elaborate network of paired strands, which form kissing points that eliminate extracellular space. It is the question if tight junctions are potential targets for drug therapy. The toxins produced by vibrio cholerae and clostridium perfringens exert their toxic effect by altering the tight junction indicating that tight junction proteins are potential targets for therapy. However, targeting the tight junction might be associated with severe adverse reactions. Recently, it was shown that anti-claudin antibodies have the potential to inhibit infection of cultured hepatocytes confirming that claudin-1 is a potential drug target despite its location in tight junctions [32]. Clinical trials and potential adverse events are not yet available.

Inhibition of Post-translational Processing (NS2, NS3/4A)

NS2

Due to our limited knowledge about the function of NS2, this protein has been neglected as a drug target until recently. However, recent advances have identified NS2 as an essential HCV protein with multiple functions. The structure of the NS2 cysteine protease shares no obvious similarities to any known protease in eukaryotes and would therefore make an attractive target for antiviral therapy. Inhibitors have not been described so far.

NS3/4A

The three-dimensional structure of NS3/4A revealed an unusually shallow substrate-binding pocket (Fig. 17.4). This structure of the binding pocket made the design of specific inhibitors difficult because long interaction surfaces are required. Nevertheless, NS3/4A is the best studied target for antiviral therapy to date. As shown in Table 17.2, multiple NS3/4A protease inhibitors have been developed and have entered clinical evaluation. The clinical development of the most advanced NS3/4A protease inhibitors telaprevir and boceprevir is described below.

The heterogeneity of HCV has a strong impact on antiviral activity of direct antiviral drugs and the development of resistance. The current NS3/4A protease inhibitors have been developed for HCV genotype 1-infected patients (Table 17.2). Compared with HCV genotype 1-infected patients, the protease inhibitor telaprevir, e.g., has a markedly reduced antiviral activity in HCV genotype 3-infected patients [33].

A major problem in the use of current NS3/4A protease inhibitors is the development of drug-resistant HCV strains. Several mutations associated with resistance were identified in vitro and in patients during treatment with protease inhibitors. The currently known mutations associated with resistance are shown in Fig. 17.6. NS3/4A inhibitors can be distinguished into linear and macrocyclic inhibitors. As shown in Fig. 17.6, the resistance profile differs slightly among the NS3/4A inhibitors of the two classes. The resistance mutation at position 168 is associated with macrocyclic inhibitors. For several protease inhibitors, escape was shown to be less frequent in genotype 1b-infected patients than in genotype 1a-infected patients which is most likely related to the requirement of two mutations for resistance development in genotype 1b compared to one mutation in genotype 1a isolates [34].

Another strategy to overcome NS3/4A protease inhibitor resistance is to target the NS4A cofactor. GS9132/ACH-806 was the first NS4A antagonist which showed promising results in a phase 1 trial but was not further developed due to renal toxicity. ACH-1095 is another NS4A antagonist for which phase I trials are planned.

Table 17.2 Post-translational processing inhibitors

NS3/4A protease inhibitors in registered clinical trials	Company	Target	Study phase (identifier)
Telaprevir (VX-950)	Vertex	NS3	Phase 3, naïve/treatment-experienced GT1 pts (NCT00627926/ NCT00703118) Triple 8–12 weeks, followed by SOC 12–36 weeks
Boceprevir (SCH 503034)	Schering-Plough	NS3	Phase 3, naïve/treatment-experienced GT1 pts. (NCT00705432/ NCT00708500) SOC lead-in 4 weeks, followed by Triple 24–44 weeks
Danoprevir (RG7227)	Roche	NS3	Phase 2 , naïve GT1 pts (NCT00963885) Triple 12–24 weeks, followed by SOC 12–36 weeks
TMC435350	Tibotec/Medivir	NS3	Phase 2, naïve/treatment-experienced GT1 pts (NCT00561353) Triple 4 weeks, followed by SOC 24–44 weeks
Vaniprevir (MK-7009)	Merck	NS3	Phase 2, naïve/treatment-experienced GT1 pts (NCT00704184/NCT00704405) Triple 4/24–48 weeks, followed by SOC 44/0–24 weeks
BI201335	Boehringer Ingelheim	NS3	Phase 2, naïve/treatment-experienced GT1 pts (NCT00984620/NCT00774397) Triple 12/24–48 weeks, followed by SOC 12/0 weeks
BMS-650032	Bristol-Myers-Squibb	NS3	Phase 2, naïve GT1 pts (NCT01030432) Triple 24–48 weeks
ABT-450	Abbott	NS3	Phase 2, naïve GT1 pts (NCT01074008) Triple 12 weeks, followed by SOC 36 weeks
GS-9256	Gilead	NS3	Phase 2, naïve/treatment-experienced GT1 pts (NCT01072695)
ACH-0141625	Achillion	NS3	Phase 2, naïve GT1 pts (NCT01180790)
MK-5172	Merck	NS3	Phase 1 (NCT00998985), monotherapy
IDX320	Idenix	NS3	Phase 1 (NCT01157104), single dose
VX-985	Vertex	NS3	Phase 1 (NCT01144936), monotherapy
Discontinued			
Ciluprevir (BILN 2061)	Boehringer	NS3	Stopped
GS-9132/ACH806	Gilead/Achillion	NS4	Stopped
Narlaprevir (SCH900518)	Schering-Plough/ Merck	NS3	On hold

Inhibition of Viral Replication

Viral Targets

NS5A

In 2010, the first small molecule NS5A inhibitor BMS-790052 was reported [35]. BMS-790052 was effective in replicons expressing a broad range of HCV genotypes and in the cell culture infectious HCV system. The half-maximum effective concentration (EC50) of the inhibitor was in the picomolar range. In a phase 1 clinical trial a single 100-mg dose of BMS-790052 reduced HCV RNA by 3.3 $\log_{10}$ within 24 h. BMS-790052 is currently being evaluated in a phase 2 trial in combination with peginterferon alfa-2a/ribavirin. As shown in Table 17.3, other NS5A inhibitors are in clinical and preclinical development.

NS5B

The RNA-dependent RNA polymerase plays a central role in the HCV replication cycle and is therefore an ideal target for drug therapy. Two classes of NS5B polymerase inhibitors, nucleoside

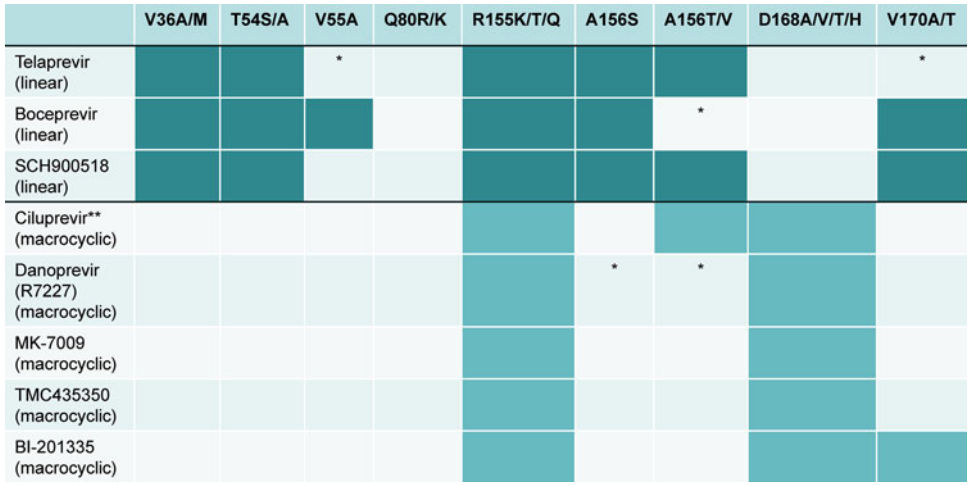

Fig. 17.6 Cross-resistance table of different NS3 protease inhibitors based on mutations selected in patients from clinical studies and/or from in vitro studies. **Mutations associated with resistance in vitro but were not described in patients. **Mutations associated with resistance in vitro. Resistance mutations of linear NS3 protease inhibitors are shown in *dark blue*, and resistance mutations described for macrocyclic NS3 protease inhibitors are shown in *light blue* (based on data from Sarrazin and Zeuzem [68])

Table 17.3 Replication inhibitors

Polymerase inhibitors	Company	Target	Study phase (identifier)
Nuc			
R7128	Roche/ Pharmaset	Active site	Phase 2, naïve GT 1/4 pts (NCT00869661) Triple 8–12 weeks followed by SOC 16–40 weeks
PSI-7977	Pharmasset		phase 2, naïve GT 1, 2/3 pts (NCT01188772) GT1: Triple 12 weeks, followed by 12–36 weeks SOC, GT2/3: Mono for 12 weeks
IDX184	Idenix	Active site	Phase 1 (NCT00807001), monotherapy
Non-Nuc			
Filibuvir (PF868554)	Pfizer	Site 2/thumb 2	Phase 2, naïve GT1 pts (NCT00987337) Triple for 24 weeks, followed by SOC 24 weeks
GS-9190	Gilead	Site 4/palm 2	Phase 2, naïve GT1 pts (NCT00743795) Triple 48 weeks Phase 2, naïve/non-naïve GT1 pts (NCT01072695) GS-9256 + GS-9190 ± ribavirin or SOC for 4 weeks
ANA598	Anadys	n.a.	Phase 2, naïve GT1 pts (NCT00978497) Triple 12 weeks, followed by SOC 12–36 weeks
ABT-333	Abbott	Site 4/palm 2	Phase 2, naïve GT1 pts (NCT00851890) Triple for 4 weeks
ABT-072	Abbott		Phase 2, naïve GT 1 pts (NCT01074008) Triple for 12 weeks, SOC for 36 weeks
VX-222 (VCH222)	Vertex (ViroChem Pharma)	Site 2/thumb 2	Phase 2, naïve GT1 pts (NCT00911963) Triple for 12 weeks, SOC for 36 weeks Phase 2, naïve GT1 pts (NCT01080222) VX-222 plus telaprevir ± SOC for 12 weeks
BI207127	Boehringer Ingelheim	Site 1/thumb 1	Phase 1, naïve GT1 pts (NCT01132313) BI 207127 + BI201335 ± ribavirin 4, 24, 48 weeks

(continued)

Table 17.3 (continued)

Polymerase inhibitors	Company	Target	Study phase (identifier)
Discontinued			
Valopicitabine (NM283)	Idenix/Novartis	Active site	Stopped
R1626	Roche	Active site	Stopped
HCV-796	ViroPharma/ Wyeth	Site 4/palm 2	Stopped
MK-3281	Merck	Site 1/thumb 1	Stopped
XTL-2125	XTL		Stopped
NS5A-Inhibitor			
BMS-790052	Bristol-Myers-Squibb	NS5A domain 1 inhibitor	Phase 2, naïve/treatment experienced GT1 pts (NCT00874770/ NCT01170962) Triple for 48/24 weeks
AZD7295	Arrow		Phase 1 (NCT00781976), monotherapy
PPI-1301	Presidio		Preclinical

and non-nucleoside polymerase inhibitors, have been developed. Nucleoside analogue polymerase inhibitors are converted into triphosphates by cellular kinases and incorporated into the elongating RNA strand as chain terminators. Generally, they show similar efficacy against all HCV genotypes and have a high genetic barrier.

Several structurally distinct non-nucleoside inhibitors of the HCV RNA-dependent RNA-polymerase NS5B have been reported to date, including benzimidazole, benzothiadiazine, and disubstituted phenylalanine/thiophene or dihydropyranone derivatives (Table 17.3). These agents target different sites within the polymerase and compared to nucleoside inhibitors have a lower genetic barrier. Different resistance profiles due to distinct target sites can be expected for the class of non-nucleoside inhibitors. In contrast to nucleoside polymerase inhibitors, a restricted spectrum of activity of non-nucleoside polymerase inhibitors against different HCV genotypes and subtypes has been described.

Host Targets

Cyclophilin

Cyclophilins are ubiquitous proteins in human cells that are involved in protein folding. Moreover, cyclophilins participate in HCV replication. It was shown that cyclophilin B binds to the HCV NS5B polymerase and stimulates its RNA-binding activity. Cyclophilin inhibitors show strong antiviral activity in vitro and in vivo. The cyclophilin inhibitor alisporivir (previously DEBIO 025) showed a 3.6 $\log_{10}$ mean decline of HCV RNA after a 14-day oral treatment with an effect against different genotypes (HCV 1, 3, and 4) [36]. For the combination of alisporivir with peginterferon alfa-2a, a decline of HCV RNA of 4.61–4.75 $\log_{10}$IU/ml and 5.89–5.91 $\log_{10}$IU/ml was reported for HCV genotype 1, 4 and genotype 2-, 3-infected patients, respectively [37]. Other cyclophilin inhibitors such as SCY-635 are in clinical development.

Inhibition of Translation (UTR, IRES)

Several strategies have been developed for the inhibition of translation (Table 17.4). The most advanced strategies are antisense DNA or RNA oligonucleotides and small molecule IRES inhibitors. Antisense oligonucleotides have a complement sequence of the target mRNA and can prevent translation of viral proteins. The antisense compounds AVI-4065 and ISIS-14803 progressed to phase 2 clinical trials. However, the development of both compounds was stopped due to lack of antiviral efficacy and/or potential hepatoxicity.

Ribozymes are RNA molecules that catalyze cleavage of a target RNA molecule based on sequence-specific recognition. Ribozymes show antiviral activity in vitro. Heptazyme, an IRES-specific ribozyme was investigated in

Table 17.4 Translation inhibitors

Translation inhibitors	Company	Target	Study phase (identifier)
TT 033	Tacere Therapeutics	siRNA	Preclinal
mi-R-122	Alnylam	RNA interference	Preclinal
RNAi	CombiMatrix	RNA interference	Preclinal
siRNA-034	Sirna Therapeutics/Merck	RNA interference	Preclinal
AVI-4065	AVI BioPharma	Antisense	Phase 2, stopped (NCT00229749)
ISIS-14803	Isis Pharmaceuticals	Antisense	Phase 2, stopped (NCT00035945)

phase 2 trials, however, the studies were halted due to cardiotoxicity in monkey animal studies.

RNA interference is a sequence-specific RNA degradation process induced by double-stranded RNA. RNA interference can be initiated by siRNA or short hairpin RNA (shRNA) that associate with various proteins to form an RNA-inducing silencing complex with nuclease and helicase activity. The siRNA or shRNA guides this complex to the complementary target RNA and the nuclease component cleaves the target RNA in a sequence-specific manner. This approach appears reasonable also in HCV treatment and in vitro experiments achieved promising results. Several compounds have been developed (Table 17.4), however, are yet at preclinical stage. The major limitation of this strategy is in vivo delivery; several approaches for in vivo delivery are currently explored.

The liver-specific micro RNA-122 has recently been shown to be required for HCV replication. SPC3649 is a micro RNA-122 inhibitor that is currently evaluated in phase 1 trials. In HCV-infected chimpanzees, SPC3649 treatment was associated with reduction in HCV RNA without severe adverse events [38].

Assembly and Release

Viral Target (Core)

HCV core proteins play a major role in the formation of viral particles. Furthermore, HCV core interacts with several cellular proteins. It is the most conserved of all HCV proteins. Organization of HCV assembly requires the oligomerization of the core protein. Recently, peptides and small molecules were identified which inhibit core–core interaction and block viral production in cell culture. Targeting the core protein is a promising anti-HCV strategy, however the development of inhibitors is still at the preclinical stage [39].

Host Target (Glycosylation)

Proper glycosylation of HCV structural proteins is required for maturation, assembly, and secretion of infective particles. Inhibition of glycosylation is another potential approach for HCV antiviral therapy. Celgosivir is a potent inhibitor of alpha-glucosidase which affects the early stages of glycoprotein processing [40]. Alpha-glucosidase is a host enzyme. A potential advantage of celgosivir is the low probability to develop drug-resistant viral mutants. On the other hand, development of adverse reactions are likely. Celgosivir showed promising results in preclinical trials and was investigated in phase 2 trials [41]. In non-responders, celgosivir in combination with peginterferon alfa-2b plus ribavirin showed a stronger decline of HCV-RNA than peginterferon alfa-2b plus ribavirin alone (−1.63 $\log_{10}$ IU/l vs. –0.92 $\log_{10}$ IU/l). Celgosivir development, however, is currently on hold.

Historical Development of the Most Advanced Direct Antiviral Agents Against the NS3/4 Protease and NS5B Polymerase

Viral Targets

NS3/4A Inhibitors

Ciluprevir

In 2003, the first randomized placebo-controlled study with the NS3/4A protease inhibitor ciluprevir in patients with chronic hepatitis C was presented [42]. Oral administration of ciluprevir to

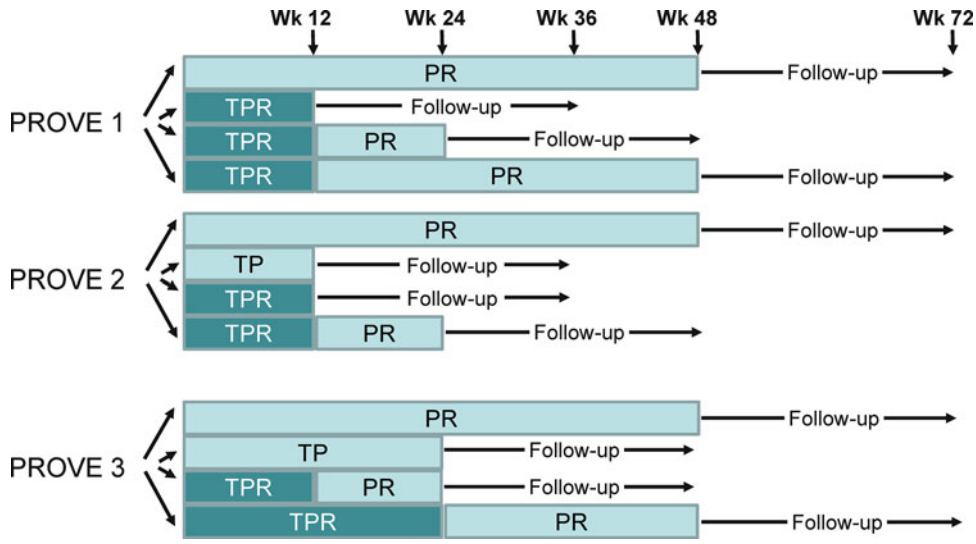

Fig. 17.7 Telaprevir phase 2 trials PROVE 1 and PROVE 2 were performed with treatment-naïve HCV genotype 1-infected patients in USA and in Europe, respectively. PROVE 3 was performed with HCV genotype 1-infected patients with prior non-response or relapse to SOC. TPR, telaprevir plus peginterferon alfa-2a, Ribavirin; TP, telaprevir plus peginterferon alfa-2a; PR, peginterferon alfa-2a plus ribavirin

patients with chronic HCV genotype 1 infection for 2 days was associated with 2–3 $\log_{10}$IU/ml decline of HCV RNA in most of the patients. The study provided proof-of-concept that HCV NS3/4A protease inhibitors are able to block HCV replication in patients with chronic hepatitis C. Due to potential cardiotoxicity, clinical development of ciluprevir was stopped.

Telaprevir

Phase 1: Monotherapy

The first monotherapy trial of telaprevir in HCV genotype 1-infected patients was started in 2004 [43]. During treatment with telaprevir, all patients (naïve and non-responders to previous SOC therapy) showed a ≥2 $\log_{10}$IU/ml decline of HCV RNA. Despite strong antiviral efficacy, telaprevir monotherapy was associated with the rapid emergence of drug-resistant HCV strains occurring in 75% of patients during telaprevir monotherapy [44]. Overall, this landmark study showed that due to rapid resistance development, only a minority of patients with chronic hepatitis C have the likelihood to be cured with telaprevir monotherapy and that combination of compounds with different resistance profiles are necessary.

Phase 1: Telaprevir in Combination with Peginterferon Alfa-2a

Subsequently, telaprevir in combination with pegylated interferon-alfa-2a was investigated in treatment-naïve HCV genotype 1-infected patients [45, 46]. The study showed a stronger decline of HCV RNA after 2 weeks of treatment in the combination arm than in the respective monotherapy arms (−5.49 $\log_{10}$IU/ml vs. −1.09 $\log_{10}$IU/ml and −3.99 $\log_{10}$IU/ml for peginterferon alfa-2a/telaprevir combination therapy vs. peginterferon alfa-2a and telaprevir monotherapy, respectively). The study provided proof-of-concept that telaprevir has at least additive antiviral effects in combination with pegylated interferon-alfa-2a.

Phase 2: Telaprevir in Combination with Peginterferon Alfa-2a with and Without Ribavirin Treatment-Naïve Patients (PROVE 1/2)

The PROVE1/2 studies (PROVE 1 was conducted in USA, PROVE 2 was conducted in Europe) were the first studies to explore whether a direct antiviral compound has the potential to increase SVR rates in treatment-naïve patients with chronic hepatitis C [47, 48].

Both studies had a similar design (Fig. 17.7). In both trials telaprevir was administered for

12 weeks (T12) in combination with peginterferon alfa-2a and ribavirin for 12 weeks (PR12) or 24 weeks (PR24), respectively. In addition, PROVE 1 contained a T12/PR48 arm that was replaced by a T12/P12 without ribavirin arm in the PROVE 2 trial. The results of the PROVE trials provided the first SVR rates of a DAA with SOC in patients with chronic hepatitis C. The highest SVR rates were obtained in the T12/PR48 (PROVE 1) and in the T12/PR24 arm. The SVR rates in these telaprevir arms were significantly higher compared with the SVR rates in the control arm (67%/69% vs. 41%/46% for triple therapy vs. SOC in PROVE1/PROVE2, respectively).

In PROVE 2, the SVR rate in patients treated with telaprevir/peginterferon alfa-2a without ribavirin for 12 weeks was lower than in patients treated with telaprevir/peginterferon alfa-2a plus ribavirin for 12 weeks (36% vs. 60%). The lower rate of SVR in the group without ribavirin was due to a higher relapse rate compared to the groups with ribavirin (48% vs. 14–29%).

Three major conclusions were drawn from the PROVE 1/2 studies: (1) protease inhibitors are able to increase SVR rates in treatment-naïve patients with HCV genotype 1 infection, (2) improved SVR rates may be achieved with shorter treatment duration, and (3) ribavirin has additive antiviral activity to telaprevir and is required to optimize SVR rates.

Phase 3: Telaprevir in Combination with Peginterferon Alfa-2a with and Without Ribavirin

The ADVANCE and the ILLUMINATE trials are phase 3 studies to investigate the efficacy and safety of telaprevir in combination with peginterferon alfa-2a and ribavirin in treatment-naïve HCV genotype 1 patients [49, 50]. Overall, the SVR rates in the telaprevir arms were superior to standard therapy (69–75% vs. 44%). The studies are discussed in detail in Chap. 19.

Retreatment of Previous SOC Non-responders

The PROVE3 trial was a randomized, placebo-controlled phase 2 study assessing safety and efficacy of telaprevir plus peginterferon alfa-2a ± ribavirin in HCV genotype 1 patients who previously failed peginterferon/ribavirin treatment [51]. The overall SVR rates were significantly higher in the telaprevir arms (peginterferon alfa-2a/ribavirin/telaprevir for 12 or 24 weeks followed by peginterferon alfa-2a/ribavirin for 12 and 24 weeks, respectively) compared with the control arm (51%, 52% vs. 14%). Subgroup analysis of previous peginterferon/ribavirin non-responders showed superior SVR rates in the triple therapy arms compared with the SOC control arm or the peginterferon/telaprevir arm without ribavirin (38–39% vs. 9–10%). Overall, the study confirmed that protease inhibitors in combination with SOC will also be a treatment option for patients who failed previous antiviral therapy.

The REALIZE trial is a phase 3 trial investigating efficacy and safety of telaprevir in combination with peginterferon alfa-2a/ribavirin in patients with prior treatment failure to SOC. The overall SVR rates following telaprevir-based retreatment were superior to retreatment with standard therapy (64–66% vs. 17%). The study is described in detail in Chap. 19.

Resistance Against Telaprevir

Mutations associated with resistance to telaprevir were identified using a highly sensitive sequencing method [52, 53]. Several mutations associated with resistance to telaprevir were identified in the HCV NS3 protease catalytic domain (Fig. 17.6). Mutations occurred either as single mutations (V36A/M, T54A, R155K/T, A156S/T/V) or as double mutations (at positions 36 + 155 or 36 + 156). The rapid occurrence of these mutations during treatment indicates that mutations are present before treatment and are selected during treatment with telaprevir. The resistance mutations can be distinguished into low-level resistance and high-level resistance mutations. Furthermore, it was shown that resistance is associated with a reduced ability of the virus to replicate (lower viral fitness).

Safety of Telaprevir

Overall, telaprevir has a good safety profile, however, premature discontinuation rates were higher in the telaprevir arms compared with the

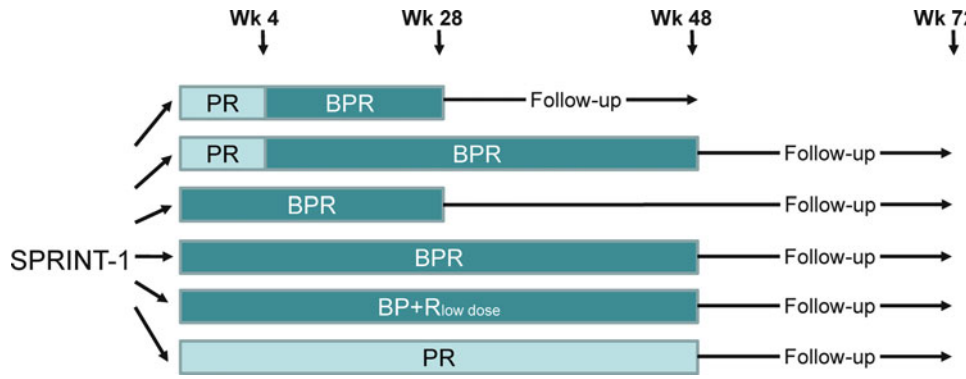

Fig. 17.8 Boceprevir phase 2 SPRINT-1 trial BPR, boceprevir, peginterferon alfa-2b, ribavirin (800–1,400 mg); PR, peginterferon alfa-2b, ribavirin; low doseR, ribavirin (400–1,000 mg)

SOC arms (12–21% vs. 4–11%). The most common adverse events, such as fatigue and influenza-like symptoms, were consistent with typical interferon-related systemic symptoms, while macropapular rash and pruritus occurred more frequently in the telaprevir study arms compared with the SOC control arms (41–60% vs. 20–41%). The skin symptoms typically occurred within 1–4 weeks after initiation of telaprevir dosage. Furthermore, a stronger decrease of hemoglobin in the telaprevir arms compared with the SOC arm was reported.

Boceprevir

Boceprevir is the second N3/4A protease inhibitor that has advanced in clinical development. Boceprevir binds reversibly to the NS3 protease active site.

Phase 1: Boceprevir Monotherapy

Boceprevir monotherapy for 2 weeks in HCV genotype 1 patients with prior failure to SOC was associated with a mean −2.06 $\log_{10}$IU/ml reduction in HCV RNA. Boceprevir was well tolerated at all doses. Similar to telaprevir, viral breakthrough with selection of resistant variants occurred during boceprevir dosage.

Phase 1: Boceprevir in Combination with Peginterferon Alfa-2b

Subsequently, a randomized, double-blind crossover study investigated boceprevir in combination with peginterferon alfa-2b in SOC non-responders. In this study, boceprevir was administered either alone for 7 days or in combination with peginterferon alfa-2b for 14 days in comparison to 14 days of peginterferon alfa-2b monotherapy. In this study, double combination therapy showed a −2.45 to 2.88 $\log_{10}$IU/ml decline of HCV-RNA compared with −1.08 to 1.26 $\log_{10}$IU/ml in patients re-treated with pegylated interferon alfa-2b alone [54].

Phase 2: Boceprevir in Combination with Peginterferon Alfa-2b with and Without Ribavirin

SPRINT-1 was a phase 2 trial in which safety, tolerability, and antiviral efficacy of boceprevir in combination with pegylated interferon alfa-2b and ribavirin was investigated in treatment-naïve patients with chronic hepatitis C [55]. Treatment with boceprevir in combination with pegylated interferon alfa-2b and ribavirin was either performed continuously for 28 or 48 weeks or after a previous 4-week lead-in phase of pegylated interferon alfa-2b and ribavirin alone (Fig. 17.8). The lead-in phase was chosen to determine a potential benefit of pre-treatment with pegylated interferon alfa-2b and ribavirin to avoid resistance development. The control group was treated with pegylated interferon alfa-2b and ribavirin for 48 weeks. SVR rates after 28 weeks of triple treatment were 54 and 56% after 24 weeks with an additional 4 weeks of pre-treatment lead in with pegylated interferon alfa-2b and ribavirin. SVR rates after 48 weeks of triple treatment were

67 and 75% after 44 weeks with an additional 4 weeks of pre-treatment lead-in with pegylated interferon alfa-2b and ribavirin. Patients in the pegylated interferon alfa-2b/ribavirin control group achieved a SVR of 38%.

Phase 3: Boceprevir in Combination with Peginterferon Alfa-2b with Ribavirin

SPRINT-2 and RESPOND-2 are phase 3 studies to investigate efficacy and safety of boceprevir in combination with peginterferon alfa-2b and ribavirin in treatment-naïve or treatment-experienced patients, respectively, with chronic HCV genotype 1 infection [56, 57]. In brief, the overall SVR rates in both trials were superior in the boceprevir arms compared with the control arm (63–66% vs. 38% and 59–67% vs. 21%, respectively). The studies are discussed in detail in Chap. 19.

Resistance

To analyze development of resistance against boceprevir, a detailed clonal analysis of mutations selected during treatment with boceprevir monotherapy was performed [54]. As shown in Fig. 17.6, mutations associated with lower susceptibility to boceprevir were similar to mutations associated with resistance against telaprevir.

Safety

Boceprevir has a good safety and tolerability profile. The most common adverse events were anemia, nausea, vomiting, and dysgeusia.

NS5B Inhibitors

Nucleoside Analogues

Valopicitabine

Valopicitabine was the first nucleoside (nuc) analogue polymerase inhibitors tested in patients with chronic hepatitis C [58]. Valopicitabine showed antiviral activity in monotherapy (mean HCV-RNA decline 0.15–1.21 $\log_{10}$IU/ml after 14 days in patients infected with HCV genotype 1 and prior non-response to interferon-based antiviral treatment) and in combination therapy with interferon alfa (mean HCV-RNA decline 3.75–4.41 $\log_{10}$ IU/ml after 36 weeks in treatment-naïve patients infected with HCV genotype 1). The development of valopicitabine was stopped due to gastrointestinal adverse events which were severe in some patients.

R1626

The nuc analogue R1626, a prodrug of R1479, was investigated in treatment-naïve patients with HCV genotype 1 infection in combination with peginterferon alfa-2a and ribavirin. After 48 weeks (4 weeks R1626 plus peginterferon alfa-2a with or without ribavirin followed by 44 weeks of peginterferon plus ribavirin) the virologic response rates were 52–84% in the R1626 treatment arms and 65% in the control arm with peginterferon alfa-2a/ribavirin [59]. Despite promising results the clinical development of R1626 was stopped due to severe lymphopenia.

RG7128

HCV genotype 3-infected patients who did not respond to SOC were retreated with peginterferon alfa-2a/ribavirin for 24 or 48 weeks in combination with RG7128 for 4 weeks or placebo. SVR rates in the R7128 group were higher than in the placebo group indicating that nuc-polymerase inhibitors have the potential to increase SVR rates in patients with chronic hepatitis C [60]. RG7128 is currently being evaluated in a phase 2 study assessing efficacy and safety in combination with pegylated interferon alfa-2a and ribavirin in treatment-naïve HCV genotype 1- or 4-infected patients. Further nuc-polymerase inhibitors are shown in Table 17.4.

Non-nucleoside Analogues

HCV-796 was the first non-nuc polymerase inhibitor that demonstrated substantial antiviral activity in patients with chronic hepatitis C [61]. Monotherapy showed a maximum antiviral effect after 4 days of treatment with a mean HCV RNA reduction of −1.4 $\log_{10}$IU/ml. Similar to monotherapy with protease inhibitors, resistant variants rapidly occurred during HCV-796 monotherapy. The combination of HCV-796 and peginterferon

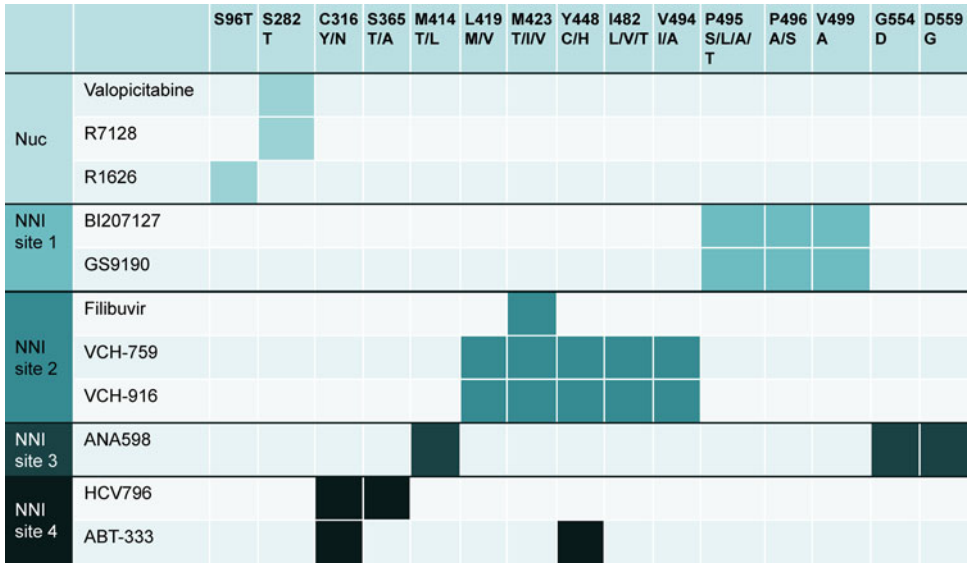

Fig. 17.9 Cross-resistance table for resistance-associated mutation in NS5B polymerase inhibitors Nuc, nucleoside analogue polymerase inhibitors; NNI, non-nuc polymerase inhibitors (based on data from Sarrazin and Zeuzem [68])

alfa-2b produced a mean viral reduction of −3.3 to 3.5 $\log_{10}$ IU/ml after 14 days of treatment compared to −1.6 $\log_{10}$ IU/ml with peginterferon alfa-2b alone. Due to clinically significant elevations of liver enzymes, the clinical development of HCV-796 was discontinued [62].

Several other non-nuc polymerase inhibitors are currently in clinical trials and are currently investigated in phase 2 clinical trials (Table 17.3). Larger trials showing that non-nuc polymerase inhibitors increase SVR rates are pending.

Resistance

Several resistance-associated mutations within the NS5B gene have been identified in vitro and in vivo (Fig. 17.9). In general, resistance-associated mutations are different between nuc and non-nuc analogue NS5B polymerase inhibitors. Furthermore, the resistance-associated mutations differ between non-nuc inhibitors targeted against different sites of the NS5B polymerase. Resistance-associated mutations against nuc-inhibitors have been identified only in vitro but not yet in vivo (most likely due to a low viral replication fitness).

Current Understanding of DAA Treatment

Among all approaches for direct antiviral treatment of patients with chronic hepatitis C, two NS3/4A protease inhibitors have progressed into phase 3 development (telaprevir and boceprevir). Both compounds show a good safety profile and high antiviral efficacy. Assuming approval by regulatory agencies, these drugs will become the new standard treatment for HCV genotype 1 patients in combination with peginterferon alfa and ribavirin. The results of the phase 3 clinical trials will be discussed in Chap. 19.

Future of DAA Treatment

Combination Therapy Without Interferon

With the introduction of the protease inhibitors telaprevir and boceprevir for treatment of chronic HCV genotype 1 infection, the major goal of recent

years to improve SVR rates in this difficult-to-treat population has been achieved. Triple therapy, however, continues to be unsatisfactory for two reasons: (1) Anti-HCV therapy still depends on peginterferon alfa and ribavirin and therefore SOC null responders are less likely to achieve SVR, (2) Triple therapy is associated with more adverse events than previous SOC. Therefore, the next goal of anti-HCV therapy will be to develop an interferon alfa-free regimen with better tolerability.

This goal can be achieved with combination of two or more direct antivirals with non-overlapping resistance profiles. The most promising drug combinations to date are protease inhibitor plus nucleoside or non-nucleoside analogue polymerase inhibitors or the combination of a protease inhibitor with a NS5A inhibitor.

Combination of NS3/4A Inhibitor Plus NS5B Polymerase Inhibitor

The INFORM-1 trial was the first study that investigated an interferon alfa-free approach for treatment of chronic hepatitis C. The nuc polymerase inhibitor RG7128 and the protease inhibitor RG7227 were administered to treatment-naïve and treatment-experienced HCV genotype 1-infected patients for 2 weeks. All patients who received combination therapy achieved profound reduction in HCV RNA without evidence of treatment emergent resistance (HCV drop after 2 weeks −4.8 $\log_{10}$ IU/ml, −4.0 $\log_{10}$ IU/ml, and −4.9 $\log_{10}$ IU/ml in treatment-naïve, relapse, and null responders to SOC) [63].

Another phase 1 trial also investigated an interferon-free combination of the NS3/4A protease inhibitor BI201335 and the NS5B polymerase inhibitor BI207127 plus ribavirin for 4 weeks in treatment-naïve HCV genotype 1-infected patients [64]. The combination therapy showed a rapid sharp decline of HCV RNA followed by second-phase decline in most of the patients. The majority of patients (11/15) and all patients (17/17) in the 400 mg TID low and the 600 mg TID high-dose arm of BI207127 had a HCV RNA level lower than 25 IU/ml at week 4. The combination will be investigated in a phase 2 trial testing different dose regimens and longer treatment with SVR as primary endpoint.

GS-9256 and GS-9190 are a NS3/4A protease inhibitor and a non-nucleoside NS5B inhibitor, respectively, that have demonstrated antiviral activity in HCV genotype 1-infected patients during monotherapy studies. GS-9256- and GS-9190-associated mutations were introduced into 1b replicons and antiviral susceptibility was tested in transient replication assays. GS-9256 maintained antiviral activity in replicons bearing mutations associated with lower susceptibility to GS-9190 and vice versa. GS-9190 maintained antiviral activity in replicons bearing mutations associated with lower susceptibility to GS-9256. The combination is now in active clinical development [65].

Combination of NS3/4A Inhibitor Plus NS5A Inhibitor

A phase 2 study investigated the efficacy and safety of the NS5A inhibitor BMS-790052 in combination with the NS3/4A inhibitor BMS-650032 with and without peginterferon alfa-2a/ribavirin in HCV genotype 1 patients with null response to prior SOC [66]. An interim analysis reported RVR rates of 63 and 60% in the arm without interferon/ribavirin and the arm with interferon/ribavirin, respectively. However, between week 4 and 12, 6 of 11 patients in the interferon-free combination arm showed a viral breakthrough, while all patients in the interferon/ribavirin containing arm maintained viral suppression.

Individualized Treatment

Virologic Response

In the phase 3 trials with telaprevir and boceprevir, it was shown that response-guided therapy is associated with similar SVR rates as fixed duration therapy for 48 weeks treatment. These trials indicate the rapidity of virologic response will remain important to guide treatment duration.

Genotype

Both protease inhibitors telaprevir and boceprevir have been developed for treatment of HCV genotype 1-infected patients. For telaprevir, the antiviral activity was shown to be lower in HCV genotype 2 and markedly reduced in HCV genotype 3- and 4-infected patients compared with HCV genotype 1 patients [33, 67]. In addition, boceprevir was reported to have lower antiviral activity in HCV genotype 2- and 3-infected patients [68]. The HCV genotype will therefore remain an important factor for individualized therapy. New N3/4A protease inhibitors such as ACH-2684 and MK-5172 currently at preclinical or in early clinical development have activity against genotypes 1–6 and genotypes 1, 3, respectively, and may overcome this restriction [69, 70]. Nuc polymerase inhibitors targeted against the active center of the NS5B polymerase have the highest potential for genotype non-1 direct antiviral therapy. NS5A inhibitors such as PPI-461 also have been shown to possess the potential for pan-genotype activity in a recently presented phase 1 study [71].

IL28B Genotype

Genome-wide association studies have recently identified single-nucleotide polymorphisms in the region of the IL28B gene on chromosome 19, coding for the interferon-λ-3 or IL28B gene which are strongly associated with treatment response to SOC treatment in patients infected with HCV genotype 1 [72–75]. The good response variant was associated with a twofold increase in the rate of cure. Allele frequencies differ between ethnic groups, largely explaining the observed differences in response rates between Caucasians, African Americans, and Asians.

The IL28B C/C genotype has been shown to be associated with improved early viral kinetics and greater likelihood of RVR and cEVR in HCV genotype 1 patients [75]. The IL28B genotype is likely to aid in clinical decision making with SOC regimens. Future studies will investigate the possibility of individualizing treatment duration and novel regimens according to IL28B type [76].

In an ongoing phase 2 trial with the non-nuc polymerase inhibitor ANA598, it was shown that ANA598 is able to improve RVR and EVR rates in IL28B CT and TT patients where SOC alone is less efficacious (23% vs. 0% and 69% vs. 50% for ANA598 plus SOC vs. SOC alone) [77]. The study shows that the IL28B genotype distribution may be also important for DAA treatment. Furthermore, the knowledge of the IL28B genotype may be relevant for designing early phase clinical trials with small patient numbers (stratification according to IL28B genotype). A modeling study showed that the probability of 10% imbalance is 31%, 18%, and 6% in trials including 60, 120, or 240 patients [78].

Treatment Beyond NS3/4A Protease-, NS5B Polymerase-, and NS5A- Inhibitors

Future Viral Targets

The recent development of DAA focused on the non-structural proteins NS3, NS5A, NS5B. NS4 inhibitors are in development and appear as an attractive approach. The only remaining non-structural protein against which no inhibitors have been presented so far is the NS2 protein. Recent studies better characterized the function and structure of NS2 making it also an attractive anti-HCV drug target.

The entry mechanism of HCV is also a promising target for anti-HCV therapy. While all other targets aim at inhibition of replication of an established infection, entry inhibitors may enable the prevention of de novo infection, i.e., to prevent HCV infection following liver transplantation or accidental needle stick injury. As chronic HCV is the result of continuous de novo infection and turnover of infected cells, entry inhibition could also strongly support the antiviral effect of replication inhibitors.

New Interferons and Immunomodulators

Albinterferon is a long-acting interferon alfa-2b which has to be administered every 2–4 weeks. The phase 3 trials showed that albinterferon has similar SVR rates compared with peginterferon alfa-2a [79, 80]. Due to unresolved safety issues the clinical development of albinterferon has been discontinued.

Interferon lambda targets a different receptor than interferon alfa and may be particularly interesting due to the recent discovery of the association between single nucleotide polymorphisms in the IL28B gene with response to interferon-based therapy. A pegylated interferon lambda recently demonstrated superior RVR rates compared with peginterferon alfa-2a (75% vs. 50%) [81]. Most importantly, interferon lambda has an improved tolerability profile compared with interferon alfa [81].

Further potential combinations are DAA with immunomodulators such as toll-like receptor agonists and/or DAA with cyclophilin inhibitors (e.g., alisporivir).

Summary

Tremendous progress has been made in the understanding of pathogenesis and replication of the HCV since its discovery in 1989. For more than one decade peginterferon alfa/ribavirin is the standard treatment for HCV and no new compounds have been approved. Due to the development of cell culture HCV models and structure determination of HCV proteins, many antiviral targets have been identified. The most promising and clinically advanced direct anti-HCV compounds are inhibitors against the NS3/4A protease and the NS5B polymerase. Further promising targets in the future are entry inhibitors against the envelope proteins E1, E2, assembly inhibitors targeted against core or p7, NS2 protease inhibitors and NS5A inhibitors. Due to the risk of selection for resistant strains, the current protease inhibitors telaprevir and boceprevir have to be administered in combination with peginterferon alfa and ribavirin. The phase 3 trials have shown SVR rates in the order of 69–75% in HCV genotype 1 patients. The future of anti-HCV therapy will focus on the development of interferon-free treatment regimens.

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Mutations and the Development of Anti-viral Resistance

18

Anna Maria Geretti and Geoff Dusheiko

Keywords
Resistance • Quasispecies • Mutation • Fitness • Replicon

Historical Perspective

The Virus Life Cycle and Targets of Antiviral Therapy

The hepatitis C virus (HCV) particle is composed of an enveloped nucleocapsid and a single stranded positive sense RNA (ssRNA+) genome, which is approximately 9.6 kb in size [1]. The viral life cycle starts with the attachment of the virus particle to the target cell through the interaction of the viral glycoproteins E1 and E2 with one or more candidate receptors including tetraspanin CD81 [2, 3] (Fig. 18.1). Following internalisation, an endoplasmic reticulum-derived membrane compartment is the likely site of HCV replication in the cytoplasm [4, 5]. The ssRNA+ serves as a template for the production of both viral proteins and genomic RNA molecules. Analogous with other ssRNA+ viruses, it is thought that the positive-strand genomic RNA serves as a template for the viral RNA-dependent RNA polymerase to transcribe a negative-strand intermediate, which in turn serves as a template to produce new genomic RNA. As there is no DNA intermediate, HCV is not believed to be able to establish a stable genetic reservoir or to integrate in the human genome. Translation of the ssRNA+ initially produces a large polyprotein of around 3,010 amino acids, which is cleaved by cellular and viral proteases into ten products. These comprise the structural core protein C and envelope glycoproteins E1 and E2, the integral membrane protein p7 (a ion channel), and the non-structural (NS) proteins NS2, NS3, NS4A, NS4B, NS5A and NS5B, which have important enzymatic and regulatory functions [1, 6] (Fig. 18.2). All the ten proteins have been considered as potential targets of direct-acting antiviral agents (DAAs) – also referred to as specifically targeted antiviral therapy for HCV (STAT-C). The viral protease encoded by NS3, the viral RNA-dependent RNA polymerase encoded by NS5B and the NS5A protein have been the main targets of drug discovery efforts to date. Other DAAs include entry inhibitors (e.g., ITX506). Furthermore, the identification of cyclophilins, particularly cyclophilin A (CypA), as

A.M. Geretti, MD, PhD
Institute of Infection & Global Health, University of Liverpool, Liverpool, and University College London, London, UK

G. Dusheiko, FCP(SA), FRCP, FRCP (Edin) (✉)
Centre for Hepatology, Royal Free Hospital, Pond Street, Hampstead, London, UK
e-mail: g.dusheiko@ucl.ac.uk

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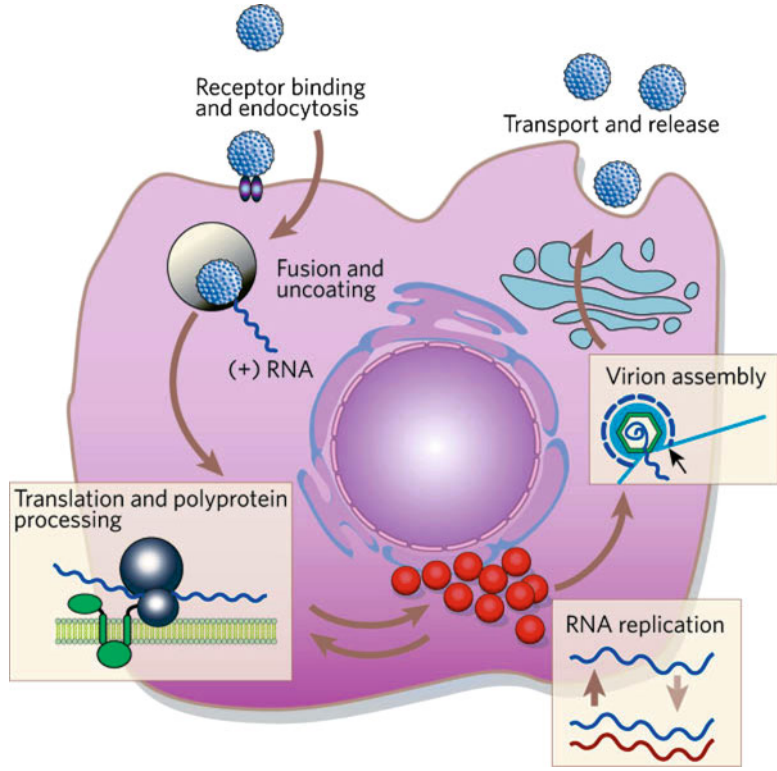

Fig. 18.1 The life cycle of the hepatitis C virus. The virus particle is approximately 60 nm in diameter. After entry into the cell and uncoating, the HCV single-stranded positive sense RNA genome functions in three main roles: translation, replication and packaging into nascent virions, which are released through the cell secretory pathway (reprinted by permission from Macmillan Publishers Ltd.: Lindenbach and Rice [3])

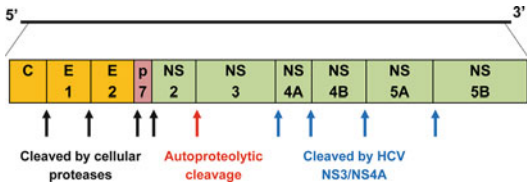

Fig. 18.2 Schematic representation of the hepatitis C virus genome. The single open reading frame is transcribed by the viral RNA-dependent RNA polymerase into a large polyprotein, which is cleaved by cellular and viral proteases into ten products. HCV also encodes a small protein, called F (frame shift) or ARFP (alternative reading frame protein), that can be produced by ribosomal frame shifting into an alternative reading frame within the core gene. Two short untranslated regions are present at both the 5′- and 3′-termini

crucial cellular cofactors for HCV replication [7–9] has led to the development of CypA analogues (Table 18.1).

The Viral Quasispecies

The first genomic sequences of HCV were obtained in 1989 [11]. Over the following two decades, several key properties of HCV were described (Table 18.2). Hepatitis C infection is characterised by high rates of virus replication, with approximately 10^{12} virions produced daily within infected hepatocytes, at a rate of 50 particles per hepatocyte per day [1, 12–14]. Extrahepatic viral reservoirs have also been proposed, including lymphocytes, intestinal epithelial cells and the central nervous system [1, 15], although their clinical significance is still debated. Newly produced virions have a half life of only 3–5 h [1]. As a result, the viral RNA load in serum, typically ranging between 10^3 and 10^7 genome copies per ml, reflects a highly dynamic equilibrium between high levels of virus production and

Table 18.1 Classes of direct acting antiviral agents against hepatitis C undergoing clinical development and their genetic barrier to resistance according to in vitro and where available in vivo studies

Target	Genetic barrier	Class	Sub-classes	Agents[a]
NS3 active site	+/++[b]	Protease inhibitors	Linear peptidomimetic ketoamide derivatives and covalent bond agents	Telaprevir (VX-950); boceprevir (SCH-503034); narlaprevir (SCH 900518)
			Macrocyclic and non-covalent bond agents	Danoprevir (ITMN191/R7227); TMC-435; vaniprevir (MK-7009); BI-201335
NS5B active site	++/+++	Nucleoside/nucleotide polymerase inhibitors		RG7128; PSI-7977
NS5B	+	Non-nucleoside polymerase inhibitors	Thumb 1	BI-207127
			Thumb 2	Filibuvir (PF-00868554)
			Palm 1	ANA598
			Palm 2	ABT-333; GS-9190[c]
NS5A	+/++[d]	N-terminus inhibitors		BMS-790052
Cyclophilin	+++[e]	CypA analogues		Alisporivir (DEBIO-025)

[a] Only representative examples are shown
[b] The genetic barrier may be higher with some compounds compared with others
[c] The binding pocket of GS-9190 appears to involve palm-2 and the β-hairpin close to the catalytic active site of NS5B [10]
[d] The genetic barrier is low in vitro but it is proposed that it may be improved by high drug levels in vivo
[e] Based upon in vitro data

Table 18.2 Key features of the hepatitis C virus infection

Feature	Note	Impact
ssRNA+ genome	Replication occurs without DNA intermediate and without integration in host genome	No genetic reservoir and no latency established
Transcription by viral polymerase	Enzyme shows typical "right hand" structure	Viral polymerase as target of antiviral therapy
Translation produces large polyprotein	Cleavage by cellular and viral proteases to yield mature virus particles	Viral protease as targetof antiviral therapy
High rates of virus replication	10^{12} virions produced daily; greater than observed with HIV or Hepatitis B virus	Dynamic equilibrium of virus production and virus clearance
Short half-life of plasma virus	$t_{1/2}$ 2–5 h	
Low fidelity of viral RNA polymerase	Incorporation of the wrong tri-phosphate nucleoside leads to mutations	Quasispecies containing spontaneously generated drug-resistant variants
Plastic genome	Mutations can enhance virus replication	Strong advantage for adaptation to a new environment

clearance by the immune system. The viral RNA polymerase has low fidelity and lacks proof reading activity. The error rate is estimated at approximately 10^{-4} mis-incorporations per nucleotide per genomic replication cycle [1]. Transcription errors may occur during synthesis of both positive and negative sense RNA. Incorporation of the incorrect tri-phosphate nucleosides leads to mutations in the viral progeny. The combined effect of a high error rate and a high viral replication rate is that all possible mutations in the viral genome, including those that engender drug resistance and immune evasion, can be produced daily in an infected host [16]. While some

mutations may deleteriously affect the virus ability to infect and replicate ("viral fitness"), others may continue to reproduce leading to a complex and continuously diversifying viral progeny – the quasispecies swarm.

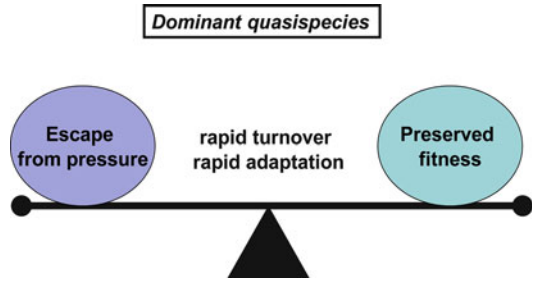

Fig. 18.3 The viral quasispecies as expression of a dynamic equilibrium between escape from selective pressure and preserved ability to infect and replicate ("viral fitness"). The best adapted strains compose the dominant population within the viral quasispecies

Selection, Emergence and Evolution of Drug Resistance

Virus variants carrying mutations in the targets of antiviral drugs are generated spontaneously during HCV replication and pre-exist the introduction of antiviral therapy. Theoretically, all possible single, double or even triple mutants may be present within the quasispecies of HCV-infected patients. The estimated odds for single and double mutants are 10^{-4} to 10^{-5} and 10^{-8} to 10^{-10}, respectively, whereas triple mutants are expected to be extremely rare. Mutations in proteins that play a key role in virus replication generally confer a reduced fitness, especially if affecting an enzyme active site. As a result, in the absence of drug pressure, drug-resistant mutants tend to exist as minority variants and are generally undetectable by routine testing methods. Once antiviral drug pressure is introduced, in the presence of continuing virus replication, the drug-resistant mutants acquire a selective advantage and emerge as dominant within the quasispecies (Fig. 18.3). If the variants continue to replicate, they accumulate further mutations leading to increasing levels of resistance and cross-resistance. Drug resistance carries a fitness cost to the virus which may initially be considerable. Many viral strains resistant to DAA compounds show a decreased fitness [17–20]. In the case of the NS3 protease inhibitor (PI) telaprevir, for example, viral replicative capacity – as an in vitro measure of viral fitness – is inversely correlated with the degree of drug resistance [21, 22]. During ongoing virus replication under drug pressure, however, adaptive mutations can emerge, either in the target of therapy or other regions of the viral genome that compensate for reduced fitness. HCV evolution under drug pressure is therefore characterised by increasing levels of resistance and adaptation towards improved fitness (Fig. 18.4). Once

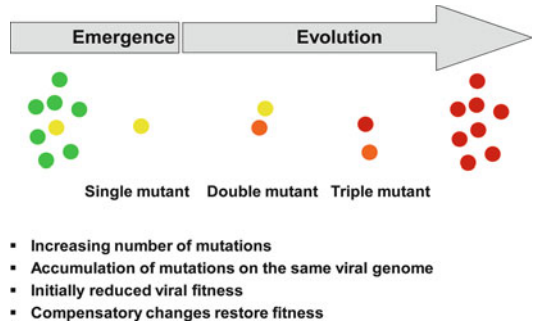

Fig. 18.4 Emergence and evolution of drug resistance. With ongoing virus replication under selective drug pressure, variants with single and double mutations evolve to accumulate further mutations, some of which increase the levels of resistance and cross-resistance, while others at least partially restore viral fitness

drug pressure is discontinued, the resistant mutants lose their advantage and are outgrown by the fitter "wild-type" virus, thus reverting to being minority variants within the quasispecies [21]. The speed of the disappearance from the dominant quasispecies is inversely correlated with the fitness of the resistant variants – the greater the fitness cost of resistance, the more rapid the disappearance of the resistant mutants as dominants species. Once drug pressure is re-introduced, if viral replication continues, the resistant species are reselected and can rapidly re-emerge (Table 18.3).

One important question in HCV resistance is related to the longevity of resistant mutants after drug-selective pressure is discontinued. Given

Table 18.3 Key principles of antiviral drug resistance

Drug-resistant mutants are selected (not created) by drug pressure acting on the pre-existing viral quasispecies when virological suppression is incomplete
Ongoing virus replication under drug pressure leads to the further evolution of resistance and cross-resistance
Resistant mutants often display reduced fitness but acquire compensatory changes over time that at least partially restore virus fitness
Once drug pressure is removed, resistant mutants are outgrown by the fitter wild-type virus and become undetectable by routine tests. The speed of the disappearance from the dominant quasispecies is inversely correlated with the fitness of the mutant
Off therapy, resistant mutants tend to persist at low frequency in plasma and can be reselected once drug pressure is re-introduced
In the absence of genetic reservoirs, the ability of previously selected resistant variants to persist as replicating species is a reflection of both virus and host factors, including viral fitness and immune clearance

the absence of a DNA reservoir, it may be hypothesised that treatment-selected resistant variants eventually disappear, thereby restoring drug susceptibility [23]. Data indicate that after discontinuation of telaprevir, wild-type strains start to increase rapidly within the first 7–10 days and by 3–7 months completely replace resistance variants in the dominant quasispecies [21]. A similar rapid decline of resistant variants has been reported after discontinuation of the NS3 PI boceprevir [24, 25]. Other studies have reported varying rates of "disappearance" of selected viral mutants after stopping DAA therapy [23]. The rates of decline of resistant mutants are likely to vary according to both host and viral determinants, including the presence of compensatory mutations in the resistant variants, which in turn are more likely to emerge during prolonged virus replication under selective drug pressure. A further key consideration is related to the sensitivity of the testing technique. PI resistant mutants such as those involving codons V36 and R155 in NS3 have been shown to persist at low to medium frequency within the quasispecies over at least 3 or 4 years of follow-up [25, 26]. Further prospective studies using sensitive testing techniques are required to address this important issue.

Systems to Study HCV In Vitro

In 1999, the first functional HCV sub-genomic replicon was described that enabled studies of HCV RNA replication in transfected cell lines [27]. The replicon consisted of a genotype 1b HCV RNA engineered to express a selectable marker gene, *Neo* (neomycin phosphotransferase), in place of the structural protein coding region; to direct expression of NS proteins, a heterologous viral IRES (internal ribosome entry site) was inserted after the neomycin resistance cassette. One important discovery was that replicons acquired adaptive mutations during passage in vitro which often affected NS5A and led to improved replicative capacity. Adaptive mutations were subsequently identified throughout the NS region [28]. In addition to the original genotype 1b construct, replicons have been established for other virus types (e.g., 1a, 2, and 1a/1b hybrid) [29]. Sub-genomic replicons typically contain all the NS proteins required for replication of viral RNA in cell lines (e.g., the human hepatoma line Huh-7), but lack the viral structural proteins. Although full-length replicons have also been established, the replicon system does not produce infectious virus and therefore does not mimic the full viral life cycle. Nonetheless, it provides an important experimental tool for the study of viral replication, evaluation of potential antiviral compounds and characterisation of drug resistance and virus phenotype in vitro. Another approach to studying HCV replication is based on the use of full-length replication-competent molecular clones (originally a genotype 2 isolate known as JFH1) which when transfected into permissive cells produce infectious virus [29–32]. A luciferase sequence inserted within the clone typically provides a read out for RNA replication, whereas the yield of infectious virus adds a measure of virus assembly and release, and overall infectiousness.

A full-length HCV cell culture system has also been described, which allows the study of the NS3 protease gene across a range of HCV genotypes [33]. An in vitro assessment of viral function and phenotype can also be obtained from measuring the viral enzymatic catalytic activity, for example, incorporation of tri-phosphate nucleosides by the viral RNA polymerase.

Describing and Detecting Genotypic Antiviral Drug Resistance

Drug resistance can be observed in terms of genotypic or phenotypic changes. Genotypic drug resistance describes the mutations in the viral genome that confer a drug-resistant phenotype. The standard nomenclature is to indicate the position of the mutated amino acid in the protein sequence, proceeded by the amino acid present in the wild-type, drug susceptible sequence and followed by the amino acid detected in the drug resistant sequence. Thus, for example, the A156T mutation in NS3 originates from a substitution of the wild-type amino acid alanine (A) with threonine (T) at position 156 of the protein. Importantly, different substitutions at the same amino acid position can have different impacts on drug susceptibility. For example, the NS3 mutation V36L in the context of a genotype 1 replicon confers only a small in vitro phenotypic effect for telaprevir, whereas V36M and V36A cause greater reductions in drug susceptibility [22]. Genotypic antiviral drug resistance (e.g., in HIV infection) is routinely determined in diagnostic settings by automated population ("bulk") sequencing, which produces a consensus sequence of the dominant variants in the quasispecies. The technique has also been widely applied to the study of HCV drug resistance, but its sensitivity is limited and virus variants present at a frequency below 10–25% escape detection. Several recent methodologies improve the sensitivity of the detection of resistant variants, including clonal analysis, pyrosequencing and ultra-deep sequencing, and allele-specific real-time PCR. It has been shown that both clonal and ultra-deep sequencing provide a more detailed assessment of HCV drug resistance compared with population sequencing [25, 34]. For example, six mutations at residues V36, T54, V55, R155, A156 and V170 of NS3 play a key role in resistance to boceprevir; however, clonal sequencing rather than population sequencing was required to appreciate the full spectrum of resistance [25]. To achieve a level of sensitivity of around 1%, 100 clones must be analysed from each sample, which is very expensive and labour intensive to be adopted in routine diagnostic settings. Other sensitive resistance testing techniques also have limited availability outside of research settings, although it is likely that ultra-deep sequencing or similar techniques will be more widely adopted in the near future. Ultra-deep sequencing automatically and relatively rapidly produces several hundreds of clonal sequences from each sample, allowing high sensitivity of detection (reliable above a threshold of around 1%), and also providing a quantitative estimate of the frequency of a mutant in the quasispecies examined. Factors currently limiting the widespread adoption of this technique include high cost and complex software requirements to allow the rapid analysis of the many viral sequences obtained from each sample. Importantly, given the high sensitivity, interpretative cut-offs are required that discriminate between resistant variants present at clinically relevant frequencies and background mutations that will not impact on virological responses.

Mutations conferring drug resistance are initially identified as changes in the viral sequence observed to emerge either in vitro during virus passage (e.g., using the replicon system) in the presence of drug, or in patients exposed to the drug in vivo. The two approaches should be seen as complementary as observations do not necessarily coincide, in part reflecting the effects of drug concentration on the selection process [21, 24, 35]. A further step is the characterization of the mutant, by studying the phenotype of laboratory strains modified by site-directed mutagenesis to contain the mutation of interest, or performing phenotypic assays with mutated viral genes derived from clinical samples. Further studies address the biochemical properties of the mutant, for example, the ability to bind the inhibitor in vitro, or predict the impact of the mutation by modelling the protein crystal structure. For example, based on the crystal

structure of the NS3 protease, a methionine substitution at position V36 is predicted to cause a loss of direct contact with the phenylalanine residue at position 43, thereby reducing interaction with telaprevir [22].

Describing and Detecting Phenotypic Antiviral Drug Resistance

The activity of a drug is described as the concentration required to inhibit virus replication in vitro by 50% (or 90%) – referred to as the inhibitory concentration $(IC)_{50}$ or the 50% effective concentration (EC_{50}). The IC_{50} of individual drugs varies according to multiple parameters related to the host, the virus and technical aspects of the test. Phenotypic drug resistance describes an increase in the IC_{50} of the resistant virus relative to a control wild-type virus, and the results are expressed as fold-changes in IC_{50} (or EC_{50}). For HCV, various systems including replicons, infectious molecular clones and enzyme assays have been used to assess phenotypic drug resistance, with moderate to good reproducibility of results across different methodologies despite significant differences between assay systems (Table 18.4) [21, 22, 24, 36, 37]. The levels of phenotypic resistance measured in vitro should not be interpreted in absolute terms, but should be seen as drug specific. Although it is intuitive that large fold-changes (e.g., >100) are likely to indicate significant levels of resistance, inferences regarding clinical significance and drug activity should not be made based solely upon the magnitude of the measured fold-change, but will require assessment in vivo. Antiviral activity may be abrogated with just a small measurable phenotypic effect (e.g., 2–3-fold), and conversely significant residual antiviral activity may persist despite relatively large fold-changes. For antiretroviral drugs, interpretation of phenotypic data is aided by the use of cut-offs. Technical cut-offs refer to the reproducibility of the measurements in a given phenotypic assay. Biological cut-offs refer to the range of phenotypic susceptibilities observed with wild-type virus strains circulating among drug-naïve individuals. The most informative cut-offs are clinically established and refer to the level of phenotypic resistance that has a demonstrable impact on treatment outcomes (Fig. 18.5). Although most relevant, clinical cut-offs are also the most difficult to establish as they require assessing drug activity in treatment-experienced

Table 18.4 Impact of single and double mutations in NS3 on phenotypic susceptibility to telaprevir, as determined by either the HCV replicon [21, 22], or the NS3 enzymatic assay [21]

	Replicon [22]	Replicon [21]	Enzyme assay [21]
Variant	Fold-change	Fold-change	Fold-change
Wild-type	1	1	1
V36M	7	7	3
V36A	7	ND	4
V36L	2	ND	ND
V36G	11	ND	ND
T54A	6	6	12
R155T	ND	20	9
R155K	ND	ND	8
A156S	ND	12	22
A156V	ND	>74	195; >781
A156T	ND	>74	285
V36M+R155K	~62	>62	71
V36A+R155K	~40	ND	ND
V36M+R155T	>62	ND	ND
V36A+R155T	>62	ND	ND
V36A+T54A	20	ND	ND
V36M+A156T	>62	ND	>781

The fold change was determined by dividing the replicon IC_{50} of a given variant by that of the wild-type reference

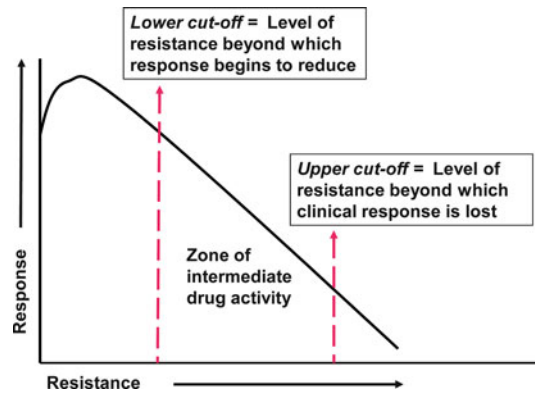

Fig. 18.5 Relationship between drug resistance and virological response. Antiviral drug resistance should be seen as a continuum, with diminishing virological responses as resistance levels increase. Breakpoints or cut-offs can be identified along the continuum to help the interpretation of phenotypic drug resistance data

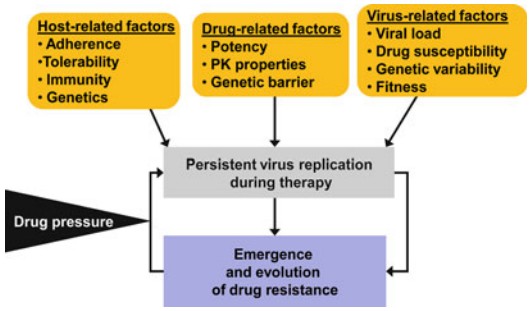

Fig. 18.6 Determinants of drug resistance. Multiple host, virus and drug-related factors influence the emergence of resistance among patients receiving antiviral therapy. Key determinants include the levels of patient's adherence and the drug potency and vulnerability to resistance ("genetic barrier"). The genetic barrier is expression of the number of mutations required to cause loss of drug activity, the magnitude of the impact of each mutation and combination of mutations on the viral phenotype including viral fitness, and drug levels. The virus genome sequence variability can also influence the genetic barrier

patients with various degrees of phenotypic drug resistance. As such, clinical cut-offs for DAAs remain largely unknown.

The Genetic Barrier to Resistance

The emergence of clinically relevant resistant variants in vivo is the result of multiple determinants (Fig. 18.6), including adherence, pharmacokinetics and genetic barrier to resistance. The genetic barrier is in turn expression of the number of mutations required to abrogate drug activity (and thus the flexibility of the inhibitor/target interaction) and the magnitude of their impact on viral phenotype and fitness, and these should be interpreted in the context of the overall level of drug exposure. Importantly, the genetic barrier should be seen as a property of the strength of the whole regimen rather than simply expression of the individual characteristics of each drug. Thus, drugs with a low or moderate genetic barrier to resistance may show sustained antiviral activity when used in the context of an optimised combination regimen. At the same time, increasing drug levels can overcome low to moderate levels of resistance. Insights into the dynamics of HCV drug-resistant variants emerging under selective pressure are offered by passaging replicons in vitro in the presence of different drug concentrations. When the HCV genotype 1b replicon is cultured in the presence of low, high, or stepwise-increasing concentrations of the NS3 PI TMC380765, distinct mutational patterns emerge depending on the concentration of the drug [34]. In these studies, culturing at low drug concentrations resulted in the selection of low-level resistance mutations (F43S and A156G), whereas high drug concentrations resulted in the selection of high-level resistance mutations (A156V, D168V, and D168A), reflecting suppression of the variants with lower levels of resistance.

Due to faster replication kinetics, HCV is considered to be more prone to developing drug resistance than HIV and indeed antiviral drug resistance is increasingly described with DAAs (Table 18.1). However, the genetic barrier to resistance varies with different anti-HCV agents according to their mechanism of action and modality of use, and is influenced by both the viral genetic sequence and drug levels. While NS3 PIs and NS5B non-nucleoside polymerase inhibitors (NIs) have demonstrated good antiviral activity in clinical trials, they have also shown vulnerability to the emergence of drug resistance [38, 39]; the genetic barrier appears to be lowest for NNIs [38–44], followed by the PIs, although potential differences between "first generation" and "second generation" PIs require further characterisation [21, 24, 45–50]. The nucleoside/nucleotide NS5B polymerase inhibitors (NIs) appear to select for drug resistance more slowly than PIs and NNIs, both in vitro and in vivo [51–55]. NS5A inhibitors, on the other hand, appear vulnerable to the selection of drug resistance in vitro [56, 57], although it has been proposed they may prove more resilient in vivo due to their high predicted drug levels relative to the IC_{50} of single resistant mutants. CypA analogues, in addition to displaying high antiviral potency, show less rapid selection of drug resistance relative to other DAA classes [58]. Importantly, variants resistant to one DAA class retain susceptibility to agents from other classes (and to pegylated interferon alpha and ribavirin), underscoring the potential utility of drug combination approaches.

Current Treatment

Pegylated Interferon Alpha and Ribavirin

The low response rates to pegylated interferon alpha (Peg-IFNα) and ribavirin (RBV) observed in patients infected with HCV genotype 1 are the likely the effect of both host and viral determinants, reflecting the multiple mechanisms of action of the two compounds. Genome-wide studies have tested the association of hundreds of thousands of single nucleotide polymorphisms (SNPs) in the human genome and response to Peg-IFNα/RBV. A number of SNPs near the IL28B locus (commonly referred to as "IL28B polymorphisms") have been shown to correlate with the outcomes of treatment [59–62]. The IL28B gene encodes IFN lambda, but the mechanism underlying the described associations has not been fully elucidated. Two SNPs – rs12979860 and rs8099917 – in particular are significantly associated with responses to Peg-IFNα/RBV, with the CC genotype for the former and the TT genotype for the latter predicting high responsiveness. The predictive value and clinical utility of IL28 polymorphism testing for decision making in the context of DAA-based therapy remains to be determined and such studies are in progress. One study reported a correlation between rs8099917 genotype TT (and substitution at amino acid 70 of the HCV core protein) and sustained virological response [63]. Classically defined antiviral resistance pathways have not been elucidated for Peg-IFNα and RBV. No single site in the HCV genome has consistently been found to be associated with complete resistance to IFN, most likely because this drug acts more indirectly through several antiviral pathways [64]. Mutations in NS5A and NS5B have been observed in patients receiving RBV monotherapy and associated with resistance to RBV in vitro [65–67], but their clinical significance is unclear as they do not appear to affect responses in vivo. Although RBV is a nucleoside analogue, it cannot be classed as a typical DAA due to its multiple proposed mechanisms of antiviral activity. These include but are not limited to effects mediated by its tri-phosphate derivate, and combine with immune-modulatory properties [68–71]. Increased viral mutagenesis, which is one proposed mechanism of action for RBV, may in theory enhance HCV ability to escape drug pressure with co-administered DAAs [67, 72]. This is an interesting line of investigation that currently has limited corroborating evidence.

Protease Inhibitors

NS3 is a multifunctional protein, with an N-terminal serine protease domain and a C-terminal RNA helicase/nucleoside triphosphatase domain. The serine protease activity of NS3 is important for the processing of the viral polyprotein, whereas the helicase function is involved in the early phase of virus assembly [73]. NS3, together with its cofactor NS4A, catalyses cleavage at the NS3-NS4A junction and downstream junctions (NS4A-NS4B, NS4B-NS5A and NS5A-NS5B), with residues H57, D81 and S139 forming the catalytic triad [74–76]. Several chemical classes of potent NS3 inhibitors have been developed, including linear peptidomimetic ketoamides such as telaprevir and boceprevir, which are now entering routine clinical use, and macrocyclic inhibitors (e.g., danoprevir, TMC-435, and vaniprevir) which are in advanced stages of development (Table 18.1). The risk of virological failure and selection of drug resistance poses a concern with NS3 PIs, particularly when used as monotherapy or in dual combination with peg-IFNα, whereas use in combination with peg-IFNα/RBV augments the activity of the overall regimen and improves the genetic barrier of the PI [21, 24, 25, 48, 77–80]. Responsiveness to the peg-IFN/RBV backbone is clearly a prerequisite of sustained activity [45, 81]. As shown with boceprevir [48], the use of a lead-in phase with peg-IFNα/RBV prior to the introduction of the PI may reduce the risk of virological failure and resistance, at least among patients responsive to peg-IFNα/RBV.

The most common PI resistance mutations detected in clinical studies to date are located at

Table 18.5 Key amino acid residues in NS3 associated with resistance to protease inhibitors[a]

NS3 codon	Telaprevir	Boceprevir	Narlaprevir	Danoprevir	TMC-435	Vaniprevir	BI-201335
V36	■	■	■				
T54	■	■	■				
Q80					■		
R155	■	■	■	■	■	■	■
A156	■	■	■	■	■	■	
D168				■	■	■	■
V170	■	■	■				■

[a]Mutations detected in vitro, in vivo, or both

positions V36, T54, R155, A156, D168 and V170 of NS3. A156V/T and R155K/T are within or close to the enzyme active site and play a key role in resistance. Mutations at codon R155 generally cause small shifts in phenotypic susceptibility and have a small impact on viral fitness, whereas those at codon A156 cause larger fold-changes and reductions in viral fitness [21, 82]. There is a significant potential for cross-resistance within the PI class, facilitated by the geometry of the enzyme active site, which offers a limited number of contact points for the inhibitors (Table 18.5). Certain PI resistance mutations appear to have a more specific effect however [83]. V170A, for example, confers a higher level of resistance to boceprevir than telaprevir, whereas A156S confers a higher level of resistance to telaprevir than boceprevir. Furthermore, mutations at codon D168 do not cause significant shifts in phenotypic susceptibility to the ketoamide inhibitors [82]. TMC-435 has been shown to remain active in vitro against replicons with certain specific mutations associated with telaprevir or boceprevir exposure, including most replicons with changes at positions 36, 54 and 170 [49]. The implications in terms of the potential for sequential NS3 PI therapy remain to be determined in clinical studies.

There is a high background of single and possibly double PI resistant mutants in the HCV quasispecies [78, 84, 85], and resistant variants can become dominant with the quasispecies within only a few days of starting PI treatment [21]. The genetic barrier of newer PIs may differ from that of first-generation compounds, as suggested by the low rates of resistance seen to emerge in patients receiving TMC-435 [86, 87], but further clinical data are awaited. In one study, using a clonal resistance testing method with a lower limit of detection of 5%, the PI resistance mutation R155K was found to be undetectable at baseline, but present at a frequency of 5–20% by day 4 of telaprevir monotherapy [21]. R155K is located in the catalytic site of the protease. The mutation confers resistance to telaprevir and cross-resistance to various other NS3 PIs (e.g., boceprevir, danoprevir, TMC435 and BI-201335) [20, 21, 45, 49, 50, 82, 88, 89].

Table 18.6 Replicative capacity of major drug resistant NS3 mutants

Replicative capacity	Mutant
Preserved	V36A/L/M, R109K, D168E, I170A
Moderately reduced	V36G, F43S, T54A, Q80R, R155K/T/G/Q, A156S, D168A/H/V/N, I170T
Severely reduced	S138T, R155G, A156T/V, D168G

Based on data from ref. [83]

Understanding the impact of PI resistance on virus fitness is important, since fitness is one critical determinant of both the background frequency of mutants within the quasispecies, and their rate of emergence during treatment. In the replicon system, PI-resistant mutants show modest (e.g., V36A and R155K) to significant (e.g., A156T) reductions in replicative capacity and some discordant results have been reported [18–20, 22, 88–90]. A comprehensive assessment of viral fitness can be obtained using infectious molecular clones. Based upon luciferase activity (as a read out for RNA replication), modest to large reductions in the replicative capacity of PI resistant mutants have been observed [83] (Table 18.6). With the same mutants, the yields

of infectious virus also vary significantly and generally correlate well with replicative capacity. However, some mutants (e.g., F43S, R155T, A156S and I170A/T) demonstrate a greater impact on yield than on RNA replication. This observation points to additional effects of PI resistance mutations downstream of RNA replication, and specifically affecting intracellular virus assembly and release [83]. The clinical significance of these observations remains to be established. Resistance mutants have the ability to acquire adaptive changes during replication, which cannot be easily appreciated in short-term in vitro experiments.

Baseline Resistance

R155K and other PI resistance mutations such as those at residues V36, T54 and D168 of NS3 have been detected in some populations of treatment-naïve patients [78, 84, 91–93]. A case report observed the occurrence of R155K as dominant species in a DAA-naïve, HIV-negative liver transplant recipient infected with HCV genotype 1a [91]. R155K was detected by population sequencing in the HCV strains recurring 1 month after transplantation, and detection was stable over a 2-month period. R155K was similarly observed as a dominant species in a DAA naïve, HIV-positive patient infected with HCV genotype 1a who was profoundly immunocompromised with a CD4 count of 62 cells/mm^3 [93]. During a follow-up lasting nearly 6 years, R155K initially persisted as either the sole dominant species or as a mixture with wild-type virus. At later time points, however, only wild-type virus was observed, coinciding with an increase in the patient's CD4 count to >300 cells/mm^3. These findings suggest that high rates of HCV replication in the setting of immune compromise may allow the emergence of R155K in the absence of drug selective pressure. Drug resistance mutations may also emerge as a result of selection by the immune response, where they occur in regions that overlap with T-cells epitopes. The 155 position, for example, is contained within an epitope recognised by CD4 and CD8 T-cells [94, 95]. However, detection of dominant R155K in the setting of profound immune compromise argues against the hypothesis of immune selection. In any case, in order to compete effectively with wild-type virus within the dominant quasispecies, drug-resistant mutants must show good levels of fitness in the absence of drug. This may reflect either a modest fitness effect of the mutation, or the presence of adaptive changes in the viral genome.

In large surveillance studies, the prevalence of R155K in treatment-naïve patients infected with HCV genotype 1a has been described as <1% overall [78, 84]. Using population sequencing, Kuntzen et al. investigated the prevalence of known resistance mutations affecting several DAA classes in 507 treatment-naïve patients infected with HCV genotype 1 from the United States, Germany and Switzerland. Mutations in NS3, NS4A and NS5B known to be associated with drug resistance were observed at individual prevalence rates ranging between 0.3 and 2.8% (Fig. 18.7). Of note, no patients showed substitutions at NS3 residue A156, consistent with their significant impact on viral fitness. Mutations in NS4A and NS5B were rare in this population. However, the NS5B mutation M423V/I, which plays a key role in NNI resistance [44, 96], occurred in 2.8% of patients with genotype 1a. Taken together, recognised resistance mutations occurred in 8.6% of patients infected with genotype 1a and 1.4% of patients infected with genotype 1b. When the analysis was more conservatively restricted to resistance variants described during treatment in vivo, 5.0% of patients with genotype 1a and 1.4% of those with genotype 1b harboured a resistance mutation. The majority of resistant variants represented sporadic cases rather than locally spread, closely related viral strains or mutants specifically selected within a certain subgroup of patients. Furthermore, viral loads were similarly high in patients with and without resistance mutations, suggesting a good fitness of the resistant variants. These findings are of concern, as the limited sensitivity of population sequencing implies that an even higher prevalence of drug resistance, particularly to PIs and NNIs, may be revealed by using more sensitive detection methods.

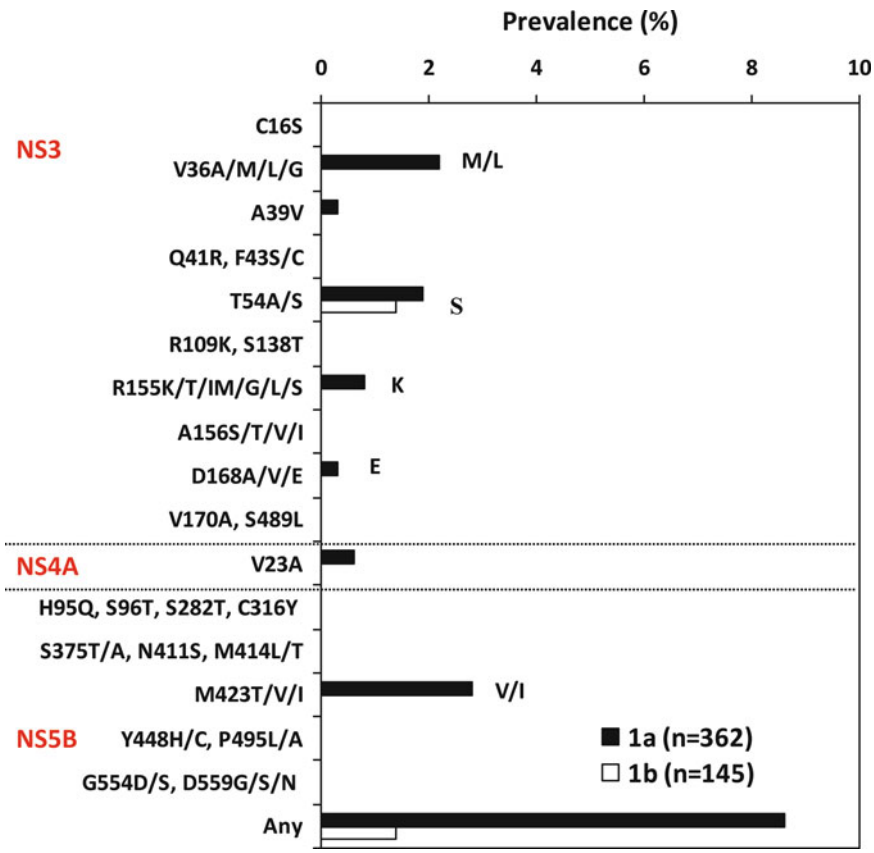

Fig. 18.7 Prevalence of resistance mutations in treatment-naïve patients as determined by population sequencing of NS3, NS4A and NS5B. The amino acid residues detected in the mutants are indicated (based on data from ref. [84])

Based upon the well-characterised impact of baseline drug resistance on treatment responses in HIV-infected patients, the observations made in treatment-naïve HCV-infected patients have led some investigators to recommend routine baseline resistance testing prior to starting DAA therapy [93]. In HIV infection, baseline resistance testing is cost effective when the prevalence of drug resistance is >1% in a given population. In this setting, resistance testing is aimed at detecting transmitted drug resistance, as the natural background of resistant mutants is generally <0.1–1% and therefore below the detection limit of routine testing methods [97]. For DAAs, baseline testing would be aimed at detecting high-frequency background resistance mutants, although the possibility of transmitted drug resistance has not been ruled out and may well become significant in the future. At a prevalence rate of 5–8.6% in patients with genotype 1a, pre-treatment screening by even a relatively insensitive method might be warranted. However, data on the impact of pre-existing resistant variants on treatment outcomes are currently limited and further studies are required to evaluate the cost effectiveness and clinical utility of routine testing and the optimal target population. One additional question relates to the preferred method for resistance testing in this setting, in order to achieve the optimal – as yet to be defined – level of sensitivity.

The Impact of HCV Genetic Variability

HCV genetic variability affords an important influence on drug activity and resistance pathways. HCV comprises at least six HCV genotypes

and more than 100 subtypes, differing by 31–33 and 20–25%, respectively, in their nucleotide sequences [98]. While most data on DAAs have been obtained in genotype 1 infection, it is becoming apparent that the overall activity of these agents varies across genotypes and to some extent subtypes, particularly for the NNIs but also the PIs [21, 24, 39, 42, 44, 45, 80, 99–107]. Telaprevir and boceprevir for example, show reduced activity against genotypes other than 1, and against genotype 1a compared with genotype 1b. HCV heterogeneity is not distributed uniformly across the genome and compounds that target conserved regions (i.e., the active site of the NS5B polymerase) are expected to show more consistent activity across genotypes and subtypes [39, 53, 55, 108–110]. HCV genetic variability also influences the emergence and pathways of resistance, and the overall genetic barrier to resistance. This is exemplified by the PI resistance mutation R155K in NS3. The arginine to lysine change at position 155 requires one nucleotide substitution in genotype 1a (AGA → AAA), whereas two substitutions are required in genotype 1b (CGA → AAA). This implies that the genetic barrier for the emergence of R155K is higher for genotype 1b compared with genotype 1a. Consistent with this observation, therapy with telaprevir or boceprevir shows higher rates of resistance mutations among patients infected with genotype 1a compared with those infected with genotype 1b [21, 45, 79, 80, 111]. The number of nucleotide changes required for a mutation to occur is only one possible mechanism that explains sequence-related difference in the emergence of resistance [112]. Additional determinants may include the molecular configuration of the drug binding site and its mode of interaction with the inhibitor. Furthermore, there is evidence that the same mutation may have different phenotypic effects depending on the sequence context into which it occurs [56]. One important implication of these considerations is that observations made in vitro or in vivo with a certain HCV genotype or subtype may not necessarily apply across the range of virus variants.

The Role of Pharmacokinetics

Antiviral drug resistance should be seen as a continuum and achieving high drug levels can both reduce the likelihood of selecting drug-resistant mutants and overcome low to intermediate levels of drug resistance. This concept was first illustrated in 1997 with PIs used in the treatment of HIV infection. A small dose (typically 100–200 mg once or twice daily) of the PI ritonavir combined with a full-dose second PI was shown to enhance ("boost") exposure to the latter, thereby preventing or overcoming drug resistance. In addition to improving the genetic barrier, the increased bioavailability of the boosted PI also reduces the potential impact of host-determined variations in drug pharmacokinetics, minimises food restrictions, and allows less frequent dosing. At the same time, the boosting effect has the potential to increase the incidence of side effects, in a dose-dependent manner. Ritonavir is a potent inhibitor of the cytochrome P-450 CYP3A4 isoenzyme and to a lesser extent of CYP2C19, and the inhibition is believed to mediate most of its boosting effects. CYP3A4 is present in the intestinal tract and liver (and other areas of the body) and is the primary enzyme involved in the metabolism of many PIs. The effects of ritonavir modify the pharmacokinetic properties of the co-administered PI, including area under the curve (AUC), maximum (peak) concentration (C_{max}), minimum concentration (C_{min}), trough concentration (at end of dosing interval and before next administration) (C_{trough}) and half-life ($t_{1/2}$). The impact on each of these parameters varies according to the pharmacokinetic properties of the co-administered PI. Overall, the optimal dosing for an antiviral drug is one that ensures drug levels remain consistently above the IC_{50}.

One important concept in resistance is that with antiviral agents showing a clear dose–effect relationship, the C_{min}/IC_{50} ratio or inhibitory quotient (IQ) summarises the relationship between drug levels and virus susceptibility. The higher the IQ, the greater the expected activity of the drug. As drug-resistant strains show increased IC_{50}, raising the C_{min} improves the IQ and can

overcome resistance. Although a very promising drug class for the treatment of HCV infection, many NS3 PIs have a relatively narrow therapeutic index and require high drug concentrations (and a demanding dosing schedule) to obtain virus suppression and prevent the emergence of resistance. Ritonavir boosting does not offer a suitable option for telaprevir or boceprevir. However, it increases the levels of other NS3 PIs including danoprevir, which is boosted by 18-fold 12 h after dosing, with an effect on C_{min} that is approximately six and three times greater than the effect on C_{max} and AUC, respectively [113]. Ritonavir also boosts the levels of the NS3 PI narlaprevir allowing administration at the dose of 400 mg twice daily rather than 800 mg three times daily without ritonavir [114]. It is anticipated that pharmacokinetics considerations will play an important role in the treatment of HCV, not only when considering the boosting effect of ritonavir, drug activity and genetic barrier to resistance, but also in the context of potential drug–drug interactions between DAAs within combination regimens, and between DAAs and concomitant medications.

Future Treatment

The development of DAAs offers new opportunities to overcome limited response rates to Peg-IFNα/RBV, particularly in genotype 1 infection [13, 21, 115, 116], and to circumvent the toxicity effects and contraindications of standard therapy. DAAs are currently being introduced as an add-on therapy to the standard of care of Peg-IFNα/RBV. While RBV may provide an option for combination regimens without Peg-IFNα [117, 118], its toxicity remains a concern. There is considerable hope that DAA combinations without Peg-IFNα and RBV may in the not very distant future offer an effective and well-tolerated strategy for treating HCV infection [119]. It is likely that accomplishing this promise will require the combination of at least two highly potent or at least three mechanistically distinct inhibitors with non-overlapping pathways of resistance and a total genetic barrier of at least four mutations, to ensure sustained antiviral activity [85, 120–122].

Polymerase Inhibitors

NS5B encodes the viral RNA-dependent RNA polymerase. The enzyme has a typical "right hand" structure [123–125], with catalytic sites in the base of the palm domain, surrounded by thumb and finger domains, together forming a channel that binds the viral RNA, and with key interactions involving the active site aspartic acid residues 220 and 318. The RNA polymerase is responsible for synthesis of both positive and negative strands of HCV RNA and it is essential for viral replication. Two main categories of NIs have been developed – compounds with a 2′ C-methyl group (e.g., RG7128, a prodrug of the nucleoside analogue PSI-6130) and compounds with a 4′ azido group (e.g., R1626, a prodrug of the nucleoside analogue R1479 no longer undergoing development). NIs, after intracellular phosphorylation to the tri-phosphate active forms, bind competitively with tri-phosphate nucleoside substrates to the active site of polymerase and, once incorporated, serve as chain terminators to block further extension of the viral RNA strand. Due to the high degree of conservation of the enzyme active site, the class offers the promise of preserved activity across HCV genotypes and subtypes, and a higher genetic barrier to resistance than NNIs and PIs. Consistent with this view, no resistant variants have been observed to emergence during NI monotherapy for up to 2 weeks using either population or clonal sequencing [41, 53, 54, 126, 127]. In vitro, the NS5B mutation S282T confers low level resistance to RG7128 [128] and other NIs (e.g., valopicitabine, no longer undergoing development), but the mutation has a significant impact on viral fitness and emerges slowly in vivo [109, 110, 126, 129, 130]. In a clinical trial, S282T emerged in around 2% of patients receiving valopicitabine monotherapy for approximately 6 months [51]. Although S282T is a key NI resistance mutation, two antagonist resistance patterns have been detected suggesting that combinations of different NIs may prove advantageous in terms of genetic barrier to resistance [41, 129, 131].

Resistance mutations affecting the NIs do not tend to pre-exist at high frequency within the HCV quasispecies and have not been commonly detected

in untreated patients [84, 127]. In one study, S282T was not detected in baseline samples of treatment-naïve patients using an assay with sensitivity >1% [127]. These observations are likely to reflect a significant impact of NI resistance mutations on viral fitness. S282T significantly reduces viral replicative capacity in genotype 1, 3 and 4 strains, and abolishes the replication of genotype 2 strains [54]. However, selection of compensatory mutations in the NS5B gene may facilitate the emergence of resistant variants in vivo during prolonged treatment [128].

NNIs are allosteric, non-competitive polymerase inhibitors that comprise several chemotypes including benzimidazole, thiophene and dihydropyranone, benzothiadiazine, and benzofuran analogues, each biding to different pockets of NS5B: thumb-1, thumb-2, palm-1 and palm-2. Key resistance mutations involve codons P495, P496 and V499 in thumb-1, L419, M423 and I482 in thumb-2, N411 and M414 in palm-1, and C316 and S365 in palm-2. In addition, imidazopyridines (e.g., GS-9190) select for drug resistance mutations in the palm domain (C316Y) and inside the beta-hairpin loop (C445F, Y448H, Y452H). The activity of some NNIs such as those binding to thumb 1 extends beyond genotype 1, but many only inhibit genotype 1 due to naturally occurring resistance polymorphisms in other genotypes [101, 102, 132]. Some compounds also show significant variation in activity in genotype 1a compared with genotype 1b [44, 100–103, 132]. HCV-796, for example, showed limited clinical efficacy due to the presence C316N as a polymorphism occurring naturally in a large proportion of genotype 1b strains [133].

In line with the above considerations, mutations conferring resistance to the NNIs exist at high frequency within the HCV quasispecies and some (e.g., those at position M423 of NS5B) may be detected as dominant variants in treatment-naïve patients [78, 84, 92, 127, 134, 135]. In one study of 92 treatment-naïve patients with HCV genotype 1, 21% showed pre-existing NNI mutations [127]. The frequency of the mutations within the HCV quasispecies was 1–3%, a relatively high background that can result in the rapid enrichment of resistant mutants under selective drug pressure. Accordingly, NNI resistance mutations have been observed to emerge within a few days of drug exposure, both in vitro and in vivo [40, 43, 44, 76, 133, 135–139]. For example, emergence of drug resistance and viral rebound were observed within 14 days of monotherapy with the NNI-4 HCV-796 (no longer undergoing development) [138]. There is overlap in the resistance profiles of NNIs that bind in the same pockets. Mutations at codon M423 play a key role in resistance to several NNIs including, for example, filibuvir, PF-00868554 and AG-021541 [43, 95]. Although it has been suggested that cross-resistance between the different subcategories of NNIs may be limited [96], there is overlap between the two palm sites, causing a high likelihood of cross-resistance.

NS5A Inhibitors

NS5A is a multifunctional phosphoprotein that has no known intrinsic enzymatic activity, but is believed to be involved in HCV replication and assembly through interactions with cellular and viral factors, including NS5B [140, 141]. NS5A is composed of a membrane anchoring N-terminus (amino acids 1–30) and three distinct structural domains: I (amino acids 37–213), II (amino acids 250–342), and III (amino acids 356–447). BMS-790052, the first DAA to target NS5A, shows potent antiviral activity by interacting primarily with the N-terminus of NS5A. In vitro passage of genotype 1a and 1b replicons with BMS-790052 leads to multiple resistance mutations in the membrane anchoring N-terminus and domain I of NS5A (key codons 28–32 and 93) [56, 57], with no evidence of cross-resistance to CypA analogues in vitro [57]. The major resistance mutations include L28T, L31F/V, P32L and Y93H in genotype 1b and M28T, Q30E/H/R, L31M/V, P32L and Y93C/H/N in genotype 1a [56]. Although the threonine substitution at residue 28 confers resistance in both genotypes, in vitro this mutation is only selected with genotype 1a, likely because two nucleotide changes are required in the 1b replicon (L28T = CTC → ACC) but only one change is required in the 1a replicon (M28T = ATG → ACG) [56]. Other important, but less easily explained, differences

are observed in resistance mutations at residues 30 and 31. In general, while the overall patterns of resistance between genotypes 1a and 1b appear to be similar, there are also important differences. Greater resistance effects are caused by most mutations in genotype 1a than in genotype 1b, indicating that the sequence context of a particular resistance mutation modulates its phenotypic effects [56, 57]. Across HCV genotypes, genetic variation is observed at several of the residues identified as important NS5A resistance sites in genotype 1 (e.g., codons 30, 31, and 93). For example, residue 31 is methionine (M) in genotype 4a. Despite this variation, in vitro BMS-790052 shows preserved activity against a range of HCV genotypes [57]. Many NS5A resistant mutants display reduced fitness in vitro, which may limit their ability to emerge in vivo. Furthermore, it has been proposed that plasma concentrations of BMS-790052 will easily exceed the IC_{50} of single mutants, which may enhance the genetic barrier and antiviral efficacy [57]. The clinical significance of these observations remains to be established.

CypA Analogues

Cyclophilin A, via its isomerase pocket, binds NS5A and also interacts with NS3 and NS5B. Disrupting these interactions stops HCV replication [142]. CypA analogues are among the most potent HCV inhibitors reported to date. Although the mechanism of action has not been fully elucidated, they are believed to act by preventing key conformational changes during virus replication [58]. Resistance to CypA analogues can occur, predominantly involving mutations in domains II and III of NS5A, indicating that the interaction of cyclophilin with NS5A is the most critical [143, 144]; D320E in particular appears to mediate most of the resistance. Alisporivir – like other members of the class (e.g., NIM811) – selects D320E in NS5A, which confer <20-fold resistance to these compounds. In addition, substitutions at other residues (e.g., 321 and 356) are involved in resistance to CypA analogues in vitro [145–147]. However, selection of resistant mutants occurs slowly in vitro [58, 147, 148]. With alisporivir, for example, selection of resistant replicons (genotype 1b) requires an average of 20 weeks, compared with the typically <2 weeks observed with PIs and NNIs [58]. This may reflect the fact that resistance mutations do not act by preventing direct binding of the inhibitor, but rather make the virus less dependent on a host factor that is otherwise essential for viral replication. Evidence is awaited to confirm whether these observations transfer to the clinical scenario.

Combination Therapy

The success of DAA combinations will depend on their ability to inhibit the replication of a broad range of viral quasispecies and prevent emergence of drug-resistant mutants. While there appears to be no cross-resistance between DAAs with different mechanisms of action, multi-drug resistance can emerge with modestly to moderately potent combinations [117, 118, 149–152]. The fitness cost of drug resistance means that treated patients may experience an initial decline in viral load through both elimination of the dominant, drug susceptible viral species and reduced fitness of the drug resistant variants. In addition, antiviral activity may be enhanced by synergistic interactions between DAAs [153, 154]. Thus, it can be proposed that, as seen with antiretroviral therapy against HIV, the combination of multiple DAAs may achieve virological control before the emergence of resistance and adaptive mutations. The DAAs currently entering clinical use or in late-stage development are inhibitors of the viral NS3 protease, NS5B polymerase and NS5A protein. Albeit to a different extent, these agents are vulnerable to the emergence of drug resistance. CypA analogues retain activity against variants resistant to other classes of DAAs [155] and offer the promise of both a high genetic barrier to resistance and a favourable safety profile, suggesting that they may offer a useful backbone of future combination therapy [148], as well as an essential component of rescuing regimens for patients who failed other DAAs treatment.

With both NS3 PIs and NS5B NNIs, the natural background of drug resistance in treatment-naïve patients and the rate of emergence of drug-resistant mutants during therapy appears to be high. Although the NS3 PIs appear vulnerable to the problem of resistance, as a class they offer the potential for augmented activity and improved genetic barrier through increased drug exposure, provided toxicity is not concomitantly enhanced. However, because PI cross-resistance appears to be extensive, there is limited scope for combining drugs from this class, whereas a combination with other DAA classes such as NS5B polymerase and NS5A inhibitors is more promising [110, 122, 156, 157]. Several proof of concept studies are in progress exploring dual combinations of a PI with an NNI (e.g. GS-9256 plus GS-9190) or an NS5A inhibitor (BMS-650032 plus BMS-790052) [117, 151]. The INFORM study investigated the combination of the PI danoprevir with the NI RG7128 in small groups of treatment-naïve and treatment-experienced patients and reported no emergence of resistance to either drug by population sequencing [110]. In contrast, viral breakthrough and dual-class resistance have been observed with the combination of GS-2956 plus GS-9190 [118] and BMS-650032 plus BMS-790052 [152]. Preliminary data from these combination studies also indicate a dose-related effect on responses. For example, with the combination of the NS3 PI BI-201335 with the NNI BI-207127 plus ribavirin, lower doses of BI-207127 (400 mg three times daily) resulted in lower response rates with subtype 1a than observed with subtype 1b, whereas at higher doses (600 mg three times daily) response rates were similar between subtypes [117]. Dual-class NS3 (R155K)/NS5B (P495L) resistance was observed in one patient showing virological breakthrough. The interactive toxicities of these agents are being intensively studied.

Clinical Considerations

A necessity for Peg-IFNα/RBV remains with the DAAs currently entering clinical practice. In this context, the merit of starting therapy with or without a lead-in phase of Peg-IFNα/RBV when using compounds vulnerable to the rapid selection of drug resistance requires further evaluation. Furthermore treatment stopping rules need to be clearly defined for patients who do not show sufficient sensitivity to either a lead in phase with PEG-IFN/RBV or to DAA combination therapy. To assist the decision process, the potential role of IL28B polymorphism testing in order to predict the activity of the Peg-IFNα/RBV backbone and guide the use of the DAA require clinical evaluation. As discussed, some DAA resistant variants may be present at high frequency prior to the introduction of therapy. As yet, there is no clear argument for baseline resistance testing, although testing may become necessary, for example in patients who show reduced genetic sensitivity to Peg-IFNα/RBV.

Data are also needed to inform stopping rules for patients showing insufficient suppression with Peg-IFNα/RBV/DAA combinations, with the aim of preventing extensive resistance and cross-resistance and the emergence of adaptive mutations that may “fix” the mutants within the quasispecies. In these patients, improving the chance of a sustained virological response with prolonged use of the DAA will require balancing against the risk of complex resistance patterns. The overall therapeutic implications of selecting drug resistance require careful consideration in patients who do not have rapidly progressive disease or advanced fibrosis. At the time of writing, patients should not be deterred from treatment but a discussion of antiviral resistance with the patient has become necessary. Adherence will also need discussion, monitoring and ongoing support. Physicians will need education regarding the inadmissibility of PI dose reductions in patients with toxicity.

Many other questions remain regarding the imminent use of DAAs in clinical practice. Which patients should be treated first, treatment-naïve or treatment-experienced patients? Among treatment-experienced patients, should one distinguish between those with prior relapse and those with prior non-response? Should patients with advanced disease be prioritised for treatment on the basis of clinical need, given the limited experience with

using DAAs in the presence of cirrhosis and the potential impact of cirrhosis on DAA metabolism? What will be the treatment options for patients who fail combination of telaprevir or boceprevir with Peg-IFNα/RBV? How should the DAAs be used in special groups such as HIV-HCV co-infected patients and liver transplant recipients? What is known about drug interactions with substitution therapy such as methadone or buprenorphine or calcineurin inhibitors? The development and implementation of rapid and cost-effective resistance testing assays, which are able to detect resistance mutants with a clinically relevant sensitivity, is a key diagnostic priority to support the use of DAA agents in clinical practice, but what additional resources will be required for resistance testing, and for increasing capacity in both clinics and laboratories? With these and many other questions unanswered at present and the critical gaps in our knowledge, it is likely that specialist centres with appropriate virological support will assume the initial responsibility for treatment with DAAs.

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Peginterferon, Ribavirin and Anti-viral Triple Therapy

19

Patrick Marcellin, Nathalie Boyer, and Tarik Asselah

Keywords
Boceprevir • Telaprevir • Pegylated interferon • Ribavirin • Protease inhibitors • Polymerase inhibitors • Interferon lambda • IL28B

Abbreviations

DAAs	Direct acting antivirals
SVR	Sustained virological response
RVR	Rapid virological response
PEG-IFN	Pegylated-interferon
RBV	Ribavirin

Historically, advances have been made in treatment with a combination of PEG-IFN and RBV. At present, in a patient with hepatitis C, therapy results in a sustained response in approximately 55% of cases [1–4]. In patients with HCV genotype 2 or 3, the SVR rates reach 80%; in genotype 1 patients, the SVR rates reach 50%. Patients achieving SVR-24 weeks after completing antiviral therapy can be considered clinically cured of viral infection. Recently, it has been proposed that a 12-week post-treatment follow-up might be as relevant as 24 weeks to determine the sustained virologic response in patients with hepatitis C virus receiving pegylated interferon and ribavirin [5]. Based on existing results, the SVR with this treatment option appears to be long lasting, associated with histologic benefit and with a reduction in the risk of cirrhosis and hepatocellular carcinoma [6].

Predictive Factors of Response to Treatment

Because a significant number of patients will fail to respond to current treatment, display a virological relapse or will have significant side effects that will need discontinuation of treatment, it is of major interest both in an economic approach and for the patient care to predict those patients who will fail to respond as early as possible, and ideally at baseline (before treatment).

The probability of SVR essentially depends on the genotype and viral load [7]. Younger age, female gender, and the absence of or minimal fibrosis are also associated with a better rate of response. In patients with HCV genotype 2 or 3, the SVR rates reach 80%; in genotype 1 patients, the SVR rates reach 50%. Recent studies allow

P. Marcellin, MD, PhD (✉)
Department of Hepatology, Beaujon Hospital, Pavillon Abrami, 100 bd Gel Leclere, Clichy 92110, France
e-mail: patrick.marcellin@bjn.aphp.fr

N. Boyer, MD • T. Asselah, MD, PhD
Service d'Hépatologie, Hôpital Beaujon, Clichy, France

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a better monitoring of patients, which allows optimization of treatment schedule according to the characteristics of the patients.

In genotype 1 patients, a reduction in HCV RNA serum levels by 2 log10 copies/ml after the first 12 weeks of treatment compared with the baseline is clearly associated with almost no chance of an SVR (negative predictive value, 97–100%). Thus, treatment can be discontinued because the probability of an SVR in these cases is approximately 0–3%. However, the positive predictive value is low, and this information is available only after 12 weeks of treatment.

RVR is a strong predictor of SVR (PPV > 96%) and failure to achieve EVR is a strong predictor of non-SVR (NPV > 75%), independent of the patients' pretreatment status [8]. Added to baseline characteristics, RVR increased the accuracy to predict SVR. The combination of RVR and EVR provided complementary information, and thus provides a key opportunity to individualize treatment and improve the benefit/risk ratio of therapy.

Furthermore, it has been shown that in non-responders, some interferon stimulated genes were highly expressed; thus, preactivation of the IFN system in patients appears to limit the effect of IFN antiviral therapy. The failure to respond to exogenous PEG-IFN in non-responders could indicate a blunted response to IFN [9].

Genetic Prediction of Treatment Response

Highly consistent data were reported by independent groups finding SNPs near the IL-28B (IFN-3k) region and associated with treatment response, thus opening a window for personalized medicine [10, 11]. All patients were from different ethnic origin, all infected by genotype 1, and received PEG-IFN plus ribavirin. Ge et al. [10] analysed 1,137 patients with HCV genotype 1 infection, and identified several SNPs near the IL-28B gene on chromosome 19 that were significantly more common in responders than in nonresponders.

A strong association of rs12979860 with both EVR and SVR in IFN-naive patients treated with Peg-IFNa-2a/ribavirin was also reported [11]. These results extend previous findings to show EVR and SVR associations in patients treated with Peg-IFNa-2a monotherapy and with conventional IFN/ribavirin. Additionally, we rank all previously described SNPs and find that rs12979860 drives the association with response. Finally, we highlight the association of rs12979860 with early HCV decline in response to IFN treatment. Although all of the identified variants lie in or near the IL-28B gene, none of them has an obvious effect on the function of this gene [12].

The Addition of a Protease Inhibitor Significantly Increased RVR, Consequently Increased SVR

New direct acting antivirals (*DAAs*) such as protease and polymerase inhibitors are under development under the name of new DAAs [13]. In genotype 1 naïve patients, highly promising results have been reported when the protease inhibitor telaprevir or boceprevir is added to the SOC.

Genotype 1, Naïve Patients

In genotype 1 naïve patients, highly promising results have been reported when the protease inhibitor telaprevir or boceprevir is added to the current SOC PEG-IFN plus RBV. It increases the SVR rates from less than 50% (PEG-IFN plus RBV) to approximately 70% (for patients treated with a combination of PEG-IFN plus RBV plus protease inhibitor). Therefore the present standard of care of genotype 1 patients consists in the addition of telaprevir or boceprevir to PEG-IFN plus RBV.

Telaprevir

The protease inhibitor NS3/4A telaprevir is being developed by the companies Vertex and Tibotec. In the randomized, double-blind, placebo-controlled phase 2 Prove-1 (USA) and Prove-2 (Europe) trials,

telaprevir is being administered for 12 weeks with PEG-IFN a-2a plus RBV [14, 15]. Data from these trials show that the triple-therapy regimen increases the rate of rapid virological response (RVR) and SVR.

In summary, the results of Prove-1 and Prove-2 demonstrate that SVR rates as high as 65% may be possible in genotype 1 patients treated with a 12-week triple-therapy regimen, followed by a 12-week standard combination therapy regimen. Telaprevir was associated with increased rates of certain adverse effects including rash, gastrointestinal events, and anemia. The rate of discontinuation for adverse events during the first 12 weeks of Prove-1 and Prove-2 was two- to threefold higher in recipients of telaprevir-based triple therapy than with the SOC. The maculopapular rash has generated the most concern, but this event resolved upon treatment discontinuation in all patients.

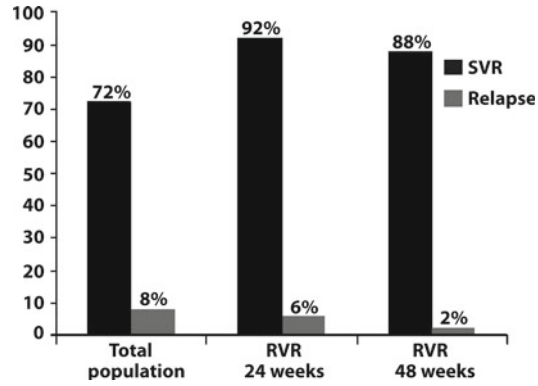

Fig. 19.1 ILLUMINATE study: the phase 3 open-label study, ILLUMINATE, evaluated genotype 1 naïve patients randomized to two durations of therapy (telaprevir plus PEG-IFN alfa2a and RBV) among those who achieved extended rapid viral response (eRVR). SVR was 92% among patients randomized to 24 weeks ($n=162$). SVR was 87.5% (Δ4.5%, 2-sided 95% C.I. = −2.1% to +11.1%) among patients randomized to 48 weeks ($n=160$). Overall, SVR was 71.9% (ITT analysis). Among patients who achieved eRVR, a 24-week telaprevir-based regimen was non-inferior to 48-week telaprevir-based regimen (92% SVR compared to 87.5%) (based on data from McHutchison et al. [17])

Illuminate Study

This phase 3 open-label study evaluated patients randomized to two durations of therapy among those who achieved extended rapid viral response (eRVR) [16]. Five hundred and forty HCV genotype 1 treatment-naïve patients were treated with telaprevir (12 weeks, 750 mg po q8h) with PEG-IFN alfa2a and RBV. Patients who achieved eRVR (undetectable HCV RNA at weeks 4 and 12) were randomized at week 20 to continue receiving PEG-IFN alfa2a and RBV for 24 or 48 weeks of total treatment. Patients not achieving eRVR were assigned 48 weeks of treatment. Seventy-two percent ($n=389$) of patients achieved RVR; 65.2% ($n=352$) of patients achieved eRVR. Three hundred and twenty two (59.6%) patients were randomized (1:1) to either a 24- or 48-week arm. SVR was 92% among patients randomized to 24 weeks ($n=162$). SVR was 87.5% (Δ4.5%, 2-sided 95% C.I. = −2.1% to +11.1%) among patients randomized to 48 weeks ($n=160$). Overall, SVR was 71.9% (ITT analysis). Thirty-six patients (6.7%) discontinued treatment due to virologic failure. Ninety-four patients (17.4%) had permanent discontinuation of all study drugs for adverse events. Fatigue ($n=22$) and anemia ($n=12$) were the most common adverse events leading to discontinuation. Treatment discontinuation due to anemia and rash were 3 (0.6%) and 6 (1.1%) patients, respectively, during the telaprevir treatment phase (Fig. 19.1).

In conclusion, among patients who achieved eRVR, a 24-week telaprevir-based regimen was non-inferior to 48-week telaprevir-based regimen (92% SVR compared to 87.5%). Response-guided treatment led to 71.9% SVR overall and nearly two-thirds of the patients were eligible for shorter duration of treatment. Permanent discontinuation of all study drugs due to adverse events occurred in 17.4% of patients. These results support response-guided therapy for telaprevir-based regimens in treatment-naïve patients.

Advance Study

This study is a 3-arm double-blind, randomized, placebo-controlled phase 3 study assessing efficacy and safety of two telaprevir-based response-guided regimens compared with PEG-IFN alfa-2a and RBV in treatment-naïve patients with chronic genotype 1 HCV infection [18]. Treatment arms were (a) Telaprevir 750 mg q8h in combination

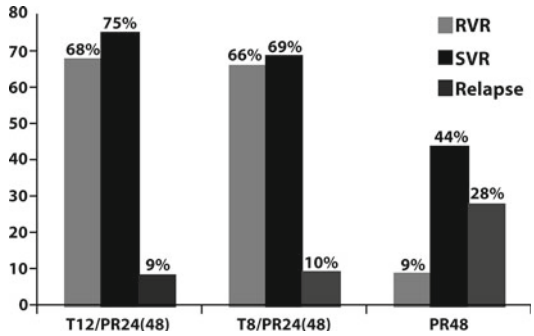

Fig. 19.2 ADVANCE study is a 3-arm double-blind, randomized, placebo-controlled phase 3 study assessing efficacy and safety of two telaprevir-based response-guided regimens compared with PEG-IFN alfa-2a and RBV in treatment-naïve patients with chronic genotype 1 HCV infection. A significantly greater proportion of patients achieved SVR with 12- and 8-week telaprevir-based combination regimens (75 and 69%, respectively) compared with PEG-IFN alfa-2a and RBV 48 weeks control arm (44%, $p<0.001$) (based on data from Zeuzem et al. [19])

with PEG-IFN alfa-2a and RBV for 8 weeks, followed by additional weeks of standard of care; (b) Telaprevir 750 mg q8h in combination with PEG-IFN alfa-2a and RBV for 12 weeks, followed by additional weeks of standard of care; (c) PEG-IFN alfa-2a and RBV for 48 weeks (control arm). Patients in telaprevir arms achieving an eRVR (undetectable HCV RNA at weeks 4 and 12) received a total of 24 weeks of therapy while those who did not receive a total of 48 weeks of therapy. A significantly greater proportion of patients achieved SVR with 12- and 8-week telaprevir-based combination regimens (75 and 69%, respectively) compared with PEG-IFN alfa-2a and RBV 48 weeks control arm (44%, $p<0.001$). The most common (>25%) adverse events in the telaprevir arms were fatigue, pruritus, nausea, headache, anemia, rash, influenza-like illness, insomnia, pyrexia, and diarrhea. Discontinuation of treatment due to adverse events occurred in 7 and 8% in telaprevir regimens and 4% in PEG-IFN alfa-2a and RBV; due to rash occurred in 0.5, 1.4, and 0.0% and due to anemia occurred in 3.3, 0.8, and 0.6% in telaprevir 8 weeks in combination with PEG-IFN alfa-2a and RBV, telaprevir 12 weeks with PEG-IFN alfa-2a and RBV; and control arms, respectively (Fig. 19.2).

In conclusion, telaprevir-based therapy improved SVR rates in genotype 1 treatment-naïve patients, including subgroups with impaired response to PR. A 12-week telaprevir-based regimen demonstrated a better benefit:risk profile than an 8-week regimen. With response guided therapy, nearly two-thirds naïve patients were eligible for 24-week treatment, and attained high rates of SVR. Discontinuation of the treatment regimen due to rash was minimized by stopping medication sequentially. Telaprevir may be used with either pegylated interferon [20].

Boceprevir

Boceprevir (Schering Plough-MSD) is a specific inhibitor of the viral protease NS3/4A.

Sprint-1 Study

Results concluded that boceprevir, when combined with SOC, appears to be safe for use up to 48 weeks and substantially improves SVR rates with 28 weeks of therapy and can nearly double the SVR compared with the current SOC (48 weeks) [21]. Use of a 4-week lead-in with SOC before the addition of boceprevir appears to reduce the incidence of viral breakthrough. The most common adverse events reported in the boceprevir arms were fatigue, anemia, nausea, and headache.

Sprint-2 Study

Sprint-2 study is a phase 3 international double-blind, randomized study including genotype 1 naïve patients (938 non-black and 159 black) and compared a 4-week lead-in treatment period with PEG-IFN alfa-2b /RBV, followed by (1) PEG-IFN alfa-2b/RBV R plus placebo for 44 weeks; (2) response-guided therapy: boceprevir plus PEG-IFN alfa-2b /RBV for 24 weeks, with an additional 20 weeks of PEG-IFN alfa-2b/RBV only if detectable HCV RNA during Week 8–24; or (3) boceprevir plus PEG-IFN alfa-2b/RBV for 44 weeks [22]. SVR in non-black patients was 40% for 48 PEG-IFN alfa-2b/RBV and significantly higher ($p<.0001$) in both boceprevir arms: response-guided therapy (67%) and lead-in phase followed by 44 boceprevir plus

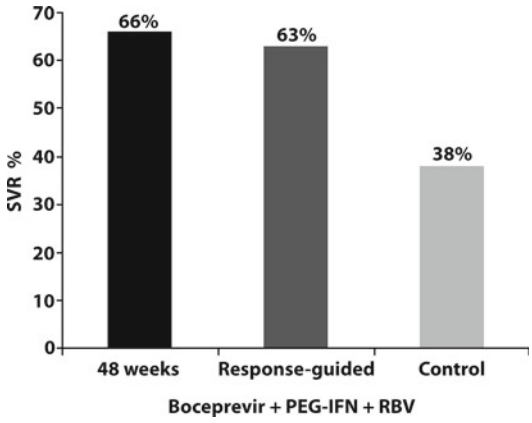

Fig. 19.3 SPRINT-2 study is a phase 3 international double-blind randomized study including genotype 1 naïve patients (938 non-black and 159 black) and compared a 4-week lead-in treatment period with PEG-IFN alfa-2b/RBV, followed by (1) PEG-IFN alfa-2b/RBV plus placebo for 44 weeks; (2) response-guided therapy: boceprevir plus PEG-IFN alfa-2b/RBV for 24 weeks, with an additional 20 weeks of PEG-IFN alfa-2b/RBV only if detectable HCV RNA during weeks 8–24; or (3) boceprevir plus PEG-IFN alfa-2b/RBV for 44 weeks). Boceprevir plus PEG-IFN alfa-2b/RBV significantly increased SVR (approximately 70%) in both arms over standard of care. Compared to 44 weeks of triple therapy after the lead-in period, response-guided therapy with lead-in plus 24 boceprevir plus PEG-IFN alfa-2b/RBV ± 20 PEG-IFN alfa-2b/RBV produced comparable SVR (based on data from Poordad et al. [22])

PEG-IFN alfa-2b /RBV (68%); corresponding SVR in black patients were 23, 42 ($p = .044$), and 53% ($p = .004$). For non-black patients receiving ≥1 dose of BOC or placebo, respective SVR were 42, 70, and 71%. Anemia was reported in 29% of controls versus 49% in the boceprevir arms, leading to dose reduction in 13 and 21% and discontinuation in 1 and 2%, respectively (Fig. 19.3).

In conclusion, boceprevir plus PEG-IFN alfa-2b/ribavirin significantly increased SVR (approximately 70%) in both arms over standard of care. Although anemia occurred more often under boceprevir, anemia rarely led to treatment discontinuation. Compared to 44 weeks of triple therapy after the lead-in period, response-guided therapy with lead-in plus 24 boceprevir plus PEG-IFN alfa-2b/RBV ± 20 PEG-IFN alfa-2b/RBV produced comparable SVR.

Ribavirin Appears to be Required in the Near Future Treatment Combination

Even though the mechanism of action of ribavirin is poorly understood, this drug appears important in future combination for several reasons: First in the standard of care, treating HCV without ribavirin or premature discontinuing, frequently missing doses of ribavirin is associated with a significant decline in virological response, and an increase in both breakthrough viraemia and relapse [23, 24].

The results of the PROVE studies clearly demonstrate that ribavirin will remain an essential ingredient in the treatment of chronic HCV. As in the PROVE 2 study, patients treated in the ribavirin-free arm (peginterferon a-2a and telaprevir) had a rapid virological response rate that was approximately 20% lower and an SVR that was approximately half of that observed in the triple combination therapy arm treated for the same duration. Patients with prior relapse had an SVR of 73% and those with a prior nonresponse had an SVR of 41% when treated with triple combination therapy compared with only 46 and 11%, respectively, when treated without ribavirin. Use of a lower dose of ribavirin in Sprint-1 was associated with lower RVR and lower SVR.

Duration of Therapy – Shorten Treatment Duration in Naïve Genotype 1 Patients Who Achieved Rapid Virological Response

A major information from the telaprevir and boceprevir studies is that approximately two-thirds of patients achieved RVR and remain HCV RNA negative through 24 weeks and benefit from 24 to 28 weeks of treatment. We will need further information regarding the predictive role of IL28B polymorphism to predict RVR. Once this is available it might be possible to decide the duration of treatment that will be required prior to initiating treatment.

Lead-In Versus No Lead-In?

Boceprevir was added after a lead-in period of treatment with peginterferon–ribavirin alone. Theoretically, a lead-in phase would serve to lower HCV RNA levels before exposure to a protease inhibitor, thereby reducing the risk of viral breakthrough or resistance to the direct-acting antiviral agent, as noted in a phase 2 study in which boceprevir with lead-in therapy was compared with boceprevir without lead-in therapy.

Patients with a poor response to interferon, defined as a reduction in the HCV RNA level from the pre-treatment baseline by less than 1 log10 IU per milliliter after the 4-week lead-in are relatively resistant to interferon and ribavirin and are at high risk to develop resistance to the DAA. These patients might be better served to stop treatment, not add the DAA, and wait for multiple DAA combinations to avoid the risk of developing resistance to the protease especially if they have mild fibrosis. In contrast, patients who are already HCV RNA undetectable after the 4-week lead-in treatment with peginterferon and ribavirin have already achieved an RVR, have a high SVR rate without the DAA and might potentially remain on pegylated interferon plus ribavirin without the DAA.

SVR Rates During Retreatment of Patients with Prior Relapse, Partial Response, and Null-Response

Genotype 1, Experienced Patients

In genotype 1 experienced patients, promising results have been reported when a protease inhibitor, telaprevir or boceprevir, is added to the current SOC PEG-IFN plus RBV. This increases the SVR rates from 10% (PEG-IFN plus RBV) to approximately 50% (for patients treated with a combination of PEG-IFN plus RBV plus protease inhibitor). Prior relapsers have higher SVR rates than previous non-responders. Null responders have less benefit from this triple therapy, and might benefit from future DAA combinations. In the very near future, standard of care for genotype 1 experienced patients will consist of a DDAs (protease inhibitor) plus PEG-IFN and RBV.

Telaprevir

Study 107 was an open-label, phase 2 rollover study of telaprevir in combination with Peg-IFN and RBV in patients who had previously received treatment with Peg-IFN and RBV in the control arms of either of the PROVE 1, PROVE 2 or PROVE 3 trials, and did not achieve SVR [25]. Patients in Study 107 were well characterized as null responders, partial responders, relapsers, or breakthroughs, based on their antiviral response documented as a result of their participation in the control arms of the PROVE clinical trials. Treatment with telaprevir-based regimens in Study 107 resulted in an overall SVR rate of 59% across all patients enrolled in the study, with 56% of the most difficult-to-treat null responder patients achieving SVR with a 48-week telaprevir-based regimen.

Prove III Study

In HCV-genotype 1 infected patients in whom initial PEG-IFN plus RBV treatment failed, retreatment with telaprevir in combination with pegiinterferon a-2a and ribavirin (approximately 50%) was more effective than retreatment with peginterferon a-2a and ribavirin alone (approximately 15%) [17].

Realize Study

Realize study is a phase 3, randomized, double-blind, placebo-controlled study conducted in 662 genotype 1 chronic hepatitis C patients who did not achieve an SVR after at least one prior treatment with IFN-based therapy [19]. There were two telaprevir-based arms (simultaneous and delayed start) and one control arm. Patients were randomized 2:2:1 to the two telaprevir arms and the control arm, respectively. As in all phase 3 studies of telaprevir, patients received no more than 12 weeks of telaprevir given in combination

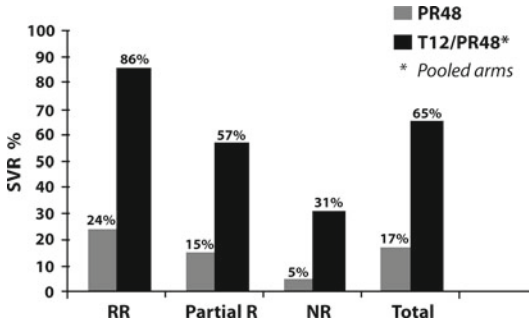

Fig. 19.4 REALIZE study was a phase 3, randomized, double-blind, placebo-controlled study conducted in 662 genotype 1 chronic hepatitis C who did not achieve a viral cure after at least one prior treatment with interferon-based therapy). There were two telaprevir-based arms (simultaneous and delayed start) and one control arm. SVR rates for the telaprevir simultaneous start arm and the delayed start arm were 64 and 66%, respectively, overall, based on an ITT analysis. For the primary analysis, the SVR rates for the telaprevir simultaneous start arm, delayed start arm, and control arm, respectively, were 83, 88, and 24% in relapsers ($p<0.001$); 59, 54, and 15% in partial responders ($p<0.001$); and 29, 33, and 5% in null responders ($p<0.001$) (based on data from Zeuzem et al. [19])

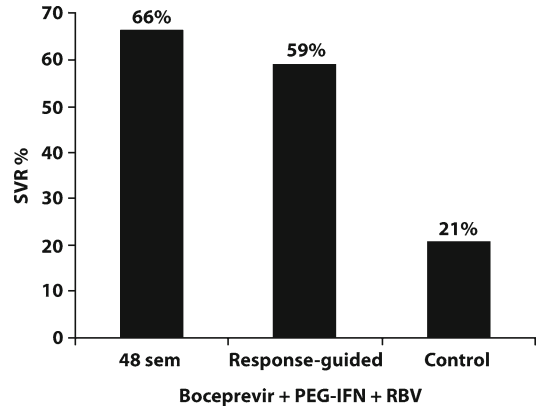

Fig. 19.5 RESPOND 2 study: this phase III randomized trial demonstrated that combination therapy with Boceprevir yields higher sustained SVR rates for patients with HCV genotype 1 who did not respond to or relapsed after treatment with PEG-IFN and RBV were reported. In this trial, three arms were randomly selected from 403 HCV genotype 1 patients who previously failed treatment – partial/non-responders or relapsers. At 24 weeks after end of treatment, the control arm achieved a SVR of 21%. Addition of Boceprevir to the treatment increased SVR to 59% for the second arm and 67% for the third arm. It was noted that previous relapsers had better SVR than nonresponders in all arms (based on data from Bacon et al. [26])

with pegylated interferon and ribavirin. In this study, the telaprevir arms included 12 weeks of telaprevir in combination with pegylated-interferon and ribavirin with 36 weeks of pegylated-interferon and ribavirin alone for a total of 48 weeks of treatment. One of the telaprevir treatment arms was designed to evaluate, whether there was any further improvement in viral cure rates when delaying the start of telaprevir by 4 weeks, during which time the patients received 4 weeks of pegylated-interferon and ribavirin alone, compared to a simultaneous start. The SVR rates between these two arms were similar and there was no clinical benefit to the telaprevir delayed start treatment arm in any of the subgroups of patients. SVR rates for the telaprevir simultaneous start arm and the delayed start arm were 64 and 66%, respectively, overall, based on an intent-to-treat (ITT) analysis. For the primary analysis, the SVR rates for the telaprevir simultaneous start arm, delayed start arm and control arm, respectively, were 83, 88, and 24% in relapsers ($p<0.001$); 59, 54, and 15% in partial responders ($p<0.001$); and 29, 33, and 5% in null responders ($p<0.001$) (Fig. 19.4).

Boceprevir

Respond 2 Study

The final results of this trial demonstrated that combination therapy with Boceprevir yield higher sustained SVR rates for patients with HCV genotype 1 who did not respond to or relapsed after treatment with PEG-IFN and RBV were reported [26]. In this trial, three arms were randomly selected from 403 HCV genotype 1 patients who previously failed treatment – partial/non-responders or relapsers (Fig. 19.5).

- Control arm received PEG-IFN alpha 2b and RBV for 48 weeks.
- Second arm received 4 weeks of lead-in therapy of PEG-IFN alpha 2b and RBV followed by response-guided therapy of PEG-IFN alpha 2b and RBV combined with 800 mg of Boceprevir three times a day.
- Third arm received 4 weeks of lead-in therapy of PEG-IFN alpha 2b and RBV followed by 44 weeks of PEG-IFN alpha 2b and RBV combined with 800 mg of Boceprevir.

At 24 weeks after end of treatment, the control arm achieved a SVR of 21%. Adding Boceprevir to the treatment increased SVR to 59% for the second arm and 67% for the third arm. SVR in patients with previous relapse achieved SVR rates of 69 and 75% for the response guided therapy and continuous 44 weeks of treatment approaches utilizing boceprevir compared to only 29% for patients retreated with only peginterferon and RBV. Non-responders retreated with boceprevir triple therapy had an SVR of 40 and 52% when randomized to groups 2 and 3, respectively. This was significantly better than a 7% SVR observed when these patients were retreated with peginterferon and ribavirin. The lead-in again assists in identifying patients who are interferon sensitive and likely to benefit from the addition of boceprevir. Patients who are interferon responsive and have more than a 1 log decline in HCV RNA during the 4-week lead-in phase had an SVR of 73–79% when treated with boceprevir triple therapy compared to 25% for peginterferon and ribavirin alone. In contrast, patients who were insensitive to peginterferon and had less than a 1 log decline in HCV RNA after the 4-week lead-in had SVR rates of only 33–34% with boceprevir triple therapy. None of the interferon patients insensitive to interferon according to the lead-in achieved a SVR if they remained on just peginterferon and ribavirin.

Can Either Interferon Be Utilized with Either Protease Inhibitor or Other Protease Inhibitors in the Future?

In a prospective, multicenter, randomized, open-label, phase 2 clinical trial study, including 161 HCV genotype 1 patients, a high proportion (>80%) of patients achieved an SVR regardless of the telaprevir dosing frequency (q8 h or q12 h) or type of peginterferon alfa used (alfa-2a or alfa-2b) [22]. Each pegylated interferon provides approximately the same SVR [4]. It might be that either interferon could be utilized with either protease inhibitor; however, we need more information regarding this issue.

PEG-Interferon lambda (IL-29) is a novel interferon in development for hepatitis C. PEG-Interferon lambda is a member of the Type III lambda interferon family, which includes IL-28A, IL-28B, and IL-29 (also known as interferon lambda 2, 3, and 1, respectively). A phase 1b clinical trial was conducted in patients with relapsed HCV, in which PEG-Interferon lambda was administered over 4 weeks in combination with ribavirin [29]. This interferon is currently being evaluated in phase 2 clinical trials. If this proves to be an effective interferon and gains approval by regulatory bodies, there is no reason why it could not substitute for peginterferon alfa-2a or 2b with either telaprevir or boceprevir.

DAAs in Development

There are many other molecules, both protease and polymerase inhibitors currently in development and undergoing various phases of testing, which could be utilized in the future to treat chronic HCV. These agents may be combined and utilized with or without peginterfeorn and/or ribavirin. How these agents will be utilized will depend upon their potency, lack of cross resistance, and safety profile.

The goals for a DAA combination should be to increase antiviral efficacy, to reduce resistance, without severe toxicity. A first study of combination DAAs in patients was the proof-of-concept INFORM-1 study [27]. In the final cohort of patients who received the highest dose of RG7227 and RG7128, 100% achieved ETR after 24 weeks PEG-IFN and RBV treatment. It will be very interesting to wait for final SVR results.

Several DAAs are being developed [28, 30–37]. Actually, several studies of combination DAAs are ongoing in patients with treatment-naïve HCV infection. All studies include an NS3/4a protease inhibitor, combined with an agent targeting the HCV polymerase complex – either a non-nucleoside NS5b, nucleoside NS5b, or NS5a inhibitor.

The following combinations are under development in phase 2a clinical studies:

- GS9256 (NS3/4a inhibitor) and GS9190 (nonnuc polymerase inhibitor) (Gilead) [28]
- BI201335 (NS3/4a inhibitor) and BI297127 (nonnuc polymerase inhibitor) (Boehringer) [30]
- BMS-650032 (NS3/4a inhibitor) and BMS-790052 (NS5a inhibitor) (BMS) [31]
- Telaprevir (NS3/4a inhibitor) with VX-222 (nonnuc polymerase inhibitor) (Vertex)
- RG7227 (NS3/4a inhibitor)/ritonovir and RG7128 (Nuc polymerase inhibitor) (Roche)
- ABT-450 (NS3/4a inhibitor)/ritonovir and ABT-072 (nonnuc polymerase inhibitor) (Abbott)
- IDX320 (NS3/4a inhibitor) and IDX184 (NS5a inhibitor) (Idenix)

Conclusion

In genotype 1 naïve patients, promising results have been reported when a protease inhibitor, telaprevir or boceprevir, is added to the current SOC, PEG-IFN plus RBV. It increases the SVR rates from less than 50% (PEG-IFN plus RBV) to approximately 70% (combination of PEG-IFN plus RBV plus protease inhibitor). The standard of care of genotype 1 experienced patients consists in the addition of telaprevir or boceprevir to PEG-IFN plus RBV.

In genotype 1 experienced patients, promising results have been reported when a protease inhibitor, telaprevir or boceprevir, is added to the current SOC PEG-IFN plus RBV. It increases the SVR rates from 10% (PEG-IFN plus RBV) to approximately 50% (combination of PEG-IFN plus RBV plus protease inhibitor). Prior relapsers have higher SVR rates than previous non-responders. Null responders have less benefit from this triple therapy, and might benefit from future DAAs combination. The standard of care of genotype 1 experienced patients consists in the addition of DDAs to PEG-IFN plus RBV.

Once several DDAs become available, treatment strategies will include a combination of several drugs with different mechanisms of action (protease inhibitors plus polymerase inhibitors) that could hopefully result in IFN- and/or RBV-sparing regimens leading to additive potency, lacking cross resistance, and with a good safety profile.

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Treating Chronic HCV Without Interferon and/or Ribavirin

20

Edward J. Gane

Keywords
Hepatitis C virus (HCV) • Sustained virologic response (SVR) • Viral genotype • IL28B genotype • Standard-of-care (SOC) • Pegylated interferon • Ribavirin • Direct acting antiviral (DAA) • Polymerase inhibitor • Protease inhibitor

Introduction

An estimated 200 million people are currently infected with the hepatitis C virus. The incidence of HCV infection has decreased by more than 50% over the last decade, reflecting reduced exposure risk [1, 2]. Although the size of the population with chronic HCV infection has been stable since 2000 [3], this is an aging cohort and the proportion of this cohort with cirrhosis will almost treble over the next three decades from 16% to over 45% [4, 5]. As a result, the incidence of both HCV-related hepatocellular carcinoma and HCV-related-mortality will also treble [6–8]. The only means to avert this projected health burden from the current HCV epidemic is to reduce the total pool of patients with chronic HCV infection by widespread eradication of chronic infection by successful antiviral therapy. However, currently less than 10% of patients have been treated with less than 5% cured. The current treatment numbers would need to increase more than tenfold in order to prevent this projected health burden [1, 8]. This would necessitate not only an improved rate of cure from current treatment regimens but also widespread community uptake of HCV screening, diagnosis, and access to treatment. The latter is significantly hindered by the poor efficacy and tolerability of the current standard-of-care (SOC) for chronic HCV infection, which is the combination of pegylated-interferon plus ribavirin for between 24 and 48 weeks duration. In global registration studies, the overall reported sustained virological response (SVR) rate in patients infected with HCV genotype (GT) 1 is 45% and in those infected with GT2 or 3 is 79%, respectively [9]. Unfortunately, in the real world where clinic follow-up and support are less intensive, SVR rates are significantly lower. In the largest cohort to-date of almost 17,000 patients treated and followed up by the US Department of Veterans' Affairs, the reported SVR rates were 35% in patients infected with GT1, 72% in those with GT2, and 62% in those with GT3 [10]. HCV GT1 is the predominant genotype globally, accounting

E.J. Gane, MBChb, MD, FRACP (✉)
Liver Unit, Auckland City Hospital,
Auckland, New Zealand
e-mail: edgane@adhb.govt.nz

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for between 55% (Australasia) and 90% (Europe and Asia) of all infections.

Baseline patient predictors of non-response to SOC other than HCV genotype include age >50 years, advanced fibrosis, high BMI, insulin resistance, and African ethnicity. Independent genome-wide association studies have recently identified several inherited single nucleotide polymorphisms on chromosome 19, upstream from the IL28B (IFN Lambda) gene, all of which are strongly associated with SVR across all patients groups, independent of all other predictors including ethnic origin [11–14]. The most commonly used SNP is the rs12979860, with two alleles termed T and C. SVR in the homozygous CC individual approaches 80% whilst it is less than 40% for both T/T and T/C. These SNPs appear to be important for the innate immunity.

In those patients with favorable baseline predictors of response, adherence to therapy is the most important determinant of outcome. Pegylated interferon is associated with significant adverse effects, from flu-like symptoms, fever, rash, anorexia, thyroid dysfunction, to dose-related life-threatening cytopaenias and mood disorders. Side effects result in dose reduction in 60–80% of patients and treatment withdrawal in 5–10%. The most frequent adverse effect of ribavirin is dose-related haemolysis. Because RBCs lack phosphorylase enzymes, ribavirin triphosphate (RTP) accumulates rapidly within RBC membranes. By directly competing with ATP, RTP may induce membrane oxidative damage, resulting in reduced red cell survival [15]. In the global registration studies for standard interferon and ribavirin, haemolysis necessitated dose reduction in almost 10% and dose withdrawal in 1% of the patients [16]. Haemolysis is particularly severe in patients with impaired renal function, reflecting rapid accumulation of ribavirin because of reduced renal clearance. Strategies to improve ribavirin adherence include supportive transfusions and erythropoietin, although the latter is expensive and has not been associated with improved response rates in non-transplant patients [17]. Adjusting ribavirin dose according to a renal function algorithm is problematic and requires regular monitoring of plasma ribavirin levels and adjusting daily dose to maintain trough plasma ribavirin levels at 10–15 mmol/L, but the HPLC assay is not widely available [18]. Taribavirin is a new liver-targeting analogue of ribavirin which has minimal systemic exposure, thereby limiting the effects on red cell fragility. Initial trials, however, suggested that the efficacy of this drug was inferior to ribavirin, and further studies are awaited. One important recent discovery, again from the GWAS project of patients at the IDEAL study at Duke University, has identified a SNP which may predict risk of haemolysis with ribavirin. The polymorphism on Chromosome 20 at rs1127354 determines the activity of inosine triphosphatase, which inversely determines the risk of haemolysis during ribavirin therapy. The A allele (and the A/A and C/A genotype) reliably predicts protection from ribavirin-induced haemolysis (vs. C/C) [19].

However, the most important barrier to successful antiviral therapy remains the small numbers of patients actually being treated. Currently less than 10% of all patients with chronic HCV infection have received SOC, reflecting both lack of diagnosis and lack of access to SOC, because of real or perceived medical or psychosocial contraindications to either interferon or ribavirin. Many more defer therapy because of anecdotal stories about severe adverse effects. Many such patients have deferred treatment until a better-tolerated, IFN-free regimen becomes available. This "warehousing" practice is widespread amongst both patients and their doctors, reflecting widespread nihilism about current treatment. More reliable methods to both stage liver disease severity (with non-invasive markers, including Fibroscan™) and predict efficacy (with IL28B genotyping) and tolerability (with IMP genotyping) of current SOC should help facilitate this discussion on whether to treat now or wait.

Finally, there is a large and growing pool of patients, largely infected with HCV GT1 with advanced fibrosis and with non-CC IL28B genotype TC or TT, who have received treatment with pegylated interferon and ribavirin but who failed to achieve SVR, including null responders, partial responders and relapsers, in whom no alternative retreatment options are currently available.

New therapeutic approaches offering improvements in efficacy, safety, and tolerability are urgently needed to address these unmet medical needs.

Direct Acting Antivirals and Triple Therapy

The most popular targets for drug development have been the HCV protease (via inhibition of NS3A4 protease), the HCV polymerase complex (via inhibition of NS5A, NS5b and indirectly through NS3A4), and viral assembly (via inhibition of NS5A). A detailed description of these targets is provided in Chap. 17. Successful development of in vitro replicon and transgenic models for HCV replication has facilitated the development of multiple DAAs against these targets. Over the last 5 years, more than 100 protease and polymerase inhibitors have entered preclinical development. Although the development of many have halted because of toxicity (BILN2061, NM283, HCV796, R1626), many more have been abandoned because of preclinical toxicity signals or lack of clinical efficacy. Despite this, more than 50 are currently in clinical trials in patients (see Table 20.1).

Two linear protease inhibitors, boceprevir and telaprevir, were the first DAAs to gain regulatory approval as add-on therapy to current SOC pegylated interferon plus ribavirin. Both were approved by the FDA in May 2011 and have become the new SOC for both treatment-naïve and treatment-experienced patients with HCV genotype 1 infection. The benefits in terms of efficacy are significant – 48 weeks boceprevir plus SOC increased SVR rates in treatment-naïve GT1 patients from 38% to 66%, while 12 weeks telaprevir plus 24 weeks SOC increased SVR rates from 43% to 75% [20, 21] Triple therapy may also offer hope in treatment experienced patients, especially previous responder-relapsers and partial responders[22–24]. Chapter 19 summarizes the available data regarding this triple combination therapy. Unfortunately, both telaprevir and boceprevir have specific toxicities (notably anemia and dysgeusia with boceprevir and anemia and rash with telaprevir), which increased the rate of treatment withdrawal in the DAA combination arms.

Although triple therapy (addition of either telaprevir or boceprevir to pegylated interferon plus

Table 20.1 Direct acting antivirals

Target	Class	Name	Phase
Post-translational processing	NS3/4a serine protease inhibitor	Boceprevir	3
		(Linear)	3
		Telaprevir (Linear)	2b
		Danaprevir	2b
		(Macrocyclic)	2a
		Vaniprevir (Linear)	2a
		BMS-650032	2a
		BMS-791325	2a
		BI201335	3
		SCH900518	2a
		TMC435	1
		ABT450	2a
		IDX320	1
		GS 9256	1
		VX-985	1
		ACH1625	1
		ACH-2684	1
		PHX1766	1
		VX500	
		MK-5712	
HCV replication complex	Cyclophillin B	Debio-025	2a
		Debio-NIM-811	2
	NS5a	AZD7295	2a
		Debio-025	2b
		BMS-790052	2a
		PPI-461	2a
		PPI-1301	2a
		GS-5885	1
		BMS-824393	1
		ACH-2928	1
	NS5B nucleoside polymerase inhibitors	RG7128	2b
		PSI-7977	2a
		PSI-7851	2a
		PSI-938	1
		IDX184	2a
		MK-0608	1
		RG7348	1
	NS5B nonnucleoside polymerase inhibitors	BI207127	2
		BMS-824393	2
		VX-222	2
		VCH-759	2
		ABT-072	2
		PF-00868554	2
		(Filibuvir)	2
		ABT-333	2
		MK3281	2
		ANA598	1
		IDX375	1
		GS9190	1
		PF-4878691	

ribavirin) has become the new standard-of-care in late 2011, this will not be suitable for patients either intolerant of or with contraindications to interferon or ribavirin, including patients with decompensated cirrhosis or following solid organ transplantation. It seems likely that the primary predictor of response to triple therapy will be the host IL28B genotype, with lowest responses seen in those non-CC patients. In addition, the efficacy of this triple therapy will probably be reduced in treatment-experienced patients who had a null response to the initial course of SOC. This is likely to be explained by the patient's non-CC IL28B genotype.

Moreover, the new triple therapy regimens will have little impact on patients infected with HCV GT 2 and 3, who comprise between 20 and 45% of the HCV-infected patient population (although telaprevir has antiviral activity against HCV GT2, this agent has no effect in patients with HCV GT3 infection) [25, 26]. All protease inhibitors and non-nucleoside polymerase inhibitors in development are active primarily against HCV genotype 1, with variable effect against GT2 and GT3. Peginterferon plus ribavirin will remain the SOC for non-GT1 HCV until nucleoside polymerase inhibitors, NS5A inhibitors, and cyclophyllin inhibitors enter clinical practice.

Finally, 30–40% of treatment-naïve GT1 patients who are treated with triple therapy will not respond because of the emergence of resistance to protease inhibitors. Follow-up virologic testing from both boceprevir and telaprevir Phase II studies suggest that the levels of these variants rapidly decline following withdrawal of the protease inhibitor because of their inferior replicative potential compared to wild-type virus. In addition, unlike HBV and HIV resistant variants, there is no hidden extrahepatic or intrahepatic reservoir for HCV resistant variants. However, it is likely that these variants will rapidly become dominant again following re-exposure to the same DAA or another linear protease inhibitor with overlapping resistance profile. Therefore, retreatment of patients who are non-responders to either boceprevir- or telaprevir-based triple therapy with another linear protease inhibitor-based triple therapy is unlikely to be successful. However, retreatment with another triple therapy regimen, containing a different DAA without cross-resistance, may be effective. Further studies will be needed to determine whether such an approach will be effective in patients who have an unfavorable (non-CC) IL28B genotype.

In summary, although triple therapy will become the new SOC for treatment of HCV infection, with improved efficacy and shortened treatment duration, this approach will fail to eradicate HCV in almost 30% of treatment-naïve and 55% of treatment-experienced patients, who will be left with resistance to protease inhibitors. This approach will not meet the needs of many patient groups who have either infection with non-1 genotypes or contraindications to interferon and/or ribavirin.

Combination of Multiple Direct Acting Antivirals Without Interferon

The reason for treatment failure in these 30% of treatment-naïve and 55% of treatment-experienced patients, who are non-responders to triple therapy, is the inability of pegylated interferon plus ribavirin to prevent the emergence of protease-resistant variants because of an inadequate antiviral effect of SOC in these patients. This will drive the development of quadruple regimens (protease and polymerase plus SOC) and also combinations of multiple DAAs without SOC.

The development of a multiple DAA regimen is very attractive as this could potentially provide an interferon-free, all-oral regimen for all treatment-naïve and treatment-experienced patients with chronic HCV infection. This approach is based on the current HIV treatment paradigm, where different direct acting antiviral agents, which target different steps of viral replication, are combined to provide both increased viral suppression and prevention of antiviral resistance. There is a rapidly increasing list of potential candidates for such a combination. The primary criteria for selection of potential components for an IFN-free DAA combination should be agents which target different steps of HCV

replication thereby avoiding cross-resistance and preventing virological breakthrough from emergence of variants resistant to both DAAs. The combination should exhibit in vivo at least additive and preferably synergistic antiviral efficacy (i.e., rather than interference as observed with telbivudine plus lamivudine in patients with HBV infection). Finally, the combination should be safe and well tolerated. The different agents should lack direct drug interactions and overlapping toxicities.

In vitro studies in the replicon model have demonstrated that the addition of either a nucleoside polymerase inhibitor, a non-nucleoside polymerase inhibitor, or an NS5a inhibitor to a protease inhibitor provides additive viral suppression and prevents or delays the emergence of phenotypic resistance to the protease inhibitor [27–30]. Similar in vitro effects have been demonstrated for the combination of two nucleoside polymerase inhibitors, which suggest that such a combination may provide an extremely high barrier to resistance, thereby removing the need for another class of DAA [31]. The first clinical evidence that combining DAAs may achieve cure in the absence of IFN was provided by a study in three chimpanzees treated with a combination of NS3/4A a protease inhibitor, MK-7009, and a non-nucleoside inhibitor MK-608 for 7 days. This combination provided rapid and sustained viral suppression and one of three animals eradicated the HCV infection [32]. The first study of combination DAAs in patients with chronic HCV infection was the INFORM-1 study completed last year [33]. In this study, 87 patients with HCV genotype 1 infection were randomized to receive up to 13 days of either oral combination therapy with RG7128, a nucleoside polymerase inhibitor, and RG7128/mericitabine, an NS3/4A protease inhibitor, or with matched placebos. Both agents had been already administered to patients for 12 weeks in combination with SOC. Direct drug interactions between RG7128 and danaprevir were considered very unlikely, due to the different mechanisms of action and routes of elimination and the lack of overlapping toxicities identified in any of the preclinical or human clinical studies. This combination achieved profound antiviral suppression, greater than the additive effects of either treatment alone. Median reduction in HCV RNA from baseline was 5 logs, falling below the level of detection in 88% in the cohort who received the highest dose of both RG7128 (1,000 mg bid) and danaprevir (900 mg bid). No evidence of the emergence of resistance to either compound was observed during the study. This combination was well tolerated with no serious adverse events, treatment-related dose modifications, discontinuations, or study withdrawals. An important observation was that antiviral efficacy was similar in treatment-naïve and treatment-experienced patients including non-responders. Because the total duration of therapy was only 13 days, all patients were treated with peginterferon and ribavirin starting on day 14. Rates of RVR, EVR, and ETR were markedly increased by 2 weeks of pretreatment. In the final cohort of patients who received the highest dose of RG7227 and RG7128, 100% achieved ETR after 24 weeks SOC. Although SVR results are still pending, the benefit of pretreatment with combination DAA on subsequent responses to SOC suggests that the strategy of combination DAA lead-in prior to starting SOC could be an alternative strategy to IFN-free DAA therapy.

However, the primary goal of combination DAA therapy in HCV infection will be to provide a safe and effective substitute for interferon regimens in all treatment-naïve and experienced patients.

Although the new treatment paradigm for HCV is based on HIV, the goals are very different. In HIV infection, cure is not achievable because it is impossible to eradicate infection from lymphocytes and macrophage reservoirs and from nuclear integration. Therefore, lifelong combination DAA therapy is needed to maintain viral suppression and prevent disease progression. In HCV infection, however, replication is entirely cytoplasmic and limited to hepatocytes. Therefore, viral eradication should be possible with short-course combination DAA.

The duration of combination DAA necessary to eradicate HCV infection is unknown. The early viral kinetic profile of single DAA therapy demonstrates a rapid Phase One decline in serum

HCV RNA levels of 3–4 logs in the initial 36 h, attributed to the clearance of free virions from the circulation. The addition of a second DAA appears to increase this initial slope, suggesting at least additive effects of both agents. This is followed by Second Phase decline in serum HCV RNA of 1–1.2 log/week, attributed to the loss of infected hepatocytes. This rate of viral decline continues until the infection is eradicated, unless DAA-resistant variants emerge. Therefore, based on the estimated total body viral burden of 10 [11] virions, between 8 and 12 weeks of DAA therapy should be sufficient to eradicate HCV infection in most patients. The addition of a second DAA targeting a different step of HCV replication and lacking cross-resistance should both increase the slope of the Phase One decline as well as prevent virological breakthrough during Phase Two.

A finite duration of combination DAA therapy without IFN assumes that viral suppression alone will eradicate HCV, which seems reasonable in the absence of evidence of either viral latency or extrahepatic reservoirs of replication (as seen in HBV). Another important factor for the maintenance of end-of-treatment response may be the indirect effect of combination DAA therapy on host immune responses. In chronic HCV infection, the HCV NS3 protease may directly impair host IFN responses through inhibition of phosphorylation of interferon regulatory factor-3 (IRF-3) [34]. Administration of the NS3/4A protease inhibitor should restore this immune responsiveness. In non-responders to SOC, interferon stimulated genes are highly expressed, signifying that preactivation of the IFN system may inhibit the effect of IFN therapy [35]. Chronic HCV infection is also associated with high levels of IP-1, reflecting endogenous interferon activation levels. High levels of IP-10 during SOC are inversely correlated with sustained virologic response [36]. In the INFORM study, viral suppression at 13 days correlated with normalization of IP-10 levels, suggesting that rapid and profound viral suppression with combination DAA therapy may restore host immune responses against HCV and prevent late relapse through immune clearance of remaining virions [37].

First Studies with All-Oral Treatments

The shift from triple therapy (single DAA plus SOC) to IFN-free combination DAA studies has been impeded by the reluctance of regulatory authorities to approve the combination of two experimental compounds still in early phase clinical development. However, such studies should be reasonable as long as safety data are available for each candidate DAA for the duration of proposed treatment. The rapid emergence of resistant variants during monotherapy with either NS3/4a or NS5b non-nucleoside inhibitors has restricted the duration of DAA monotherapy studies to 3–5 days, thus longer duration safety data must be obtained from studies of DAA in combination with SOC. An additional requirement prior to embarking on combination DAA studies in patients should be data from preclinical and human clinical studies for each candidate DAA, confirming lack of cross-resistance, lack of overlapping toxicities and a low likelihood of any drug–drug interactions, which could affect antiviral activity, bioavailability, or clearance. INFORM-1 fulfilled all of these requirements but was performed in Australia and New Zealand, because the conservative regulatory environment of the FDA and EMEA prevented performing this study in either the US or Europe at that time. The success of this proof-of-concept and widespread enthusiasm in the HCV field over these results will push the regulatory authorities to modify their previously conservative position.

Following the INFORM proof-of-concept study, seven Phase 2 studies of combination DAA studies are already entering Phase 2 clinical trials in patients with treatment-naïve HCV infection (see Table 20.2), with many more planned. All studies include an NS3/4a protease inhibitor with or without ritonavir boosting, combined with another agent which targets the HCV polymerase complex, either a NS5a inhibitor, a

Table 20.2 Combination DAA clinical studies in 2010/2011

Company	DAA (1)	DAA (2)	Phase
Vertex	Telaprevir (NS3/4a inhibitor)	VX-222 (nonnuc polymerase inhibitor)	2a
BMS	BMS-650032 (NS3/4a inhibitor)	BMS-790052 (NS5a inhibitor)	2a
Gilead	GS9256 (NS3/4a inhibitor)	GS9190 (nonnuc polymerase inhibitor)	2a
Boehringer	BI201335 (NS3/4a inhibitor)	BI297127 (nonnuc polymerase inhibitor)	2a
Idenix	IDX320 (NS3/4a inhibitor)	IDX184 (NS5a inhibitor)	2a
Abbott	ABT-450 (NS3/4a inhibitor)/ritonovir	ABT-072 (nonnuc polymerase inhibitor)	2a
Roche	RG7227 (NS3/4a inhibitor)/ritonovir	RG7128 (Nuc polymerase inhibitor)	2a

non-nucleoside NS5b inhibitor or a nucleoside NS5b inhibitor. Most of these experimental protocols have retained ribavirin as a third oral agent based on the results of a Phase II studies with telaprevir, where patients randomized to combination of telaprevir plus pegylated interferon without ribavirin experienced higher rates of both on-treatment breakthrough and post-treatment relapse [38]. Breakthrough (after completion of 12 weeks telaprevir) was observed in 24% of patients who did not receive ribavirin compared to only 3% in those who did. Post-treatment relapse was observed in 48% of patients who did not receive ribavirin compared to only 23% in those who did. The mechanism of this benefit is not yet understood but presumably reflects both weak direct antiviral and indirect immunomodulatory properties. In vitro studies have demonstrated that the addition of ribavirin to direct acting antivirals provides antiviral synergism and reduces emergence of DAA resistance [39]. However, the impact of ribavirin on the efficacy and tolerability of combination DAAs will need to be evaluated. The use of ribavirin will remain a problem in certain "difficult-to-treat" patient groups such as those with renal impairment and haemoglobinopathies.

In a recent landmark study, 11 patients with HCV GT 1 infection, who were all previous null responders to SOC, received 24 weeks combination NS5A inhibitor BMS-790052 and NS3A/4 protease inhibitor BMS-6500324. Although 7 developed rapid viral rebound (from emergence of dual NS5A/NS3A4 resistant mutants), the remaining 4 patients were cured, thereby providing the proof of concept that HCV can be cured without pegylated interferon provided DAA resistance can be avoided.

As outlined above, the likely duration of SOC-free therapy needed to eradicate HCV infection will be greater than 8 weeks. All current and planned IFN-free combination DAA studies are looking at a minimum of 12 weeks of therapy with longer duration in those patients who fail to achieve complete early virologic responses. This response-guided therapy will utilize the 2 or 4 week RVR, rather than 12 week EVR as a predictor of efficacy (and shortened treatment duration).

It is not clear how many DAAs will be necessary in order to maximize response and minimize resistance. Nucleoside polymerase inhibitors possess a relatively high genetic barrier to resistance with no resistance seen in up to 42 weeks monotherapy and 24 weeks combination with SOC. The prevalence of spontaneous mutations conferring resistance to nucleoside polymerase inhibitors is also very low, with no baseline S282T mutations detected in a recent survey of untreated patients with chronic HCV infection GT1 (compared to 2–8% prevalence of mutations conferring resistance to either NS3/4a inhibitors or non-nucleoside polymerase inhibitors) [40]. The application of ultrasensitive pyrosequencing techniques may in fact detect these mutations in many more patients. Current studies should determine whether the addition of a single nucleoside polymerase inhibitor will be enough to prevent protease resistance and if so a dual DAA combination (nucleoside polymerase inhibitor plus protease inhibitor with or without ribavirin) may be sufficient to eradicate HCV GT1. An alternative approach for the future could incorporate a triple DAA combination including a nucleoside polymerase inhibitor, a protease inhibitor, and either an NS5a inhibitor or a non-nucleoside poly-

merase inhibitor. In HCV GT2 and 3, potential candidates are currently limited to the nucleoside polymerase inhibitors, some of the newer NS5A inhibitors, the cyclophyllin B inhibitors, and of course ribavirin. It is therefore possible that the combination of two nucleoside polymerase inhibitors plus ribavirin may also replace peginterferon and ribavirin for treatment of patients with genotypes 2 or 3 in the future.

Summary

Although triple therapy with a protease inhibitor and pegylated interferon plus ribavirin will increase the cure rate and decrease duration in both treatment-naïve and treatment-experienced patients, there will still remain a large "unmet medical need," in previous null-responders to SOC and patients unable to or unwilling to receive interferon or ribavirin therapy. The availability of multiple DAAs targeting different steps of HCV replication and lacking cross-resistance should facilitate the rapid achievement of what was previously thought to be an unattainable goal – a short duration, interferon-free oral combination, with excellent tolerability and efficacy in both treatment-naïve and treatment-experienced patients.

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Maintenance Therapy with Oral Antiviral Agents

21

Andrew Aronsohn and Donald Jensen

Keywords
Hepatitis C treatment • Direct acting agents • Maintenance therapy • Protease inhibitors • Polymerase inhibitors

Introduction

Direct-acting antiviral agents (DAA) will likely represent a new era of hepatitis C (HCV) therapy. With early clinical trials showing significant improvements in rates of sustained virologic response using new agents such as protease inhibitors, the treatment algorithm for patients with chronic HCV will most certainly change [1, 2]. Attempts at long-term suppression of HCV viral loads using maintenance, lower dose peginterferon (PEG) and ribavirin (RBV) have been largely ineffective in improving clinical outcomes. Although no trials evaluating the efficacy of DAAs as maintenance therapy have been published to date, this may represent a potentially viable therapeutic alternative for some who have not achieved a sustained virologic response. This chapter will review mechanistic differences between current standard of care therapy and new DAAs as well as explore the concept of long-term viral suppression in HCV and the potential role of DAAs in maintenance therapy.

A. Aronsohn, MD • D. Jensen, MD (✉)
Center for Liver Disease, Section of Gastroenterology, Hepatology and Nutrition, University of Chicago Medical Center, 5841 South Maryland Avenue, MC 7120, Chicago, IL, 60637, USA
e-mail: djensen@medicine.bsd.uchicago.edu

Mechanistic Differences Between Standard of Care Therapy and Emerging Direct Antiviral Agents

The hepatitis C virus contains a 9.6-kB reading frame that encodes a single 3,000 amino acid polyprotein which is processed during and after translation into ten mature structural and non-structural proteins. Current standard of care therapy includes PEG and RBV which are unique antiviral agents since they have little direct antiviral activity, instead relying on host immune factors to halt viral replication [3, 4]. Therapy with PEG and RBV is expensive, has many side effects, and has limited efficacy – resulting in sustained virologic response rates of less than 50% in genotype 1 patients [5]. Alternatively, newer agents such as protease inhibitors and polymerase inhibitors have direct antiviral effects and when used in combination with RBV and PEG have been shown to significantly improve response rates [1, 2, 6].

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Interferon

Interferon inhibits HCV replication by stimulating the host innate antiviral response rather than via direct antiviral activity [3]. Endogenous type 1 interferons have immunomodulatory, antiproliferative, and antiviral activities [7]. The mechanism of antiviral action involves induction of IFN-stimulated genes (ISGs) which activate an intracellular antiviral host response [8]. When interferon is given exogenously, it elicits a similar response as endogenous IFN; however, the antiviral effect is enhanced due to higher serum concentrations [8]. Interferon monotherapy is minimally effective in treating chronic HCV, with SVR rates of 16–20% seen after 12 months of therapy [9].

Ribavirin

Ribavirin is a guanosine analog which may be a weak inhibitor of viral polymerase; however, it also interferes with viral replication through immunomodulation, depletion of GTP needed for viral RNA synthesis, and increasing the mutation frequency of HCV creating lower quality replication of virus. Early clinical trials evaluating efficacy of ribavirin monotherapy in chronic HCV showed some improvement in aminotransferase levels; however, there was no significant decrease in HCV RNA levels after 1 year of therapy [10]. These findings have been supported by a more recent pharmacokinetic study of genotype 1 patients treated with RBV alone. Significant viral load decrease occurred in only half of the patients and this effect was transient lasting only 2–3 days despite adequate serum concentrations of drug [11]. Although ineffective as monotherapy, RBV was later found to significantly improve SVR rates when used in combination with PEG; however, the mechanism of synergistic efficacy of these two agents is largely unknown [12].

HCV Protease Inhibitors

The viral NS3/4a protein complex contains a protease enzyme which cleaves the viral polyprotein in multiple sites, playing a crucial role in viral replication [13]. Several agents specifically targeted to inhibit NS3/4a protease activity have been shown to have direct antiviral activity. When given alone, these agents show early reduction of HCV viral load; however, this is usually followed by rapid viral breakthrough secondary to emergence of multiple resistance mutations [14]. When given in combination with PEG and RBV, significant improvements in sustained virologic response have been reported. Triple therapy consisting of the protease inhibitor telaprevir along with RBV and PEG has been studied prospectively in the PROVE 1 and PROVE 2 trials which has shown SVR rates of 60–69% compared to controls of standard therapy with SVR rates of 41–46% [1, 6]. Patients enrolled in telaprevir arms did have higher rates of discontinuation due to adverse events (most commonly rash) [1]. Adding telaprevir to RBV and PEG has also been shown to significantly improve rates of SVR in previous nonresponders and relapsers to standard therapy with PEG and RBV [15]. Bocepravir, an NS3 protease inhibitor, has also been shown to improve response rates when compared to standard HCV therapy. In a recent mulitcenter study consisting of 520 treatment-naïve HCV patients, treatment regiments containing boceprevir resulted in SVR rates of 54–75% vs. 38% in the standard therapy control group [2]. The most common adverse events reported with the use of boceprevir included anemia (34%) and dysgeusia (27%) [2].

Polymerase Inhibitors

The HCV NS5B RNA-dependent RNA polymerase plays a critical role in synthesis of viral RNA and subsequent viral replication. Since this polymerase is not expressed in mammalian cells, polymerase inhibitors represent a highly selective and targeted therapy that may improve HCV therapeutic response rates [16]. Currently, there are two classes of polymerase inhibitors: nucleos(t)ide inhibitors (NI) and nonnucleos(t)ide inhibitors (NNI). NI interacts at the active site of NS5B as a chain terminator and in both in vitro

studies and clinical trials has been shown to have potent antiviral effects and a high barrier to resistance mutations. Early clinical trials using some NI have been limited by hematologic toxicity; however, newer agents demonstrating less adverse events are currently being investigated [17, 18]. Rather than binding directly to the catalytic site, NNIs inhibit viral replication by binding to one of four secondary allosteric sites on the NS5B polymerase which inhibits activity by causing a conformational change in the active site [16]. NNIs studied to date have been relatively well tolerated; however, results in monotherapy treatment have been hampered by virologic breakthrough secondary to emergence of resistant variants [19]. NNIs combined with PEG and RBV are now undergoing investigation.

Other Investigational Agents

Cyclophilin inhibitors and siRNA represent two classes of direct acting agents that are currently undergoing evaluation for therapeutic efficacy in HCV. Cyclophilin B is a cellular protein involved in HCV RNA replication [20]. Inhibition of cyclophilin has demonstrated anti-HCV effects both in vivo and in phase I clinical trials [21, 22]. Long-term clinical trials will be needed to determine safety and efficacy of this agent both as monotherapy and as an adjunct to standard therapy. Short interfering RNA (siRNA) inhibits HCV replication at a posttranslational level by targeting mRNA which blocks structural protein production [23]. Although antiviral efficacy has been demonstrated in vitro, in vivo studies are underway aimed at determining optimal mRNA targets and delivery mechanisms [24].

Maintenance Therapy in Viral Infections

Long-term administration of antiviral therapy as a means to reduce viral load and improve clinical outcomes is used in the setting of many chronic viral infections where complete viral eradication is impossible. Maintenance therapy is the cornerstone of treatment in chronic infections such as HIV and HBV; however, to date, has been ineffective in improving outcomes in HCV [25].

Success of Maintenance Therapy in Other Viral Infections

Unlike HCV, which is an RNA virus that replicates independently of the host genome, HIV is a retrovirus that requires a DNA copy of its RNA to integrate into the host genome as part of its replication cycle [26]. With currently available antiretroviral medications, complete viral eradication from the host is impossible; however, significant suppression of viral load is attainable in most patients. Multiple classes of antiviral therapies are used in treating HIV which include nucleoside (and nucleotide) reverse transcriptase inhibitors (NRTIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), integrase strand transfer inhibitors (INSTIs), and CCR5 antagonists [27]. Current guidelines recommend initiation of therapy using a combination of three drugs with a primary goal to reduce HIV viral load to <50 copies/mL [27]. Since effective therapy suppresses but does not eradicate the virus, discontinuation of therapy usually results in relapse of viral load and long-term therapy is almost always indicated. As expected, long-term antiviral therapy has been associated with viral resistance which can be effectively treated by modification of therapeutic regiments [27]. Persistent reduction of HIV viral load had been shown to restore immune function, measured by CD4 count, and subsequently improve clinical outcomes. Successful HAART therapy reduces the incidence of opportunistic infections, improves quality of life, and results in an overall survival benefit [28–30]. In 2005, Sterne and colleagues examined patients treated with HAART therapy after 1996 and reported an 86% decrease in progression to AIDS or death compared to patients who did not receive therapy [30].

Hepatitis B virus is a DNA virus that includes an envelope and a nucleocapsid which contains an incomplete double-stranded DNA virus, viral polymerase, and a core protein. After entry into the host cell, HBV integrates into the host genome

and produces a covalently closed circle DNA (cccDNA) which is crucial for viral replication [31, 32]. Similar to HIV, complete viral eradication from the host is often impossible; however, improved clinical outcomes have been repeatedly demonstrated in patients on long-term antiviral therapy with suppressed viral loads. Oral antiviral agents such as lamivudine, adefovir, entecavir, and tenofovir have been shown to be effective as monotherapy in dramatic reduction of HBV viral load [33]. In a 3-year follow-up of patients with chronic HBV on lamivudine therapy, 56% of patients demonstrated histologic improvement on therapy and only 11% showed worsening of disease [34]. Increased viral load has been shown to be an independent risk factor for hepatocellular carcinoma and treatment with oral antiviral agents has been shown to reduce this risk [35, 36]. Finally, Liaw et al. demonstrated a reduced risk of hepatic decompensation among patients with advanced fibrosis or cirrhosis who were on long-term antiviral therapy [35]. Clearly, in both HIV and HBV, clinical benefit is achieved with the use of antiviral medication aimed at producing long-term viral suppression.

Maintenance Therapy in HCV

Conversely, maintenance therapy aimed at suppression rather than viral eradication has not been shown to be beneficial in HCV. The largest study evaluating the effect of maintenance therapy in chronic HCV came from the HALT C group which evaluated 1,050 patients with HCV who had not responded to previous therapy with interferon and ribavirin. Five hundred seventeen patients received 90 μg of peginterferon alfa-2a for 3.5 years. Compared to controls, those receiving peginterferon did not have significant reduction of primary end points which included death, hepatocellular carcinoma, hepatic decompensation, and increase of Ishak fibrosis score of 2 or more points [25]. Further analysis of the HALT C data did show reduction in adverse clinical outcomes in a small subset of patients who were able to maintain a greater than 4 log decrease in viral load [37]. Although only a small number of patients were able to attain this degree of viral suppression, this is an important finding since it shows that a persistently suppressed viral load without eradication may lead to improved outcomes. Finally, ribavirin maintenance therapy has also been studied and failed to show improvement of symptoms, HCV RNA levels, and hepatic fibrosis scores [38].

There are several possible explanations for the lack of efficacy of maintenance therapy in HCV. One explanation may be related to the relatively small degree of viral suppression observed during maintenance therapy in HCV. Unlike HIV and HBV, where chronic antiviral therapy often leads to undetectable viral loads, HCV trials such as HALT C only yielded a 0.71 log decrease in viral load during 3.5 years of therapy. It is likely that a more robust and persistent decrease in viral load would be necessary to significantly reduce any adverse clinical outcomes as was seen in the subset analysis of HALT C patients who maintained suppressed viral loads [37]. In addition, published trials of maintenance therapy have only been performed using agents such as interferon and ribavirin which do not have direct antiviral activity but rather rely on the host immune response. Use of more directly acting agents could, theoretically, produce a more potent and sustained viral suppression. Finally, most trials evaluating efficacy of maintenance therapy have studied cohorts of patients who have failed previous attempts at HCV treatment. In light of recent data identifying the genetic variation of *IL28B* as a predictor of treatment response, it is plausible that most patients enrolled in maintenance trials had unfavorable genetic predispositions to respond to therapy and more success may have been seen if patients with the favorable genotype were enrolled [39].

The HCV life cycle differs from HIV and HBV in that HCV is an RNA virus without reverse transcriptase and there is no HCV integration into the host genome. These characteristics make HCV eradication a possibility, thus making cure, not control, the goal of therapy. In contrast to patients with HCV who have received maintenance therapy, those who achieve a sustained virologic response have demonstrated significantly reduced rates of liver-related morbidity and mortality [40].

Table 21.1 Patient groups who may benefit from long-term DAA therapy

Patients unable to tolerate or have contraindications to PEG or RBV
• Cardiopulmonary disease
• Psychiatric disease
• Severe immune-mediated disorders
Viral suppression posttransplantation
• Potentially stop or delay histologic progression of HCV
Cirrhosis
• Suppress virus with less risk of decompensation when compared to current standard of care therapy
Control person-to-person spread
• Especially among high-risk groups
Viral suppression in patients with sensitive wild-type virus
• Patients who relapse after PEG/RBV therapy

The Potential Role of DAAs in HCV Maintenance Therapy

Although there is currently no published data, there may be a *theoretical* role for direct antiviral agents in maintenance therapy for chronic HCV (Table 21.1). Previous trials using low-dose interferon with or without ribavirin in prior treatment failures have failed to persistently produce sustained viral load suppression and clinical benefit [25, 38]. Newly developed agents such as protease inhibitors, polymerase inhibitors, and other small molecule inhibitors have more potent antiviral activity and may be able to more consistently suppress virus over longer periods of time, particularly if given in combination. Early trials of combinations of protease and nucleoside polymerase inhibitors, without interferon and ribavirin, have demonstrated potent viral suppression without viral breakthrough out to 2 weeks (INFORM-1) [41]. Persistent suppression without the emergence of resistant mutations may lead to reduction of adverse clinical outcomes as is seen in HBV, such as cirrhosis progression and hepatocellular carcinoma. Furthermore, many patients are unable to tolerate interferon and/or ribavirin due to concomitant medical conditions, and have been excluded from current treatment paradigms. These patients may also be candidates for long-term combination DAA therapy. In the absence of interferon and ribavirin, duration of therapy may need to be extended (perhaps indefinitely) to ensure effective viral eradication. Patient groups where this may be a viable strategy include: solid organ posttransplant patients, those with severe cardiopulmonary disease, severe psychiatric disease, and those with severe immune-mediated disorders.

Direct antiviral agents may also be of potential use in the setting of emergence of resistant mutations with low virulence which may occur during attempts at eradicative therapy. Rather than discontinuing therapy in patients with no other rescue therapy, DAAs could potentially be used to chronically suppress these resistant strains and improve clinical outcomes. This could be especially useful in patients at high risk of hepatic decompensation such as those with cirrhosis and those in the posttransplant setting. The concern, of course, is the ultimate development of double or triple viral mutations which could potentially eliminate a class of antiviral agents.

Other potential uses of DAA maintenance therapy may include viral suppression as a means to control person-to-person spread (especially in patients with high-risk behaviors) and in patients who repeatedly relapse with a sensitive wild-type virus. Direct acting antiviral agents may not only improve rates of sustained virologic response, but they may also represent a viable option to improve outcomes in patients who are unable to completely eradicate the virus.

Conclusions

In conclusion, since cure is the goal of therapy in HCV treatment, there is a limited role for maintenance therapy. New DAA agents will increase the number of patients who will completely eradicate the virus, and in some circumstances, may also prove useful in chronic viral suppression. Previous trials attempting long-term viral suppression using interferon and ribavirin have been largely unsuccessful, which was likely due to their relatively weak antiviral effects. Newer, more potent agents may offer improved long-term viral suppression. Further study will be needed to determine if these agents are capable of producing

long-term suppression without significant emergence of additional resistant mutations and, more importantly, if suppression without eradication results in improved long-term clinical outcomes. Since direct antiviral agents will likely offer a relatively high probability of cure, it will be difficult to construct ethically permissible clinical trials aimed at viral suppression and we may look to case reports and small series of high-risk patients who have failed standard therapy to determine the efficacy of DAAs as maintenance agents.

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Part IV

Liver Transplantation for Chronic HCV

Natural History of Chronic HCV After Liver Transplantation

22

Bart J. Veldt and Michael R. Charlton

Keywords
HCV • Natural history • Recurrence • Immunosuppression • Antiviral therapy

The Natural History of Posttransplant HCV Infection

HCV infection of the allograft occurs at the time of transplantation, with negative-strand HCV RNA detectable in the first postoperative week. HCV RNA is cleared rapidly from serum during the anhepatic phase. Following reperfusion, the rate of decrease in HCV RNA accelerates, almost certainly reflecting HCV binding to its obligatory hepatic receptors. HCV RNA levels typically increase rapidly from week 2 posttransplantation, peaking by the fourth postoperative month. At the end of the first postoperative year, HCV RNA levels are, on average, 10–20-fold greater than pretransplant levels [1]. Histological features of hepatitis develop in ~75% of recipients in the first 6 months following liver transplantation [2]. By the fifth postoperative year up to 30% have progressed to cirrhosis [2]. A small proportion of patients (4–7%) develop an accelerated course of liver injury (cholestatic hepatitis C, associated with very high levels of viremia) with subsequent rapid allograft failure. Early post-LT histology, e.g., at 1 year, has been consistently predictive of subsequent fibrosis progression [1].

The impact of recurrence of HCV on the allograft has led to long-term graft survival for recipients with HCV infection that is relatively lower than that of recipients undergoing liver transplantation for most other indications [3].

There is an interplay of factors, such as immunosuppression, infectious complications, donor and recipient risk factors, and antiviral therapy, that can affect the course of outcomes following LT for HCV-associated liver disease (Table 22.1).

B.J. Veldt, MD
Department of Gastroenterology and Hepatology, Erasmus MC University Medical Center, Rotterdam, The Netherlands

M.R. Charlton, MD, MBBS (✉)
Mayo Clinic, Division of Gastroenterology and Hepatology, 200 First Street SW, Rochester, MN 55905, USA
e-mail: charlton.michael@mayo.edu

Immunosuppression: Impact on Viremia and Recurrence

Corticosteroids

Pulsed intravenous methylprednisolone treatment for acute cellular rejection is associated with transient 1–2 log increases in HCV RNA

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Table 22.1 Risk factors for more severe recurrence of HCV

Factor	Strength of evidence
Pretransplant	
Donor age (linear for age >35, HR 1.01/year)	+++
Donor/recipient HLA matching	+
Genotype 1B	+
HIV coinfection	++
IL28B donor and recipient TT genotype	++
Operative	
Longer cold ischemic time (>12 h)	++
Donor genetic factors	+
Posttransplant – recipient	
Advanced age (>50 years)	+++ (Patient/graft) survival
Nonwhite race/ethnicity	++ (Patient survival)
Lack of antiviral treatment of HCV	+++
Virological variables	
Higher pretransplant viral load	+++
Higher posttransplant viral load	+++
Immunosuppression	
OKT3, pulsed corticosteroids	+++
Short time to recurrence	+++
Treated cytomegalovirus infection	+++

levels [4, 5], probably due to the suppression of the HCV immune response [6].

In addition to being proviral, treatment of acute cellular rejection with corticosteroids is associated with increased mortality and graft loss in LT recipients with HCV infection (relative risk = 2.7–2.9, $P = 0.04$) [7].

In an Italian study, patients receiving a higher daily prednisone dose, 12 months after transplantation, the proportion of recurrent hepatitis C was reduced by 50%, and it was suggested that a long-term treatment with corticosteroids, slowly tapered off over time, may prevent the more aggressive forms of recurrent liver disease [8]. Indeed, Berenguer et al. showed that avoiding abrupt variations in immunosuppression by slow steroid tapering over more than 6 months may lead to reduced incidence of severe recurrent hepatitis C infection [9].

Lladó et al. have shown that immunosuppression with basiliximab and cyclosporine without prednisone is feasible, without an increase in overall rejection rate [9]. Moreover, it has been suggested that a regimen consisting of tacrolimus, MMF, two doses of basiliximab, and only 6 days of steroids is feasible and may lead to lower rates of posttransplant diabetes mellitus and lower rates of histologic HCV recurrence compared to historic controls, with no increased risk of rejection [10]. However, in a small randomized study, Vivarelli et al. found that fibrosis stages at 1 year after transplantation were higher in patients undergoing rapid steroid tapering within 3 months, compared to patients with slow tapering of steroids [11].

Finally, a large ($n = 312$) randomized controlled study, which included a steroid free arm of immunosuppression, has, to date, found no difference in the rate of recurrence of HCV nor in patient or graft survival between steroid free and steroid utilizing arms [12].

There is thus no compelling basis for avoiding corticosteroids in the early postoperative period.

Calcineurin Inhibitors

In the nontransplant setting, cyclosporine A treatment does not produce changes in HCV viremia. Although no studies of the independent impact of tacrolimus on HCV viremia have been reported, posttransplant HCV levels are similar among patients receiving tacrolimus and patients receiving cyclosporine A. The relative impact of the choice of calcineurin inhibitor on posttransplant outcomes merits detailed consideration. In a prospective randomized controlled study of 495 recipients with HCV infection, no difference was seen in the histological recurrence rate of hepatitis C at 12 months posttransplantation between patients receiving cyclosporine vs. tacrolimus [13]. A meta-analysis of studies comparing the two calcineurin inhibitors, however, found a patient and graft survival benefit associated with tacrolimus as maintenance immunosuppression (graft loss: hazards ratio 0.73, 95% CI 0.61–0.86) [14]. There is emerging evidence that cyclosporine may have an impact on HCV biology that requires

concomitant administration of interferon. The binding of NS5B to cyclophilin B is inhibited by cyclosporine A (cyclophilin B is a functional regulator of the NS5B-RNA-dependent RNA polymerase). In the nontransplant setting, the combination of IFN and cyclosporine results in significantly higher virological and biochemical response rates than IFN monotherapy.

The potentiation of the antiviral effects of cyclosporine was strongly implied in a study in which 21 liver transplant recipients with recurrence of HCV were treated for 6 months with peginterferon alpha and ribavirin while maintained on tacrolimus monotherapy for maintenance immunosuppression [15]. Eight patients who had not achieved a virological response after 6 months of antiviral therapy were switched from tacrolimus to cyclosporine. Five of these eight became HCV RNA negative after the conversion from tacrolimus to cyclosporine. These intriguing data need to be confirmed in a large randomized trial.

In a small randomized controlled trial, 48 patients were either switched to cyclosporine or continued on tacrolimus during antiviral therapy with peginterferon and ribavirin for HCV recurrence. Seven patients out of 20 (35%) in the tacrolimus group vs. seven out of 18 patients who switched from tacrolimus to cyclosporine (39%) ($P=0.80$) achieved a sustained virological response [16].

Further evidence of the importance of cyclophilin B inhibition in the treatment of HCV has been demonstrated by the antiviral effects of a nonimmunosuppressive, potent inhibitor of cyclophilin, Debio-025, in HCV/HIV-coinfected patients [17]. The availability of nonimmunosuppressive cyclophilin inhibitors is eagerly awaited in the transplant arena. Meanwhile, a theoretical case could be made for using tacrolimus as initial (e.g., for the first 2 postoperative months) maintenance immunosuppression for recipients with HCV infection, changing to cyclosporine during interferon-based antiviral therapy. Such an approach would take advantage of cyclophilin inhibiting properties of cyclosporine during antiviral therapy, and the greater immunosuppressive potency of tacrolimus for maintenance immunosuppression, minimizing the frequency of acute cellular rejection.

Mycophenolate Mofetil

Mycophenolate mofetil (MMF), a potent inosine monophosphate inhibitor (as is ribavirin), has been shown to have antiviral properties against flaviviruses. In vitro, it has been shown that mycophenolic acid inhibits hepatitis C virus replication and acts in synergy with cyclosporine A and interferon-alpha [18]. Neither HCV RNA levels nor liver biochemistries are thought, however, to be affected significantly by MMF treatment in vivo.

Although MMF has been reported to be associated with more severe recurrence of HCV, a negative impact of MMF on recurrence of HCV has been refuted by analyses of the UNOS/SRTR database [19] and large randomized controlled trials [20], which found MMF triple therapy was associated with a reduced risk of death (hazard ratio [HR] = 0.77, $P<0.001$) and graft loss (HR = 0.81, $P<0.001$) [19].

Moreover, in a large retrospective study comparing 4,946 patients on a three-drug regimen including MMF to 3,884 patients on a two-drug regimen without MMF, treatment with MMF was associated with a 6% lower adjusted risk of progressive renal dysfunction [21].

Based on the aggregate of these reports, the impact of MMF on recurrence of HCV appears to be neutral or beneficial to long-term outcomes.

T-Cell Depleting Therapies

OKT3 administration has consistently been identified as a significant risk factor for both the time to development of and the severity of histological recurrence of hepatitis C. The notion of a negative impact of T-cell depletion on posttransplant outcomes in recipients with HCV infection is supported by the potent effect of alemtuzumab (Campath) in exacerbating recurrence of HCV [22]. Data concerning the impact of rabbit antithymocyte globulin (ATG), an increasingly popular induction agent, are less clear. Outcomes in patients with HCV infection who received induction ATG have been reported to be similar to controls who did not receive ATG, with an analysis

of outcomes from three centers further suggesting that induction with ATG is associated with less severe fibrosis progression [23]. The interpretability of any of these studies is greatly limited by the lack of protocol biopsies and the use of historical controls.

The results of a prospective randomized study including 93 liver transplant patients suggest that the addition of thymoglobulin to a triple immunosuppressive regimen (tacrolimus, MMF, and steroids) does not modify the incidence of acute rejection episodes or long-term survival and is responsible for increased leukopenia rates [24].

Therefore, thymoglobulin should probably be used cautiously, if at all, in liver transplant recipients with HCV infection.

Interleukin-2 Receptor Inhibition

Large ($n = 300$) randomized, controlled studies of Interleukin-2 receptor antibody-based therapy in liver transplant recipients with HCV infection suggest a neutral impact on medium-term outcomes, including allograft histology [12, 25].

Post-transplant Diabetes Mellitus (DM)

HCV infection may contribute to posttransplant insulin resistance through effects of HCV on insulin signaling on a molecular level. Proteins such as insulin receptor substrates mediate signaling of the insulin receptor. Tyrosine phosphorylation of the insulin receptor substrate by insulin is a crucial step in insulin action [26]. Interestingly, in a mouse model harboring the HCV core gene, this step in the insulin pathway is affected [27].

Disturbances in insulin action and sensitivity may affect different pathways eventually leading to liver fibrosis [28]. One of the most important features of insulin resistance is impaired suppression of hepatic glucose production [29]. Loss of control over hepatic glucose output leads to hyperglycemia and compensatory hyperinsulinemia, which in turn may exert mitogenic and proliferative effects on hepatic cells [30, 31].

In contrast to hepatic glyconeogenesis, pathways involved in hepatic lipogenesis initially remain insulin sensitive [32, 33]. However, hyperinsulinemia may stimulate expression of tumor necrosis factors-alpha (TNF-α) and interleukin (IL)-6, eventually leading to increased lipid synthesis and hepatic steatosis [34, 35].

Risk Factors for Post-transplant DM

Several retrospective studies have shown that hepatitis C infection is an independent predictor of posttransplant DM [36–38].

A large retrospective study including 15,463 liver transplant recipients without pretransplant DM showed that other risk factors beside hepatitis C were recipient age older than 50 years, African American race, body mass index, recipient cirrhosis, donor age older than 60 years, diabetic donor, tacrolimus and steroid treatment at hospital discharge. Living donor transplant and induction therapy were associated with a decreased risk of posttransplant DM [39].

Although treatment with tacrolimus is a risk factor for posttransplant DM, it is a potent imunnosuppressant, and as mentioned earlier, the current evidence suggests that tacrolimus be used as initial maintenance immunosuppression for recipients with HCV infection, changing to cyclosporine during interferon-based antiviral therapy.

Consequences of Post-transplant DM

Fibrosis progression: Posttransplant DM is a risk factor for fibrosis progression in the graft. Foxton et al. investigated 163 patients undergoing LT for HCV and found that donor age and both pretransplant and posttransplant DM were risk factors for progression to severe fibrosis [40]. Indeed, Veldt et al. confirmed that fibrosis progression is more rapid in HCV-positive liver transplant patients with overt DM as well as in patients with elevated Homeostasis Model Assessment of Insulin Resistance (HOMA-IR). Thus, HOMA-IR can be used as an early predictor to identify insulin-resistant patients at risk for rapid fibrosis progression [40].

Hepatocellular carcinoma (HCC) development: The incidence of HCC is estimated to be 25 per 100,000 person-years among liver transplant recipients, and both HCV infection and DM

are risk factors for development of HCC. In a large study among liver transplant recipients in the USA, the incidence of HCC was 5 times elevated for HCV-positive liver transplant recipients compared to the general population and 6.2 times for recipients with DM [41].

Mortality: Steinmüller et al. showed that posttransplant DM, but not pretransplant DM is associated with decreased 5-year survival rates [42].

In a study comparing long-term outcomes between 47 HCV-positive and 111 HCV-negative liver transplant recipients, Baid et al. found that posttransplant DM was more prevalent among HCV-positive recipients and was an independent predictor of mortality, with a hazard ratio of 3.7 [43].

Infectious Complications and Coinfections

Cytomegalovirus (CMV)

CMV is a DNA virus that commonly causes infection in immunocompromised patients, including liver transplant recipients. CMV has been shown to have direct immunosuppressive effects that increase the risk for other opportunistic infections. Moreover, posttransplant CMV infection has been repeatedly and strongly associated with increased severity of recurrence, even after adjusting for covariables such as degree of immunosuppression [44, 45], resulting in poorer graft survival rates for patients with CMV disease than for patients without CMV [46].

While these data suggest that targeted prophylaxis against CMV might reduce the impact of CMV infection on posttransplant outcomes in HCV-infected liver transplant recipients, a randomized controlled trial directly addressing this question has never been performed.

Human Herpesvirus 6 (HHV-6)

HHV-6 and CMV infection and viral load are not associated with increased overall rates of HCV recurrence or HCV viral load after liver transplantation but might be associated with more severe forms of recurrence [47]. Several small studies have suggested that HHV-6 viremia in HCV-positive liver transplant recipients is associated with an increased risk for early fibrosis upon HCV recurrence [48]. However, a larger study including 92 liver transplant recipients did not show any effect of HHV-6 reactivation on graft survival or mortality [49].

Human Immunodeficiency Virus (HIV) Coinfection

Until 2004 HIV infection was considered an absolute contraindication to liver transplantation [50]. The introduction of highly active antiretroviral therapy (HAART) rendered restoration of the immune function possible and nowadays HIV coinfection is no longer an absolute contraindication for liver transplantation in HCV-infected patients.

Nevertheless, HIV/HCV-coinfected patients have higher HCV RNA loads and show more rapid progression of fibrosis than do monoinfected patients [51]. Also, HCV/HIV-coinfected patients have about a threefold greater risk of antiretroviral therapy-associated hepatotoxicity than patients with HIV only.

In a study comparing 35 HCV/HIV-coinfected patients to 44 HCV-monoinfected patients undergoing liver transplantation, the 5-year survival rates were 81 and 51% in coinfected patients and monoinfected patients, respectively [52]. However, coinfected patients had higher initial MELD scores than monoinfected patients, and in multivariate analysis initial MELD score was the only predictor of survival.

Hepatitis B Coinfection

There are no large studies describing the outcome of HBV–HCV coinfection after liver transplantation. However, a study including 204 liver transplant patients, among whom 9 patients with HBV–HCV coinfection, suggested that graft survival was not worse and even better in the coinfected patients [53].

Another study including 13 HBV–HCV coinfected patients suggested that concomitant hepatitis delta infection may be beneficial, leading to suppression of HCV replication after LT [54].

Recipient Genetic Factors

Gender

Although male gender has been identified as an independent risk factor for development of HCC after transplantation [41], a recent multicenter cohort study showed that graft survival might be slightly lower in female than in male HCV patients undergoing LT [55].

Interestingly, not only recipient gender per se seems to influence the outcome of liver transplantation, but also mismatches between donor and recipient gender seem to be important.

A large study comparing 13,992 gender-mismatched patients with 18,552 gender-matched patients showed a small but statistically significant increase in the risk of graft failure of 12.2 vs. 11.3%, respectively [56].

Human Leukocyte Antigen (HLA) Type

HLA matching is, at present, not used for the allocation of cadaveric hepatic allografts because the liver is generally believed to be less susceptible to HLA-mediated rejection.

There has been one retrospective study that suggested an advantage of HLA type I-matched donor–recipient pairs compared to non-HLA I-matched pairs [57]. This differs from renal transplant recipients, who are matched for HLA type II. A more recent study among 321 pediatric liver transplant recipients found no supportive evidence of beneficial effects of HLA matching in this group [58].

Ethnicity

The prevalence of HCV infection is not equal among patients with various ethnic backgrounds. HCV infection is more prevalent among African Americans, with a higher rate of detectable viremia, predominance of genotype 1, and a higher viral load. Moreover, African Americans exhibit lower response rates to antiviral therapy [59]. Among Asian patients, the risk of developing HCC is increased compared to other ethnicities [60].

A study including 166 HCV-positive liver transplant patients showed that graft survival was poorer for nonwhites than for whites, the cause of death being recurrent HCV in 19% of whites compared with 44% of nonwhites [61]. An `explanation for this ethnic difference in HCV recurrence may be that the IL28b TT-genotype, which is associated with HCV recurrence, is more prevalent among nonwhites (see below).

IL-28B Genotype

Polymorphism in the *IL28B* gene region, encoding interferon-lambda(λ)-3, is strongly predictive of response to antiviral treatment in the nontransplant setting. There is emerging evidence that *IL28B* genotype is also important in the liver transplant setting. *IL28B* genotyping of the polymorphism rs12979860 was performed on DNA from all donors and recipients in a cohort of 189 consecutive patients undergoing liver transplantation for HCV-associated liver disease. Sixty five of these recipients received IFN-based antiviral therapy posttransplantation. Several important observations were made in this study. The first is that the CC *IL28B* variant was less common in the LT recipients with HCV infection than in non-HCV donor livers (33 vs. 47%, $P=0.03$). This is consistent with a role for the CC variant in spontaneous clearance of HCV, with enrichment for the non-CC variants in the chronic hepatitis C population. Indeed, a role for the CC variant in promoting natural clearance has recently been established. The second observation was that *IL28B* recipient genotype was significantly predictive of fibrosis stage, with TT genotype being associated with more rapid fibrosis (Pearson Chi-square $P=0.024$ for the comparison CC vs. TT). Finally, donor and recipient *IL28B* genotype were independently associated with SVR ($P<0.005$). The presence of *IL28B* CC variant in either the recipient (R) or donor (D) liver was associated with increased rate of SVR (D-non-CC/R-non-CC = 3/19 (16%) vs. D-CC/R-non-CC = 11/22 (50%) vs. D-non-CC/R-CC = 5/12 (42%) vs. R-CC/D-CC = 6/7 (86%), $P=0.0095$). *IL28B* genotype was not significantly associated with survival (overall/liver related).

These results demonstrate that recipient *IL28B* genotype is associated with more severe histological recurrence of HCV. Recipient and donor liver *IL28B* genotype are strongly and independently associated with IFN-based treatment

response in patients post-OLT. The data suggest that CC donor livers might be preferentially allocated to patients with HCV infection.

In summary, recipient variables, including recipient gender, HLA type, and ethnicity, lack sufficient sensitivity and specificity to be used in determining eligibility of patients for liver transplantation or identifying candidates for preemptive antiviral therapy [45]. Recently, a single nucleotide polymorphism (SNP) upstream of the *IL28B* gene has been discovered, which was found to be associated with response to therapy with pegylated interferon and ribavirin, with spontaneous clearance of acute hepatitis C and also with recurrence of HCV after LT [62, 63]. In the near future, *IL28B* genotyping might allow for improved selection of donor livers to be used preferably in HCV-positive recipients.

Donor Factors

Donor factors are potentially modifiable or selectable and are thus of particular interest.

Factors of interest include donor age, preservation injury or steatosis of the donor liver, living donor liver transplantation, and donor ethnicity.

Donor Age

An association of advancing donor age with more rapid and severe histological progression of HCV recurrence has been very reproducible [45]. The effect is nonlinear, with donor age greater than 65 years associated with more rapid progression of fibrosis and allograft failure.

Donor Steatosis

It has been stated that the use of steatotic grafts does not exacerbate the progression of fibrosis or affect patient survival in HCV-positive recipients. However, the study these conclusions are based on included only five HCV-positive recipients who received a liver with moderate or severe steatosis [64].

An earlier study of 120 liver transplantations with steatotic livers (72 mild, 25 moderate, and 23 severe steatosis) has shown that both initial poor graft function and 1-year graft loss were more common in recipients of donor livers with moderate or severe steatosis [65].

However, moderate steatosis of the donor liver alone does not necessarily lead to worse outcomes when used as a graft in otherwise optimal conditions. In combination with other unfavorable criteria such as high Model for End-Stage Liver Disease (MELD) score, donor or recipient age >50 years, or prolonged ischemic time, the use of fatty livers leads to higher rates of graft loss [65–67]. Complying with these restrictions will limit the use of steatotic donor livers, since donors of steatotic livers are generally older.

Ischemic Times

Cold ischemia time is the time interval that begins when the donor liver is cooled with perfusion solution after procurement surgery and ends when the liver is implanted.

In a study including 120 liver transplantations for HCV-related end-stage liver disease, a cold ischemia time of more than 12 h was an independent predictor of mortality, as determined by multivariate analysis [67].

The warm ischemia time is defined as rewarming time between beginning of the vena caval anastomosis and portal vein reperfusion during the recipient operation. A study including 56 HCV-positive liver transplant recipients showed that a prolonged warm ischemic time was associated with more severe HCV recurrence after transplantation [68].

In an analysis of the United Network for Organ Sharing (UNOS) database, including 5,640 HCV-positive liver transplant recipients, warm ischemia was among others an independent predictor of graft survival [69]. Ghobrial et al. also found that both cold and warm ischemia times are important predictors of graft survival in HCV-positive recipients [70].

Preservation Injury

Preservation injury, also known as harvesting or reperfusion injury, is suspected when abnormal liver enzymes occur in the early postoperative period. Histological findings include neutrophilic infiltration, microvesicular steatosis, hepatocyte

cytoaggregation that progresses to centrilobular necrosis, hepatocyte swelling, and cholestasis. These histologic changes suggestive of preservation injury may be a result of perioperative factors, such as prolonged ischemic times and donor factors, such as age and steatosis.

Preservation injury may therefore be a surrogate marker which helps to identify the subgroup of HCV patients at highest risk for poor outcomes [71].

Living Donors

Recurrence of HCV is not affected by the use of living donor organs, when compared to deceased donor organs, provided total center volume exceeds 20 [72].

In a long-term follow-up study including 202 patients with adult-to-adult living donor liver transplantation and 69 liver transplant patients with deceased donors, patients receiving a graft from a living donor showed less acute rejection episodes and had similar rates of histological HCV recurrence [73].

Donor Ethnicity

Rustgi et al. showed that mismatches between donor and recipient ethnicity, especially between Afro-American donors and Caucasian recipients, may increase the risk of graft failure (11.5% in white-to-white compared to 15.1% in black-to-white transplantations) [56].

These findings have been confirmed by Pang et al., who also showed that ethnic mismatches between Caucasian donors and Afro-American recipients lead to worse survival rates. The crude 5-year survival rate for black recipients who had a black donor was 14% higher than the 5-year survival rate for black recipients who had a white donor [74].

Different immunologic factors such as racial variations in antigen-presenting cells and an increased proliferation to phytohemagglutinin in purified T cells of blacks have been proposed as mechanisms that might be responsible for some of these ethnic differences [75]. It has also been shown that a subgroup of black kidney transplant recipients who lack DR3 antigen has decreased graft survival in comparison with black recipients who do express DR3 antigen [76]. Ethnic variations in the recently described IL28b gene may also be responsible for some of the above-mentioned differences in outcome after transplantation.

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Use of HCV-Positive Organs in Patients With and Without Chronic HCV

23

Elizabeth C. Verna and Robert S. Brown Jr.

Keywords
Hepatitis C virus • Liver transplantation • Extended criteria donation

Introduction

The shortage of organs for transplantation of all types has led to broadening of the criteria used to identify acceptable organ donors, including those from donors seropositive for hepatitis C virus (HCV) infection. The importance of HCV serologies in organ donation has been clear since the virus was first identified, and several early studies confirmed that the vast majority of recipients of HCV-positive organs went on to chronic HCV infection [1, 2]. These organs have been primarily utilized for recipients with pre-existing chronic HCV infection, but in selected cases they are also placed into recipients without HCV. The majority of these patients will become viremic and have the potential to develop sequelae of chronic HCV infection. The clinical course of the disease is generally accelerated in the setting of post-transplant immunosuppression. The clinical manifestations of chronic HCV following solid organ transplantation are twofold: chronic hepatitis and progressive hepatic fibrosis; and the indirect effects of chronic immune activation and cytokine release which may contribute to a variety of pathologic effects ranging from rejection to accelerated atherosclerosis [3]. In addition, HCV treatment after transplant of any organ with interferon-based therapies likely increases the risk of graft rejection, especially in kidney transplantation [4–6].

Patient and graft outcomes in transplantation of HCV positive liver, heart, and kidney grafts have now been studied on the national and center levels. We herein review the literature on the transplantation of HCV-positive grafts into recipients with and without chronic HCV and speculate on future developments as anti-HCV therapy improves over the coming years.

HCV-Positive Liver Allografts

Although transplantation of HCV-positive liver allografts was avoided for many years because of the fear of more significant graft dysfunction with viral transmission, HCV-positive organs are now among the more common extended criteria donor categories, and are primarily used in recipients with chronic HCV. The number of

E.C. Verna, MD, MSc • R.S. Brown Jr., MD, MPH (✉)
Center for Liver Disease and Transplantation,
Columbia University College of Physicians
and Surgeons, 622 West 168th Street,
New York, NY 10032, USA
e-mail: rb464@columbia.edu

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HCV-positive donors has risen steadily in recent years, from less than 50 per year in 1994 to almost 200 by 2007 [7].

The early studies to evaluate this population included several single-center studies showing no significant difference in short-term patient and graft survival [8–13]. Subsequently, long-term outcomes in a single center were published with 10 years of follow-up, showing similar patient survival and severe HCV recurrence rates in HCV-positive recipients who received HCV-positive or HCV-negative grafts [14]. In this study, Testa et al. reviewed outcomes in 22 patients who received HCV-positive grafts and 115 who received HCV-negative grafts between 1985 and 1995, and found similar rates of biopsy proven recurrence (54.55 vs. 41.74%), patient survival (83.9 vs. 71.9%), and graft survival (79.1 vs. 76.2%), respectively.

In contrast, at least one other large single-center cohort suggested that HCV-positive grafts do have a detrimental effect on rates of post-transplant fibrosis [15]. In this study, Khapra et al. analyzed 39 recipients of HCV-positive allografts and 580 recipients of HCV-negative grafts and found that patients who received HCV-positive allografts from older donors (age 50 or older) had higher rates of graft failure [hazard ratio (HR), 2.74] and death (HR 2.63) compared to recipients of HCV-negative grafts of similarly aged, older donor livers. In addition, matched case–control analysis showed that recipients of HCV-positive grafts experienced more severe fibrosis post-transplantation than recipients of HCV-negative livers, regardless of donor age.

Larger cohorts of patients in nationwide databases have also been studied to better assess graft and patient outcomes [16, 17]. In the largest and most recent of these studies, Northrup et al. analyzed over 56,000 transplants between April 1994 and February 2008 through the US Organ Procurement and Transplantation Network Scientific Registry [7]. HCV-positive grafts were used in 934 (1.7%) liver transplants during that period, the majority (79.3%) of which were transplanted into HCV-positive recipients. No significant difference in 5-year survival was observed between HCV-positive recipients transplanted with HCV-positive (67.0%) or HCV-negative organs (67.8%, $p=0.08$). Adjusting for multiple known risk factors, donor HCV status was not an independent predictor of mortality after liver transplantation (HR 1.07, $p=0.24$).

HCV-positive liver allografts are less commonly placed into HCV-negative recipients. Velidedeoglu et al. [17] identified 29 HCV-negative patients in the UNOS database who received HCV-positive grafts between 1995 and 1999. Survival in these patients was similar to that of HCV-positive patients who received HCV-positive grafts. Northrup et al. [7] subsequently re-evaluated survival in this group after longer follow-up and added patients transplanted between 1994 and 2008. The 193 patients without HCV who received an HCV-positive graft were compared to patients with HCV in the registry. Recipients without HCV who received an HCV-positive graft were more likely to be males, have hepatocellular carcinoma (HCC), be older, and have grafts from older donors with longer cold ischemia times. In addition, many of the HCC patients were not allocated these organs under the formal MELD exception system, and it is likely that many of these HCV-positive organs were transplanted into patients with HCC beyond the Milan criteria. In this study, the recipient negative-donor positive group had the lowest survival of all donor–recipient groups, with a 5-year survival of only 55.1%. Because there was no control group, it is impossible to prove that these outcomes would have been better than not transplanting these patients. Despite this, concern about disease transmission and poor outcomes has led to a general avoidance of transplanting HCV-positive organs into HCV-negative recipients.

Given the available data to date, it is likely that HCV-positive liver allografts will continue to be used in HCV-positive but not in HCV-negative recipients. However, HCV-positive older liver donors should be used with caution, even in HCV-positive liver recipients because of data suggesting a reduced survival. It is unclear whether this reduction in survival is due simply to the well-recognized overall impact of donor age on post-transplant HCV progression or

whether older HCV donors have more advanced baseline fibrosis than younger HCV donors. In addition, as it is generally not possible to obtain records to define the HCV genotype of a potential donor and the high prevalence of genotype 1 virus in the United States, HCV-positive organs should be transplanted with caution or not at all into HCV genotype 2 or 3 recipients. Finally all potential HCV-positive donors should have a predonation biopsy and the graft should only be utilized if there is mild necroinflammatory activity and little or no fibrosis.

HCV-Positive Kidney Donation

The prevalence of HCV is significantly higher in patients with end-stage renal disease (ESRD) compared with the general population [18]. Small single-center studies have suggested that HCV-positive recipients may have diminished graft and patient survival compared with HCV-negative recipients [19–21]. A larger study utilizing data from the UNOS database confirms these observations [22]. In addition, epidemiologic data and clinical evidence have demonstrated that HCV infection can cause glomerular disease [23, 24]. However, HCV-positive kidney transplant recipients still have improved outcomes compared with those patients who remain on long-term hemodialysis [25, 26] and the availability of HCV-positive organs for these patients significantly decreases their time on the wait list [22, 27, 28]. As in liver transplantation, the growing disparity of patients on the waiting list and the number of organs available have forced the kidney transplant community to expand their donor pool by using HCV-positive renal grafts in HCV-positive recipients. Kidney and liver function outcomes have been evaluated in a smaller number of studies, and the results are somewhat more variable than those in liver transplantation [29].

Early small single-center studies with short-term follow-up failed to show an effect of donor HCV status on outcomes [27, 28, 30]. However, a larger single-center study with longer follow-up by Gentil et al. [31] evaluated 300 deceased donor renal transplants in which patients with either donor or recipient HCV were compared with controls. They found that these HCV-positive patients had a higher incidence of proteinuria, worse kidney function, and lower patient (87 vs. 96% at 5 years) and graft survival rates (68.3 vs. 84.7% at 5 years) compared with HCV-negative patients. In addition, HCV seropositivity after transplant was an independent predictor of death and graft failure. Conversely, a single-center study from Kasprzyk [32] et al. reviewed the results of 765 transplants performed between 1994 and 2006, 259 of which were performed in recipients seropositive for HCV, 60 of whom received kidneys from HCV-positive donors. Compared with HCV-negative grafts and HCV-negative recipients, no significant difference was observed between the groups with respect to liver function tests, serum creatinine at 5 years, and patient or graft survival. These studies may have significant selection bias. HCV-positive organs are not placed at random and are often not allocated by the standard UNOS algorithm. Thus there may be significant baseline differences between recipients of HCV-positive and HCV-negative grafts that confound outcome analyses.

Larger nationwide databases have also been used to address this question. Bucci et al. [33] analyzed 20,111 adult recipients transplanted between 1994 and 1998 in an historical cohort study including 484 HCV antibody-positive kidneys, 165 (34%) of which were given to recipients with confirmed negative HCV serology. Unadjusted 3-year recipient survival was diminished in HCV-positive grafts (85 vs. 93%). This decrease in survival was persistent in multivariable analyses and when looking only at HCV-positive recipients.

In the largest study to date from the OPTN database, 76,787 patients transplanted between 2001 and 2006 were analyzed [22]. Serologic tests showed that 6.25% of cadaveric kidneys and 2.97% of living donor kidneys were anti-HCV positive. HCV donor seropositivity was associated with decreased waiting time for HCV-positive recipients (mean of 440.5 vs. 735 days). However, HCV-positive donors were also associated with diminished recipient (adjusted HR 1.43) and graft survival (adjusted HR 1.48). It is

not clear whether this has to do with donor quality or factors in the recipient not measured in their analysis. The alternatives for an HCV-positive candidate on the waiting list are either to accept an HCV-positive kidney or to remain on the waiting list for a potential HCV-negative kidney at some time in the future. Thus, the appropriate survival analyses should adjust for the shorter waiting time in the patient who receives an HCV-positive kidney and/or to begin the survival analysis from the time the patient enters the waiting list, so the impact of pre-transplant mortality associated with remaining on the waiting list could be assessed. It is believed that this "intent-to-treat" analysis would likely show an overall mortality benefit for HCV-positive candidates on the waiting list receiving an earlier transplant with an HCV-positive donor graft, especially in regions of the country where waiting times are long. Similar analyses have demonstrated the impact of earlier transplantation in recipients of a living donor graft on overall survival compared to remaining on the waiting list for a potential deceased donor [34].

In summary, HCV is common in patients with ESRD. These patients gain a survival benefit from renal transplantation compared to remaining on hemodialysis and the willingness to accept an HCV-positive graft for an HCV-positive recipient does reduce waiting time to undergo transplantation. However, long-term survival and graft function are likely to be diminished in HCV-positive recipients who receive HCV-positive grafts compared with patients who receive an HCV-negative graft. The causes of death in these patients are difficult to assess in nationwide cohorts, and there are several potential mechanisms of poor outcomes including possibly more rapid progression of liver disease and HCV-related renal disease. This potential for increased post-transplant morbidity and mortality must be weighed against the potential for pre-transplant morbidity and mortality. As in liver transplant recipients, it is probably best not to utilize HCV-positive organs in recipients who are HCV genotype 2 or 3 or in any patient who is HCV RNA negative.

HCV-Positive Heart Donation

Historically, 4–28% of heart transplant recipients may be HCV seropositive when tested in the post-transplant period, depending on the era in which the transplant occurred [35, 36]. However, less data are available in heart transplantation to separate the effects of donor and recipient HCV status. Despite early evidence of equivalent outcomes in recipients of HCV-positive and HCV-negative organs in very small series [37–40], practices have varied widely by center [41] and case reports of severe, rapidly progressive liver fibrosis and the cholestatic variant of HCV in recipients of HCV-positive hearts led to skepticism about the widespread use of these organs [37, 40, 42–45].

Additional concern regarding the use of HCV-positive donors was generated by the report from Haji et al. [3] that donor HCV seropositivity was associated with accelerated vasculopathy after heart transplantation. In this study, 438 patients who underwent heart transplantation between 1993 and 1998 at a single center were studied, 10.5% of which received HCV-positive hearts. The development of coronary vasculopathy was defined as the development of at least one new stenotic lesion in any of the three major blood vessels on angiogram, and moderate-to-severe vasculopathy as at least one stenosis of greater than 50%. After a mean follow-up of 3.4 years, the unadjusted risk of vasculopathy and moderate-to-severe vasculopathy was 3.08- and 9.4-fold higher in patients who received HCV-positive hearts compared with controls, respectively. This study was performed in part because of the reported association between HCV and atherosclerosis [46], in which, after adjustment for confounding risk factors, HCV seropositivity was found to be associated with an increased risk of carotid artery plaque (odds ratio 1.92) and carotid intima-media thickening (OR 2.85). CMV infection in heart transplantation has similarly been associated with vasculopathy and death [47, 48]. In addition, these authors point to the kidney transplant literature which suggests that patients with HCV have higher rates of cardiovascular

mortality than HCV-negative controls [49]. The exact mechanism by which viral infection, and specifically HCV, would lead to accelerated atherosclerosis is unclear and likely multifactorial. Proposed contributors may include heightened alloimmune responses, viral activation of inflammatory and adhesion signaling, and mixed cryoglobulinemia-associated immune complex vasculitis [50] which can occur in patients with HCV. It is also unclear whether there is an independent effect of the donor's HCV status when used in heart recipients who are already HCV-positive and viremic.

The impact of HCV-positive donor hearts on overall recipient mortality has also been investigated. Early series found similar survival in patients with HCV-positive and HCV-negative grafts in both single-center experiences and with a UNOS database query [39]. In the single-center series by Haji et al. [3] mentioned above, seropositive donor HCV status was associated with an unadjusted 2.8-fold greater risk of death compared to controls at a mean follow-up of 4.3 years. Most recently, Gasink et al. [51] reviewed the US Scientific Registry of Transplant Recipients for 10,915 adults who received a heart transplant between 1994 and 2003. Two hundred and sixty-one of these patients received HCV seropositive hearts, and 1-, 5-, and 10-year mortality were higher in this group than in controls (16.9 vs. 8.2%, 41.8 vs. 18.5%, and 50.6 vs. 24.3%, respectively). Donor HCV seropositivity was associated with death from liver disease and coronary vasculopathy, and after adjustment by propensity matching, receipt of an HCV-positive organ was associated with a hazard ratio of 2.1 for death compared to an HCV-negative organ, and this finding was independent of recipient HCV status. Why donor HCV status would have a greater impact than recipient HCV status remains uncertain. Because both groups will be viremic post-transplant, this association may reflect a selection bias in patients who were willing to accept an HCV-positive graft.

As a result of these data, the enthusiasm for the use of HCV-positive donors in heart transplantation remains low. However, given the life-saving nature of transplantation in patients without other temporizing options such as mechanical assist devices, transplantation of these organs will likely to continue, particularly as they will offer shortened waiting time and a decreased risk of pre-transplant mortality. The 2001 American Heart Association consensus conference report stated that HCV-positive donors "may be appropriate in selected higher-risk recipients," [52] and most authors advise that the use of HCV-positive hearts should be restricted to "the critically ill who would not survive without immediate transplant" [53]. Further work in the pathogenesis of HCV-associated vasculopathy, the potential reasons for a greater impact of donor than recipient serostatus [51, 54], and the use of direct acting antivirals (DAA) for HCV in these patients is greatly needed. Once again the only way to correctly assess the impact of accepting an HCV-positive heart on long-term survival is to initiate the survival analyses from the time of listing instead of the time from transplantation. This would account for any decrease in pre-transplant mortality associated with accepting an HCV-positive heart and the possible reduction in overall mortality from this decision.

Other HCV-Positive Organ Donation

Very little data are available about the role of HCV in lung transplant donors and recipients. In one of the largest single-center experiences, 291 lung transplants performed between 2000 and 2005 were evaluated (6 HCV-positive and 285 HCV-negative recipients). No difference in survival or the incidence of rejection in these patients was found. However, no large series have evaluated the role of lungs from HCV-positive donors and no longer follow-up or larger series of HCV-positive recipients are available.

Although corneal transplantation is thought to carry a low risk of viral transmission because of the low vascularity of this tissue, several reports are now available regarding impact of HCV seropositive corneal donors. In contrast to other organ donors, deceased cornea donors are tested for HCV with blood samples collected after death. These sera which may be collected at variable

times after death are often of poor quality and often yield false-positive results [55]. In one study of all 851 corneas procured between 1993 and 1997 from 438 donors, antibodies to HCV were found in 29 donors (6.6%) and 57 corneas (6.7%) were discarded because of this positive test. The time from donor death to cornea procurement was significantly longer among HCV-positive patients, raising the possibility that corneal ischemia might be associated with high rates of HCV antibody false-positivity [56]. Serologic testing in cornea donors should therefore be interpreted with caution in donor samples investigated more than 12 h after death [55], and new more accurate methods to evaluate postmortem serum for HCV infection (e.g., nucleic acid testing (NAT) testing, see below) are needed to ensure efficient use of the deceased corneas available.

HCV Testing in Organ Donors

Accurate determination of donors with active HCV infection remains a major challenge to this field. As with HCV testing in the general population [57], the current method of determining HCV exposure in organ donors is by testing for anti-HCV antibodies. However, it is now known that some donors with positive HCV serologies have cleared infection or have false-positive tests, and several authors have advocated for molecular methods to detect viral genomes routinely or in antibody-positive samples [58]. In addition, several donors have been identified with negative HCV antibodies but positive HCV RNA testing [59, 60], which puts recipients at risk of acquiring infection from serologically negative donors [61]. The imperfect sensitivity and specificity of HCV serology in the identification of chronic HCV infection and risk of transmission impacts much of the literature cited above as HCV antibody testing alone are used to define groups thought to be at risk.

Nucleic acid testing (NAT) is sensitive and specific for active HCV infection [62–64], and may reduce the risk of HCV transmission in the setting of blood transfusion [64, 65]. Whether NAT testing should be done with transcription-mediated amplification or polymerase chain reaction-based methods and whether screening all potential donors would be cost effective remains uncertain. The impact of lost donors as a result of false-positive NAT testing in low-risk individuals remains unclear. As technology improves the efficiency, sensitivity, and specificity of this testing, it is likely that the routine use of a combination of serologies and NAT will provide the best margin of safety for recipients.

Potential Impact of Direct Acting Antivirals

It is likely that the upcoming approval of novel HCV treatments including DAA may alter the use of HCV-positive organs in a number of ways. First, if more HCV-infected patients on the transplant waiting list can be effectively cured of HCV prior to organ transplantation, the pool of potentially acceptable recipients for HCV-positive grafts may shrink considerably, and timing viral clearance with living donation of liver or kidney grafts, for example, might significantly reduce the burden of HCV disease in many transplant patients.

Second, as these DAA medications gain widespread use, treatment in the general population may significantly affect donor selection. Confirmation of persistent chronic infection in a donor with positive HCV antibody with NAT may be of even greater importance as the proportion of effectively treated and cured individuals may rise. It is likely that donors in whom HCV has been eradicated can be used with a low or even zero rate of transmission, even in HCV-negative recipients. This is supported by the observation that transplantation of patients with prior HCV who have cleared virus and achieved a sustained virologic response is rarely, if ever, associated with recurrence of HCV after transplantation. On the other hand, concerns about transmission of resistant virus through transplantation from donors who failed prior DAA therapy may further complicate allocation of these organs.

Therefore, not only will genotype matching be of potential benefit to predict post-transplant treatment response, but sequencing to identify specific mutations may be required. Given the limited time available between the identification of a potential donor and transplantation of the organs into the recipient, it is unlikely that sequencing of HCV to allow the selection of appropriate recipients could be accomplished.

Lastly, the availability of DAA regimens will likely affect HCV recipients in the post-transplant setting. There is little doubt that treatment of HCV in transplant recipients with a protease inhibitor, pegylated interferon, and ribavirin will enhance sustained virologic response compared with treatment with pegylated interferon and ribavirin alone. However, the high potential for interaction with immunosuppression medications, in particular calcineurin inhibitors (tacrolimus and cyclosporine), will make such treatment difficult and require close monitoring. In addition, there will be hematologic toxicity and other side effects that will complicate therapy. Nevertheless, the promise of effective post-transplant treatment will likely change our approach to utilizing organs from HCV-positive donors. The ability to eradicate HCV post-transplant may allow some patients without HCV to accept an HCV-positive organ, particularly if the recipient has the IL28B-CC genotype and has a high likelihood of achieving a sustained virologic response with treatment. However, even this must be balanced against the adverse effects of pegylated interferon and ribavirin, which are clearly more severe in transplant recipients. Concern for rejection precipitated by interferon in non-liver transplant recipients will certainly dampen the enthusiasm for utilizing HCV-positive donors in HCV-negative patients undergoing heart, kidney, and lung transplantation.

Eventually, several DAA will be available to treat chronic HCV. Whether a combination of DAAs without interferon and/or ribavirin could yield a sustained virologic response in patients with chronic HCV remains to be demonstrated. However, if this becomes possible the doors would be opened to utilize HCV-positive organs in HCV-negative recipients followed by treatment of HCV with multiple DAA to eradicate the infection acquired from the donor organ. This would be analogous to utilizing an organ from a hepatitis B core antibody-positive donor for a patient that is hepatitis B surface antigen negative and then utilizing an oral nucleos(t)ide agent to suppress the hepatitis B virus and infection. The current limitation to DAA treatment of HCV after transplantation of a positive donor is the genotype specificity of the current DAA. The vast majority of these agents are specific for genotype 1 HCV and either less potent or ineffective against other genotypes. Thus, the HCV genotype of the potential donor must either be known or the use of HCV-positive donors in recipients with HCV genotypes 2 and 3 should be avoided. So, though it is conceivable that improved treatment might lead to more liberal use of HCV-positive organs in HCV-negative recipients in selected cases, this is unlikely to occur within the next several years.

Summary and Conclusions

The growing organ shortage in all areas of transplantation will continue to encourage the use of extended criteria and marginal donors to expand the donor pool and decrease mortality on the waiting list. HCV seropositive donors are likely to remain an important pool of extended criteria organs in the near future, especially in liver transplantation for HCV-positive recipients.

Many questions remain unanswered about the use of HCV-positive organs, and it is clear that HCV seropositive organs and their recipients are heterogeneous groups. The current literature on outcomes is significantly limited by inadequate power to see potential differences in outcomes at the single-center level and sensitivity for significant outcomes such as biopsy-proven fibrosis and cause of death at the national database level. In addition, in all organ transplantations, HCV-positive grafts are generally placed into HCV-positive recipients or older, sicker patients, and this non-random allocation of organs continues to cloud mortality comparisons between these groups. It is likely that maybe as much as 20% of these organs represent

cleared HCV infection or false-positive antibody tests, and as more rapid testing becomes available, the addition of routine HCV NAT to the algorithm may become a reality. In addition, even among those with chronic infection, the virologic consequences are not uniform and little is known about the impact of donor HCV genotype or how the interaction of different pools of virus affects outcomes in HCV-positive recipients. Approval of DAA for the treatment of HCV will significantly impact both donor and recipient selection in the use of HCV-positive organs, but will require more rapid and accurate knowledge of donor and recipient viral data including genotype. More work is needed to elucidate the mechanisms of poor outcomes in recipients of HCV seropositive heart and kidney grafts and to understand the true impact of the utilization of these grafts on overall (pre- and post-transplant) survival to effectively shape clinical decision making and organ allocation policy.

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Treating HCV Prior to Liver Transplantation

24

Alvaro Martinez-Camacho, Brett E. Fortune, and Gregory T. Everson

Keywords

Hepatitis C virus • Cirrhosis • Liver transplantation • Allograft hepatitis C • Sustained virologic response • Post-transplant virologic response • Management • LADR • Peginterferon • Ribavirin • Telaprevir • Boceprevir • Direct acting antivirals

The main goals of treating patients with chronic hepatitis C virus (HCV) infection who are listed for liver transplantation (LT) are: (1) to stabilize disease to reduce the need for LT, (2) to prevent HCV recurrence in the allograft, and (3) to improve graft survival. However, antiviral treatment of HCV-infected patients with advanced disease is limited by poor tolerability and the risk for treatment to increase the risk for hepatic decompensation and even death. Current guidelines suggest that patients with MELD ≤18 could be treated with antiviral therapy by clinicians experienced with treatment in the setting of advanced liver disease [1, 2]. On the basis of the recent OPTN data, more than half of the 5,627 patients infected with HCV currently listed for transplantation have a model for end-stage liver disease (MELD) score of ≤18 and would be potentially eligible for pre-transplant antiviral therapy. In this chapter, we describe the benefits and risks of pre-transplant antiviral therapy for HCV-infected patients who are waiting for liver transplantation.

A. Martinez-Camacho, MD • B.E. Fortune, MD
Department of Gastroenterology and Hepatology, University of Colorado Denver, 12631 East 17th Avenue, Aurora, CO 80045, USA

G.T. Everson, MD (✉)
Department of Internal Medicine, University of Colorado Denver, 1635 Aurora Court, B-154, Aurora, CO 80045, USA
e-mail: greg.everson@uchsc.edu

Goal 1: Stabilizing Disease

Rates of SVR: Treatment-Naïve Patients with Cirrhosis

Twelve to 30% of patients enrolled in the large, randomized-controlled trials of pegylated interferon (Peg-IFN)-based therapy had either advanced fibrosis or cirrhosis [3–5]. All patients with cirrhosis were well compensated with normal or nearly normal biochemical profile and no history of clinical complications, such as ascites, variceal hemorrhage, encephalopathy, or spontaneous bacterial peritonitis. Rates of sustained virologic response (SVR) in patients with advanced fibrosis or cirrhosis were 5–15% lower than rates of SVR in patients with minimal or no fibrosis. Helbling et al. treated 124 HCV-infected patients with advanced fibrosis or compensated

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cirrhosis with Peg-IFN and ribavirin [6]. Observed SVR was 58% for patients infected with HCV genotypes 2 or 3 (G2/3), and 32% for patients infected with HCV genotype 1 (G1). Baseline platelet count greater than 150,000/μL and Genotype 2/3 infection were independent predictors of SVR. A combined analysis of three large, randomized-controlled trials compared 99 G1 and 380 G2/3 patients with advanced fibrosis or cirrhosis to 242 G1 and 1,147 G2/3 patients with minimal fibrosis [7]. Stage of fibrosis predicted the rates of SVR. In patients with G1 HCV rates of SVR were 60% in patients with minimal fibrosis, 51% in those with advanced fibrosis, and 33% in patients with cirrhosis. Rates of SVR in patients with G2/3 HCV for these stages of fibrosis were 76, 61, and 57%, respectively. Rapid virologic response, defined as undetectable HCV RNA at week 4 of therapy, and higher cumulative dose of ribavirin were associated with increased chance of SVR. Rates of SVR were lower and relapse higher in patients with G2/3 HCV infection when they were treated for 16 weeks compared to 24 weeks (SVR: 48 vs. 57%; relapse: 49 vs. 32%, respectively). Tolerability to therapy appeared to be acceptable and comparable to patients without cirrhosis.

Rates of SVR: Treatment-Experienced Patients with Cirrhosis

During the "lead-in phase" of the Hepatitis C Antiviral Long-Term Treatment against Cirrhosis (HALT-C) Trial, 1,145 prior non-responders to standard interferon with or without ribavirin and advanced fibrosis or cirrhosis were retreated with pegylated interferon alpha-2a and ribavirin [8]. The overall rate of SVR was 18%. History of treatment with interferon monotherapy, infection with HCV G2/3, serum HCV RNA level less than 1.5 million IU/mL, AST:ALT less than 1.0, and lower stage of fibrosis (Ishak 3 or 4 vs. 5 or 6) were independent predictors of SVR [8].

Patients in the HALT-C cohort were stratified by histology and platelet count into four groups: advanced fibrosis and platelet count greater than 125,000 mm^3 (least severe disease), advanced fibrosis and platelet count less than 125,000 mm^3, cirrhosis and platelet count greater than 125,000 mm^3, and cirrhosis and platelet count less than 125,000 mm^3 (most severe disease). In this comparison, SVR declined from 23 to 9% from patients with the least to those with the most severe disease. Even though over 50% of patients required dose reductions in either Peg-IFN or ribavirin, the decline in SVR was independent of dose reductions [9]. Thus, patients with cirrhosis are not only "difficult to treat" secondary to poor tolerability to therapy, but also "difficult to cure" due to resistance to antiviral therapy.

In the Evaluation of PegIntron in Control of Hepatitis C Cirrhosis (EPIC [3]) Trial, 2,333 prior nonresponders to standard interferon with or without ribavirin were retreated with pegylated interferon alpha-2b and ribavirin [10]. Overall, SVR was 22%, but SVR was higher in relapsers (38%) compared with nonresponders (14%) to prior therapy. Independent predictors of SVR were prior therapy with standard interferon, HCV G2/3, baseline viral load less than 600,000 IU/mL, and lower fibrosis score.

Impact of SVR on Clinical Outcomes

Although rates of SVR are lower in advanced fibrosis and cirrhosis, SVR halts fibrosis progression. Camma et al. studied three randomized controlled trials ($n = 1{,}013$) that examined the change in histology between paired (pre-treatment and 6 months post-treatment) liver biopsies from patients achieving SVR after antiviral therapy [11]. Improvement by at least one fibrosis stage (scale 0–4) was seen in 67 of 198 (33.8%) cirrhotic patients, of which 9.6% regressed to stage 1 fibrosis. In cirrhotic patients, SVR was the only significant predictor for improvement of fibrosis stage (OR 2.16; 95% CI 1.04–4.47).

Achieving SVR in HCV-infected patients with compensated cirrhosis reduces the risk of progression to hepatic decompensation and hepatocellular carcinoma (HCC). A multinational, retrospective study on 479 patients with advanced fibrosis or cirrhosis examined post-treatment

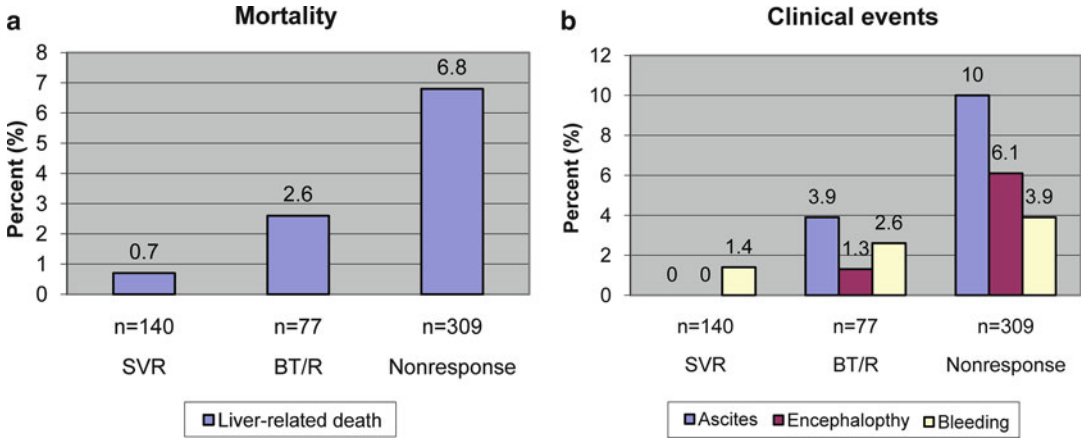

Fig. 24.1 SVR reduces clinical outcomes in patients with compensated cirrhosis: results from the HALT-C Trial (**a**, **b**). *SVR* sustained virologic response, is defined as HCV RNA negative 6 months or more after discontinuation of treatment; *BT/R* breakthrough/relapse, is defined as achieving negative HCV RNA during treatment, but then developing positive HCV RNA either during treatment (BT) or after treatment has stopped (R). Nonresponse is defined as detectable HCV RNA at all times (based on data from Morgan et al. [14])

clinical outcomes, including hepatic decompensation, HCC, and death [12]. In this cohort, 142 patients achieved SVR and only 4 (2.8%) experienced a clinical outcome – three developed HCC and none had hepatic decompensation. In contrast, 83 of the 337 (24.6%) patients who did not achieve SVR experienced a clinical outcome. Sustained virologic response was associated with a significant reduction in the risk for clinical events (HR, 0.21; 95% CI 0.07–0.58). Another retrospective, multicenter study followed 902 patients with cirrhosis for an average of 96.1 months after treatment [13]. Patients who achieved SVR had lower rates of liver-related death and liver failure.

In the only prospective trial, the HALT-C investigators compared 140 patients achieving SVR to 307 patients with nonresponse and 77 patients with breakthrough/relapse (BT/R). In this study, the period of follow-up extended beyond 7 years [14]. Patients who achieved SVR had a significantly reduced rate of all-cause mortality, and reduced rates of any liver-related outcome, hepatic decompensation, development of HCC, and liver-related death or LT compared with the nonresponse group (Fig. 24.1). A similar improved outcome was noted comparing patients achieving SVR to patients with BT/R; however, it did not reach statistical significance. One additional finding was the continued risk for HCC despite SVR – emphasizing the need for continued surveillance for HCC in cirrhotic patients.

A Markov decision model examined the most cost-effective approach for combination antiviral therapy in HCV genotype 1-infected patients with advanced liver disease awaiting transplantation [15]. The researchers compared three antiviral strategies, pre-transplant therapy of compensated disease, pre-transplant therapy of decompensated disease, and post-transplant therapy for recurrent HCV. Pre-transplant treatment of compensated cirrhotics resulted in 119 fewer deaths, 54 fewer HCCs, 66 fewer transplants, increased QALYs by 0.950, and saved $55,314 per patient compared with no-treatment. Outcomes after pre-transplant treatment of patients with compensated cirrhosis were superior to those with either pre-transplant treatment of patients with decompensated cirrhosis or post-transplant therapy.

We conclude that the patient with cirrhosis may be "difficult-to-treat" and "difficult-to-cure," but treatment is clearly warranted because of the potential for a significant clinical benefit and favorable cost-effectiveness.

Table 24.1 SVR in decompensated cirrhosis

References	Patients (*N*)	RNA negative Rx	EOT (%)	SVR (%)
Iacobellis et al. [17]	66	PEG/RBV	49	20%
Carrion [20]	51	PEG/RBV	29	20
Tekin et al. [24]	20	PEG/RBV	45	30
Annicchiarico et al. [19]	15	PEG/RBV	47	20
Everson [22]	124	IFN/RBV	46	24
Forns et al. [23]	30	IFN/RBV	30	20
Thomas et al. [25]	20	IFN	60	20
Amarapurkar et al. [18]	18	IFN ± RBV	61	38
Crippin et al. [21]	15	IFN ± RBV	33	0
Totals	359		44	24

SVR sustained virologic response is defined as HCV RNA negative 6 months or more after discontinuation of treatment; *EOT* end-of-treatment; *PEG* peginterferon; *RBV* ribavirin; *IFN* nonpegylated interferon

Treating Decompensated Cirrhosis: Selection Criteria

The onset of clinical complications (decompensation) suggests a change in the course of liver disease associated with poorer response to antiviral therapy and higher risk of complications during treatment. These patients are at increased risk for further decompensation or death. The AASLD practice guidelines recommend that HCV-infected patients experiencing hepatic decompensation be evaluated for liver transplantation, and, if candidacy is confirmed, they should be listed [2]. The Consensus Development Conference on Liver Transplantation and Hepatitis C suggested that patients on the waiting list with MELD scores 18 or less should be considered for combination treatment [1]. In addition, the AASLD practice guidelines state that patients referred for liver transplantation with mild degree of hepatic compromise could be considered for antiviral therapy, initiated at low dose, "as long as treatment is administered by experienced clinicians, with vigilant monitoring for adverse events." [2]

Currently, 35% of patients listed for liver transplantation in the United States have either a primary or secondary diagnosis of hepatitis C (OPTN data, http://www.OPTN.org/LatestData/rptData.asp). Ideal candidates for pre-transplant antiviral therapy with the current combination of peginterferon plus ribavirin have MELD ≤18, are living donor recipients, or have received MELD upgrade for HCC. In the United States, the average MELD score at time of transplantation of the liver from a deceased donor is approximately 25 which limits the access to transplantation for patients with MELD under 18. In the Adult-to-Adult Living Donor Liver Transplantation (A2ALL) Cohort Study of pre-transplant antiviral therapy, patients undergoing living donor liver transplantation (LDLT) typically had an average MELD score of 11 at time of transplantation [16]. Hence, HCV-infected candidates for LDLT represent a group of patients that potentially can receive pre-transplant antiviral therapy. In the same A2ALL study, patients who received MELD upgrade points for early HCC had an average disease-related MELD score of 12.

Decompensated Cirrhosis: Results of Antiviral Therapy

The published experience with antiviral therapy of decompensated cirrhosis is given in Table 24.1. Except for the study by Iacobellis [17], most series represented single-center experiences, were nonrandomized or uncontrolled trials, and patients selected for treatment were candidates or listed for transplantation [18–25]. Sustained virologic response ranged from 20 to 30%, regardless of the interferon used and despite the heterogeneous nature of these studies.

Lacobellis and colleagues randomized 129 patients with decompensated cirrhosis due to HCV,

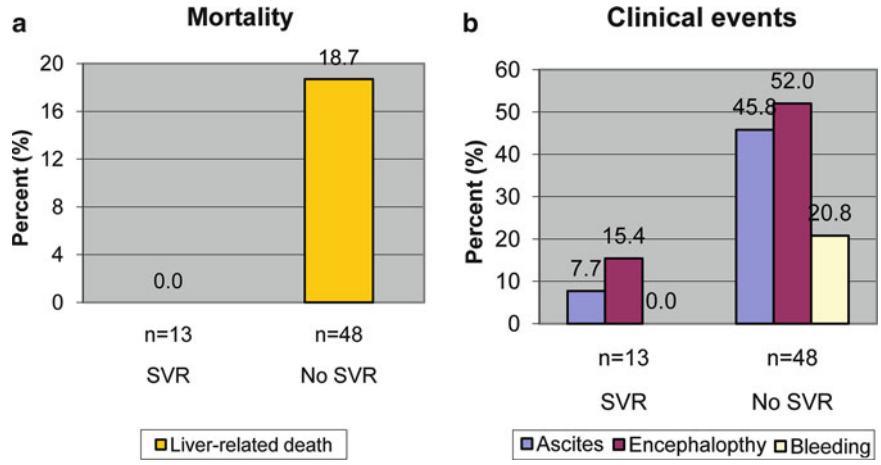

Fig. 24.2 SVR reduces clinical outcomes in patients with decompensated cirrhosis. (**a**, **b**) *SVR* sustained virologic response, defined as HCV RNA negative 6 months or more after discontinuation of treatment (based on data from Iacobellis et al. [17])

who were not candidates for liver transplantation, to either pegylated interferon with ribavirin ($N=66$) or no treatment ($N=63$). Patients were followed up for clinical outcomes over 30 months [17]. The patients selected for this trial had had hospital admissions for ascites, variceal bleeding, or encephalopathy, and were naïve to standard or pegylated interferon and ribavirin. Approximately 75% were classified as Child-Pugh class A or B and average MELD score was 14. Two-thirds were infected with HCV genotype 1 and average platelet count was 86,000/μL. Rates of SVR were 43.5 and 7.0% for patients infected with HCV G2/3 and HCV G1, respectively. Figure 24.2 shows that SVR was associated with a significant reduction in progression to hepatic decompensation (23.1%) and liver-related mortality (0%) compared with patients who did not achieve SVR (68.7 and 18.7%, respectively). The reduction in clinical outcomes in patients who achieved SVR was associated with a significant reduction in Child-Pugh (7.8 ± 1.1 vs. 6.4 ± 0.7; $p=0.001$) and MELD (14.1 ± 2.9 vs. 10.5 ± 2.3; $p=0.0005$) score. For comparison, those patients that did not achieve SVR had an increase in Child-Pugh (8.0 ± 1.3 vs. 8.7 ± 1.5; $p=0.02$) and MELD (14.3 ± 3.8 vs. 15.8 ± 4.7; $p=0.4$) score. These results suggested that clearance of HCV with antiviral therapy halts disease progression and is potentially life-saving in patients with decompensated cirrhosis. However, the low response in HCV genotype 1 infection coupled with adverse and serious adverse events (SAEs) has limited application of this strategy.

The aforementioned Markov decision model also identified a benefit of treating decompensated cirrhotics on the transplant waiting list. Compared to no-treatment, therapy during decompensated cirrhosis would potentially prevent ten deaths, decrease costs by $5,511 per patient, and increase QALYs by 0.044 [15]. Treatment of decompensated cirrhotics was more cost-effective compared with treatment of patients with post-transplant recurrence of HCV when the two strategies were directly compared. More specifically, treatment during the pre-transplant decompensated period decreased cost by $2,288 and increased QALYs by 0.017 compared with deferring treatment until post-transplant recurrence.

Patients with decompensated cirrhosis may be difficult-to-treat and difficult-to-cure, but an attempt at antiviral treatment is warranted because of the benefits of SVR and potential cost-effectiveness. Treatment of decompensated patients should be limited to experienced hepatologists who practice at or are in contact with a liver transplant center.

Goal 2: Prevention of Post-transplant Recurrence of Hepatitis C

The second major goal of antiviral therapy for HCV patients with decompensated cirrhosis who are on the transplant waiting list is to prevent recurrence of HCV after transplantation. The data in Table 24.2 show that 20–30% of patients with decompensated cirrhosis can achieve SVR. Rates of SVR were low, but during treatment the rate of viral clearance ranged from 29 to 61%. The latter finding raises the possibility that pre-transplant antiviral treatment could render a sizeable proportion of patients negative for HCV RNA by the time of transplantation. Both SVR and on-treatment clearance of HCV RNA are associated with reduced rates of post-transplant HCV recurrence [26–28].

Definition of Post-transplant Virologic Response

Efficacy of pre-transplant treatment in achieving post-transplant virological clearance requires a new definition for virological outcome. Some patients prior to liver transplant have achieved SVR, but could theoretically relapse under immunosuppression. Other patients taking antiviral treatment up to the time of transplant could be HCV RNA negative but harbor low-level HCV and relapse post-transplant. In the LADR-A2ALL study, post-transplant virologic response (pTVR) was defined as negative HCV RNA in serum 12 weeks after transplant. No patient achieving pTVR relapsed in subsequent follow-up [29].

Uncontrolled Descriptive Studies

Reports, primarily from single centers, demonstrated rates of pTVR ranging from 20 to 28% (Table 24.1). In these studies, the on-treatment rate of viral clearance ranged from 29 to 60%. Forns and colleagues from Barcelona, Spain, demonstrated that on-treatment viral clearance can reduce rates of HCV recurrence [23]. Thirty patients with decompensated cirrhosis were treated with interferon alpha-2b and ribavirin. While on treatment, nine patients (30%) achieved clearance of HCV RNA prior to LT and 6 of those patients (20%) achieved pTVR. Rapid virologic response (≥2 log drop at week 4) was the strongest predictor of SVR. All patients who were HCV RNA positive prior to liver transplant experienced HCV recurrence.

Everson et al. used a low accelerating dose regimen (LADR) of interferon alpha-2b and ribavirin to treat 124 patients with HCV and decompensated cirrhosis (70% HCV genotype 1 and 63% having Child-Pugh class B or C) [30]. HCV RNA cleared in 46% at the end of treatment and 26% achieved pTVR (13% among genotype 1 patients and 50% among non-genotype 1). Forty-seven patients were transplanted. Twelve of 15 patients who were HCV RNA negative prior to LT achieved pTVR. All 32 patients who were positive at time of transplant developed HCV

Table 24.2 Prevention of post-transplant recurrence

		RNA negative	
References	Patients (*N*)	Day of LTx (%)	pTVR (%)
Carrion et al. [20]	51	29	20
Everson et al. [30]	47	32	26
Forns et al. [23]	30	30	20
Thomas et al. [25]	20	60	20
Everson et al. (LADR-A2ALL) [29]	40	60	28
Totals	148	39	24

LADR-A2ALL low accelerated dose regimen of peginterferon/ribavirin, was conducted as a substudy of the NIH-sponsored Adult-to-Adult Living Donor Liver Transplantation (A2ALL) Study. Patients infected with genotypes 1, 4, 5, and 6 were randomized 2:1, treatment: control, and patients infected with genotypes 2 or 3 were treated. pTVR is post-transplant virological response

recurrence. These findings have been replicated in other studies [21, 25].

Another study from Barcelona highlighted some of the complications in treating patients prior to liver transplantation [20]. Fifty-one patients with HCV and decompensated cirrhosis were treated with pegylated interferon alpha-2a and ribavirin and 29% achieved on-treatment viral clearance. Ten of 15 patients (20%) who were HCV RNA negative at transplantation achieved pTVR. Serious side effects, primarily bacterial infections such as spontaneous bacterial peritonitis, occurred more frequently ($p=0.0016$). As a result, the authors suggested the use of antibiotic prophylaxis, such as norfloxacin, during antiviral therapy.

LADR-A2ALL: Randomized Controlled Trial

Centers within the Adult-to-Adult Living Donor Liver Transplantation Cohort Study (A2ALL) conducted the only randomized controlled trial of pre-LT antiviral therapy [29]. Patients enrolled in this study were candidates for living donor liver transplantation or were candidates for deceased donor liver transplantation who had received MELD exception points for HCC. Genotypes 2 and 3 were assigned to treatment. Genotypes 1, 4, and 6 were randomized, 2:1 to treatment vs. control to assess safety. Treatment consisted of peginterferon-2b, 0.75 μg/kg/week increasing to 1.5 μg/kg/week as tolerated and ribavirin, 600 mg/day increasing to 1,200 mg/day as tolerated. Growth factors, such as erythropoietin analogs (EPO) or granulocyte colony-stimulating factors (G-CSF), were allowed. Overall pTVR was 28% (18% for genotypes 1/4/6 and 39% for genotypes 2/3) (Fig. 24.3). Duration of treatment was the key variable defining likelihood for pTVR; pTVR was 44% in patients receiving 16 or more weeks of treatment compared to 18% in patients who received 10–15 weeks and 15% in patients who received less than 10 weeks ($p=0.04$).

SAEs were more common in treated patients ($p=0.04$) but mortality rates were similar ($p=0.68$). In this randomized controlled trial, as in the study by Carrion, infections were more common in treated patients ($p=0.09$).

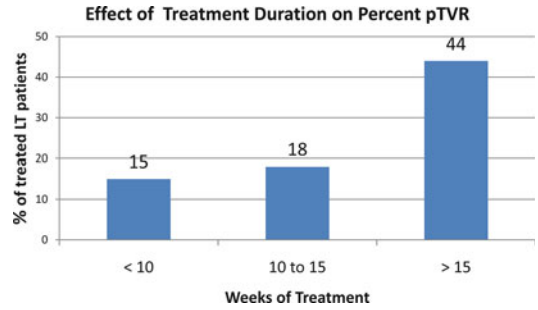

Fig. 24.3 Rates of post-transplant virologic response in the LADR-A2ALL study. *pTVR* post-transplant virologic response, is defined as HCV RNA negative at 12 weeks post-transplantation; *LT* liver transplantation; *LADR-A2ALL* low accelerated dose regimen of peginterferon/ribavirin conducted as a substudy of the NIH-sponsored Adult-to-Adult Living Donor Liver Transplantation (A2ALL) Study. Patients infected with genotypes 1, 4, 5, and 6 were randomized 2:1, treatment: control, and patients infected with genotypes 2 or 3 were treated (based on data from Everson et al. [29])

Goal 3: Improving Patient and Graft Outcomes by Clearance of HCV RNA Prior to Liver Transplant

In the previous sections, we have highlighted virologic responses and some of the pros and cons of treating patients on the transplant list. One key question is whether pre-transplant clearance of HCV RNA benefits patients in terms of post-transplant graft and patient survivals. There are no published reports addressing this question. We accessed the Scientific Registry of Transplant Recipients (SRTR) database to investigate whether HCV RNA status prior to transplant in patients listed with a primary diagnosis of chronic hepatitis C had a significant impact on graft and patient survivals (OPTN data based as of September 17, 2010). This unadjusted data revealed that patients who were HCV RNA negative prior to transplant had significantly higher 5-year graft (67.3 vs. 61.8%, $p=0.0424$) and patient survivals (73.7 vs. 67.3%, $p=0.0166$) compared to patients who were HCV RNA positive. Although additional studies are needed, the improved post-transplant survivals suggest that the strategy of using antiviral therapy to clear HCV RNA prior to transplant could positively impact post-transplant outcomes.

Current Management of Antiviral Treatment for Patients on the Transplant List

Selection Criteria

Treatment is associated with significant side effects, some of which, such as infection, may even be life-threatening [20]. For this reason, patients considered for treatment should be sufficiently stable so that they can tolerate side effects and cytopenias associated with treatment. Patients with G2/3 HCV or G1 HCV with low viral load (under 400,000 IU/mL) are more likely to achieve SVR. There are three types of candidates that could be considered: (1) patients who are listed with MELD scores less than 18 points, (2) living donor recipients, and (3) patients with MELD upgrade for HCC and stable liver disease.

Low Accelerating Dose Regimen

In our center, we use the LADR (Fig. 24.4). Initial treatment consists of either peginterferon alfa-2a

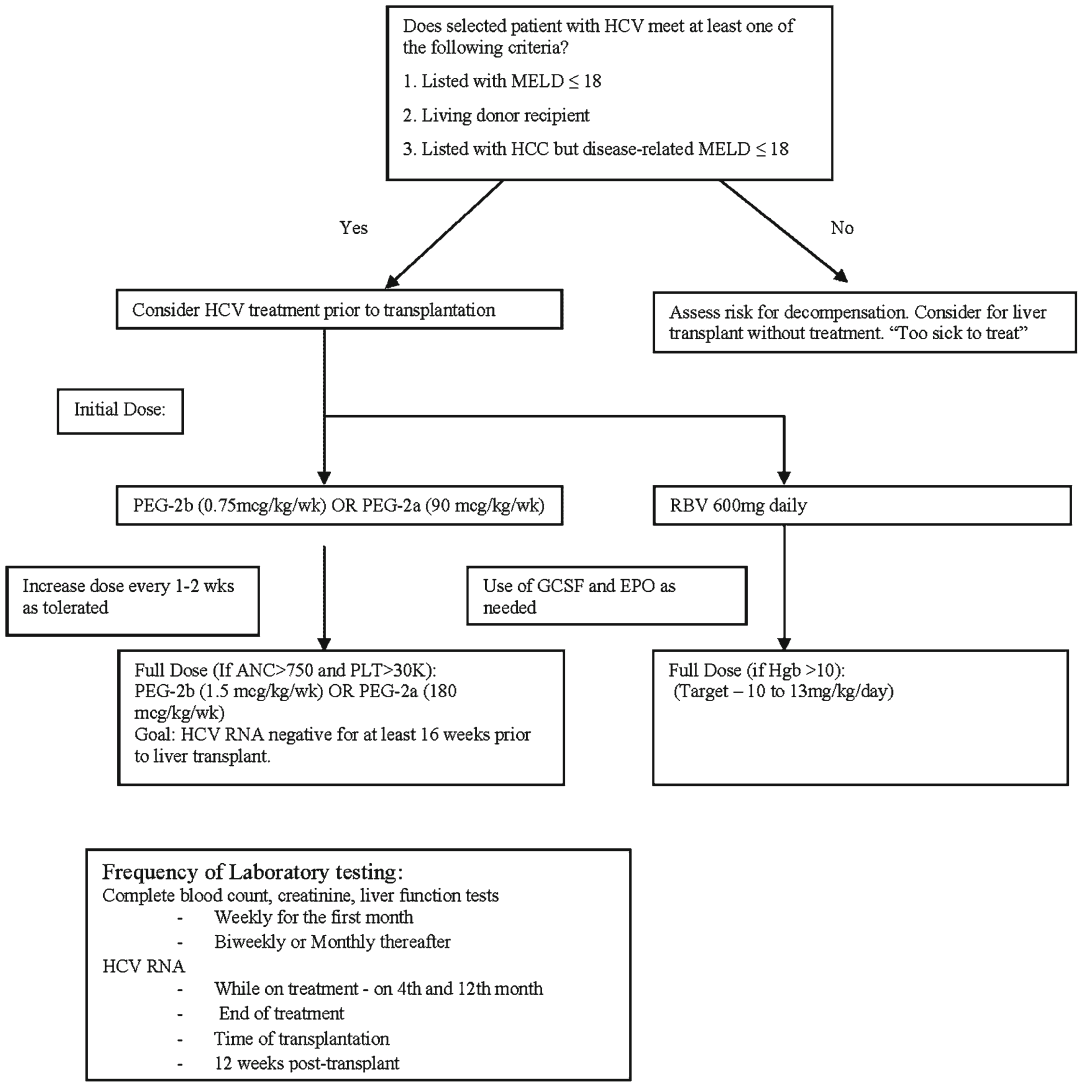

Fig. 24.4 Algorithm for the LADR protocol for treatment of HCV patients prior to transplantation

(Pegasys, 90 μg/kg/weekk) or peginterferon alfa-2b (Pegintron, 0.75 μg/kg/week) combined with ribavirin (Rebetol or Copegus, 600 mg/day). Titrations in dosing should be made every 2 weeks based on patient tolerance to reach maximally tolerated or target dosing (Pegasys, 180 μg/kg/week; Pegintron 1.5 μg/kg/week; Rebetol or Copegus 10–13 mg/kg/day) [29].

Virological response defined by change in HCV RNA should be performed on treatment weeks 4, 12, 24, and at end of treatment. HCV RNA should also be obtained at the time of liver transplantation to assess if HCV RNA clearance was achieved. HCV RNA is measured at 3 months post-transplant to define pTVR. HCV RNA should also be quantified at 6 and 12 months post-transplant to define late relapse.

Dose reductions or discontinuation of treatment depend on patient tolerance and side effects to the peginterferon or ribavirin. In the event of serious side effects or decompensation of the patient's liver disease treatment should be discontinued. Erythropoietin analog may be required for patients with hemoglobin <10 g/dL, and granulocyte colony-stimulating factors may be needed in patients with absolute neutrophil count (ANC) <750/μL. Patients are treated up to the time of transplantation or for 48–72 weeks based on virologic response.

Future Directions for Antiviral HCV Therapy

Goals of emerging therapies for chronic hepatitis C are to improve virologic responses, particularly in patients with G1 HCV, while decreasing side effects and improving the tolerability of the treatment regimen. Direct-acting antivirals (DAAs) represent new classes of drugs designed to target HCV-specific replication pathways. Pre-transplant treatment with DAAs could potentially increase rates of SVR and on-treatment virologic clearance, and reduce the rate of HCV recurrence.

Protease Inhibitors

Two protease inhibitors (PIs), telaprevir and boceprevir, have completed Phase 3 trials (using G1 HCV patients) and may be available for clinical use in 2011. Triple therapy with peg-interferon, ribavirin, and a PI has three potential advantages over dual therapy with peg-interferon and ribavirin: rapid decline in HCV RNA [31–34], higher rate of viral clearance [35–37], and reduced rate of relapse. These effects could enhance the efficacy of pre-transplant therapy in preventing post-transplant recurrence.

In the Phase 2 trials of telaprevir (PROVE1 and PROVE2) maximum RVR was 79%, SVR was 69%, and relapse was less than 7%. SVR was not affected by the severity of fibrosis or lower platelet counts, although no patient had histologic cirrhosis [38]. In the Phase 2 studies of boceprevir (SPRINT1) maximum SVR was 75% [35–37]. Seven percent of treated patients had histologic cirrhosis without clinical decompensation and 67% of those patients achieved SVR.

The LADR-A2ALL study demonstrated that duration of therapy of 16 weeks or longer was associated with a significantly higher rate of pTVR [29]. Use of triple therapy could eliminate HCV viremia more rapidly and yield a higher percentage of patients with an undetectable viral load for a longer period of time. These antiviral effects would likely reduce rates of recurrence of HCV post-transplantation. No studies using telaprevir or boceprevir in patients awaiting liver transplantation have yet been reported.

Disadvantages of triple therapy are added complexity of the treatment regimen, side effects, and cost. Peg-interferon is one injection per week and ribavirin is dosed twice daily, and both PIs are dosed three times a day. Telaprevir treatment is associated with a greater frequency of skin rash (PROVE1 59.4 vs. 41%; PROVE2 52.3 vs. 35.3%). Boceprevir treatment is associated with dysgusia (27 vs. 9%; SPRINT-1) and anemia requiring the use of EPO or reduction in the dose of ribavirin or boceprevir. In the Phase 2 studies the rates of study drug discontinuation as a

result of adverse events during triple therapy and standard-of-care were 21 vs. 11% in PROVE1, 12 vs. 7% in PROVE2, 15 vs. 4% in PROVE3, and 30 vs. 15% in SPRINT1 [35–37, 39]

Viral Resistance

Two factors increase the risk of viral resistance – inadequate blood concentrations of the antiviral medications, and high rates of viral replication. Resistance to telaprevir and boceprevir occurs within 14 days when these drugs are administered as monotherapy [40, 41]. Although peginterferon and ribavirin protect against emergence of these viral variants, maintaining adequate blood concentrations of antiviral drugs is critical. An analysis of PROVE1 and PROVE2 indicated that emergence of viral variants was linked to low blood concentrations of both peginterferon and telaprevir [38]. With reductions or interruptions in drug therapy blood concentrations of antiviral drugs diminish and viral replication increases. This issue may be particularly relevant to patients with cirrhosis who are relatively more intolerant of peginterferon and ribavirin and are more likely to experience dose reductions or interruptions in medications. However, these same patients have relatively lower blood concentrations of HCV RNA, possibly related to impairment of viral replication by the diseased liver. Thus, resistant viral variants may be less likely to emerge during dose reductions or interruptions in antiviral medications in patients with cirrhosis. Carefully controlled trials will be required to define the risks.

Emerging Treatments Beyond Telaprevir and Boceprevir

The liver is preferentially enriched in receptors for interferon-lambda. A pegylated form of interferon-lambda has been produced and has been evaluated in early phase studies of patients with chronic hepatitis C. In these trials, peginterferon-lambda has demonstrated similar or enhanced early virologic responses compared to peginterferon-alfa [42, 43]. The main advantages of peginterferon-lambda over peginterferon-alfa include fewer side effects, such as flu-like symptoms, less bone marrow suppression, and lower rates of neutropenia, anemia, and thrombocytopenia. These properties of peginterferon-lambda would be particularly advantageous in the treatment of patients with cirrhosis.

Patients with advanced liver disease may be more susceptible to ribavirin-induced hemolytic anemia. In published reports, only a minority of patients with decompensated cirrhosis tolerated full doses of ribavirin. Dose reduction or discontinuation of ribavirin was frequent, and erythropoietin analogs were required in approximately 50% of patients. Taribavirin (viramidine) is a precursor of ribavirin that is converted to ribavirin in the liver – systemic exposure to ribavirin is reduced, reducing risk for hemolysis, whereas hepatic exposure is enhanced, preserving ribavirin's antiviral efficacy [44]. Although studies are lacking, the reduced risk for hemolytic anemia with taribavirin would be advantageous in patients with cirrhosis.

Peginterferon-alfa is the Achilles' heel of the current treatment regimen in patients with cirrhosis. DAAs may provide sufficient suppression of HCV so that future treatment regimens might be free of interferon or allow reductions in doses of peginterferon without compromising efficacy. Although most of our comments regarding DAAs have centered on telaprevir or boceprevir, several new agents are under investigation. These include second-generation protease inhibitors, polymerase inhibitors, inhibitors of NS5a, matrix metalloproteinase inhibitors, therapeutic vaccines, and cyclophillin inhibitors. As the palette of therapeutic options expands, it is highly likely that the treatment of patients will be individualized and dependent upon unique viral and host genetic characteristics. It is entirely possible that most, if not all, of the patients undergoing liver transplantation for chronic hepatitis C could be successfully treated prior to transplantation and avoid the excess morbidity, mortality, and cost of recurrent hepatitis C.

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Treating HCV After Liver Transplantation

25

Michael Chang, Hubert Sung, and Sammy Saab

Keywords
Hepatitis C recurrence • Liver transplantation • Antiviral therapy

Background

Hepatitis C is currently the most common indication for liver transplantation in the United States, accounting for approximately 40–45% of all transplants [1, 2]. Recurrent Hepatitis C after liver transplantation can be an enormous burden on patients, physicians, and the healthcare system. Early experience among recipients of liver transplant for hepatitis C appeared promising. There were no significant differences in survival in patients transplanted for hepatitis when compared to other indications for liver transplantation 2–3 after surgery [3, 4]. However, decreased survival has been demonstrated with longer-term follow-up [5–8].

Recurrent hepatitis C infection is universal and occurs early after transplantation [9]. Early hepatitis C recurrence has been documented to occur as early as during reperfusion of the newly transplanted liver [10]. In one of the largest cohort studies in patients with hepatitis C, Ghobrial et al. found that the median time to Hepatitis C virus (HCV) recurrence was approximately 36 months [3]. Other studies have demonstrated that 20–30% of the patients progressed to cirrhosis, leading to graft failure and need for retransplantation or death in within 5 years of transplant [6, 11].

Several factors appear to affect the severity and time to recurrent infection. In particular, donor characteristics, pretransplant viral load, recipient viral co-infection, and immunosuppression after transplantation appear to be significantly associated with time to recurrence and severity of recurrence [1, 3, 5, 9, 12–18] (Table 25.1). Graft donor age has been shown to affect the severity of HCV recurrence and progression to fibrosis, particularly in graft donors >50 [19, 20].

Pre- and post-transplant viral loads have also been shown to affect HCV recurrence. The cut-off values for pre- and post-transplant viral loads associated with worse outcomes as demonstrated by Charlton et al. and Roche et al. are $>1 \times 10^6$

M. Chang, MD
Department of Medicine, University of California at Los Angeles, Los Angeles, CA 90095, USA

H. Sung, MD
Department of Surgery, Pfleger Liver Institute, University of California at Los Angeles, 200 Medical Plaza, Suite 214, Los Angeles, CA 90095, USA

S. Saab, MD, MPH, AGAF (✉)
Department of Medicine, University of California at Los Angeles, Los Angeles, CA 90095, USA

Department of Surgery, Pfleger Liver Institute, University of California at Los Angeles, 200 Medical Plaza, Suite 214, Los Angeles, CA 90095, USA
e-mail: SSaab@mednet.ucla.edu

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Table 25.1 Factors affecting HCV Recurrence

Characteristics	Significance
Graft donor age >50 [19, 20]	Increased severity or recurrence, increased progression to fibrosis
Pretransplant viral load $>1\times10^6$ IU/mL or Post-transplant viral load $>1\times10^7$ IU/mL [14, 15]	Decreased overall survival
HIV co-infection [17, 18]	Decreased overall survival
CMV co-infection [16]	Decreased graft survival
Pulse dosed corticosteroid use [12]	Increased severity of recurrence, decreased graft survival, and increased overall mortality
HCV-positive donor organ [29]	Possibly protective or no difference
Prolonged cold and/or warm ischemia time [28]	Negative effect on outcomes is not readily reproducible
Recipient age >50 [27]	
Co-incident diabetes type II [26]	
HCV genotype 1 [14]	
Female gender [14]	
Non-White race [14]	

IU/mL and $>1\times10^7$ IU/mL (4 months after transplantation) respectively [14, 15]. These values were associated with decreased 5-year survival, 57% in the $>1\times10^6$ IU/mL cohort vs. 84% in the $<1\times10^6$ IU/mL cohort.

Viral co-infection with Human immunodeficiency virus (HIV), as well as with cytomegalovirus (CMV), has also been associated with more severe HCV recurrence and more rapid progression to cirrhosis [16–18]. With regard to HIV coinfection, Duclos-Vallee et al. observed that 2- and 5-year survival rates were 73 and 51% in HIV/HCV coinfected patients vs. 91 and 81% in HCV monoinfected patients, respectively. Burak et al. observed that graft failure was significantly more common in CMV coinfected patients when compared with CMV-negative patients, 52 vs. 19.1% respectively. They also reported that fibrosis as defined by stage 2 or greater on the 4-month liver biopsy specimen was more common in CMV coinfected patients, 45 vs. 16.4%, respectively.

Immunosupression is also believed to affect HCV recurrence and severity. Particularly, pulse dosed corticosteroids are associated with 1–2 log increases in HCV RNA levels, decreased graft survival, and increased overall mortality [12, 21, 22]. Also there is early data to suggest that cyclosporine-based immunosuppressive regimens, when used in conjunction with interferon-alpha and ribavirin to treat HCV, may lead to a greater likelihood of virological response to therapy [23]. However, it has also been shown that treatment with tacrolimus-based immunosuppressive maintenance therapies may be associated with prolonged graft survival and prolonged patient survival [24, 25]. Therefore, there may be merit in treating patients with tacrolimus-based therapies until HCV recurrence at which point the patient may be switched to cyclosporine-based therapy during HCV antiviral treatment. This data will need to be reconfirmed before being accepted as standard practice.

Other factors that may be associated with worse outcome in patients transplanted for hepatitis C include prolonged cold and warm ischemia time (>90 min), recipient age >52, diabetes mellitus, HCV genotype, female gender, and non-White race [3, 14, 26–29]. Emerging data suggest that the recipient IL28b genotype may also be associated with rapid histological recurrence [30]. However, the IL28b genotype results do not appear to impact overall or liver-related survival. All these factors require further study to confirm their clinical impact.

Monitoring for Recurrence

There are different strategies such as assessing HCV viral load, serum AST, ALT, and bilirubin used to screen for recurrent disease. Serum transaminases and HCV RNA viral load do not correlate well with clinical disease nor overall

hepatic function [12, 31]. Currently the generally accepted treatment strategy for recurrent hepatitis C after transplantation requires the histologic confirmation of recurrent infection and fibrosis on consecutive biopsies. Typically patients should undergo liver biopsy at least annually to monitor for fibrosis [19]. Patients should also be considered for biopsy for flares in liver function tests [32]. The specific timing of anti-viral therapy has not been well established; however, it is widely accepted that anti-viral treatment should be initiated in patients with stage II fibrosis or higher. In addition to histologic evidence of recurrent disease, other considerations when initiating treatment should include patient age, renal function, immunologic status, and psychiatric status, particularly evidence for depression.

Quality of Life

Another significant burden accompanying recurrent HCV infection after OLT is quality of life. Patients with recurrent HCV demonstrate lower overall health-related quality of life scores, greater amounts of depression, higher psychological distress, higher total mood disturbance, and overall lower physical functioning when compared with patients without HCV recurrence [33, 34]. Some of these aforementioned psychological effects of recurrence, particularly depression, occur even in the absence of physical manifestations of recurrent disease. This demonstrates the need for a multi-disciplinary approach to treatment of HCV recurrence which might include psychological intervention and patient education of the implications of HCV recurrence. The initiation and timing of such interventions have not been well established.

Anti-viral Therapy

Prophylactic/Preemptive Therapy Post-transplant

Prophylactic or preemptive therapy is defined as the use of antiviral therapy against hepatitis before there is histological evidence of recurrent hepatitis C disease, which is generally within 6 months of transplantation. Treatment entails interferon-based therapy, with or without ribavirin.

Initial studies suggested that there was potential for preemptive treatment as a means of preventing recurrent hepatitis C disease, achieving sustained viral response (SVR), defined as undetectable viral load 24 weeks following antiviral therapy, and delaying fibrosis progression. Preemptive treatment studies utilizing a combination therapy involving interferon-based therapy in cadaveric transplant recipients resulted in SVR of less than 20% in patients with genotype 1 [35, 36]. Chalasani et al. demonstrated a 48% reduced risk of progressing to a fibrosis score greater than or equal to 2 when using preemptive therapy [36]. Preemptive antiviral therapy may slow disease progression and need for subsequent HCV therapy [37]. Moreover, similar predictors of response found when treating the general population have been described when treating liver transplant recipients preemptively [38–43].

Tolerability is limited in liver transplant recipients treated with preemptive antiviral therapy. Immediately after transplantation, recipients are recovering from surgery and require extensive immunosuppression. Few recipients are considered candidates for treatment early after transplantation. In one study, only 41% of 124 consecutive patients were candidates for empiric antiviral therapy within the first 6 weeks after transplantation [44]. Dose reduction and early discontinuation are common. Shergill et al. also demonstrated that 40% of the patients who received at least one dose of interferon discontinued treatment [44]. Common reasons for drug discontinuation included acute rejection, pancytopenia, and depression unresponsive to medications. Ultimately, only 15% of patients achieved full-dose therapy and only 23% of patients were able to achieve at least 80% of the treatment doses for at least 80% of the treatment duration.

A controversial area is the risk of acute rejection with interferon therapy [38, 40]. Interferon-induced rejections are often mild to moderate, and can be treated by temporarily discontinuing interferon therapy and/or by increasing the immunosuppressive regimen [45]. Moreover, the

frequency and severity of acute cellular rejection are not significantly greater in patients undergoing anti-viral therapy when compared to patients not receiving anti-viral therapy [38, 40].

Empiric Therapy for Patients with Established Disease

The current regimen used as standard of care therapy to treat recurrent HCV after transplantation consists of pegylated interferon plus ribavirin [1, 18, 46, 47]. Lower SVRs are achieved pegylated interferon monotherapy [38, 48]. Ribavirin monotherapy is ineffective in achieving an SVR [38, 49, 50].

The recommended dosing regimen of pegylated interferon and ribavirin differs among centers. The generally accepted starting regimen, as recommended by the International Liver Transplant Society, consists of pegylated interferon alfa-2b at 0.5 μg/kg/week or pegylated interferon alfa-2a 90 μg/week plus Ribavirin 600 mg/day [19]. The pegylated interferon regimen should be up titrated as tolerated to full dose within 2–4 weeks, with a goal dose pegylated interferon alfa-2b of 1.0–1.5 μg/kg/week. Ribavirin should be up titrated every 2 weeks as tolerated until a goal dose of 10–11 mg/kg/day is achieved [19]. Again, many other studies have used slightly varying dosing regimens of both pegylated interferon (0.5–1.5 μg/kg/week) and ribavirin (400–1,200 mg/day), all with slightly varying, but similar, results in attaining SVR [11, 18]. Adverse effects associated with antiviral therapy are similar to those experienced in non-transplant patients. However, the severity and frequency of several adverse effects are common in liver transplant recipients such as neutropenia and anemia [11, 51]. In addition, there is controversy regarding several potential unique adverse effects such as interferon causing acute and chronic rejection [22, 52].

Patients should be monitored with routine metabolic panels and complete blood counts at least every 2 weeks during the titration phase, and monthly once stable doses have been achieved. HCV RNA should be measured at least every 3 months and, as previously mentioned, if the patient fails to achieve an early viral response (EVR) by 12 weeks continuing treatment should be re-evaluated.

Factors Affecting Sustained Viral Response

The probability of reaching an SVR appears to be affected by multiple factors, including HCV genotype, pretreatment HCV viral load, patient age, ability to maintain recommended target doses, and duration of therapy, as well as ability to achieve an EVR to treatment [11, 18, 41, 53]. The CC polymorphism variant of the IL28b gene region has been recently been associated with increased SVR rates in non-transplant genotype 1 patients [54]. Likewise, both recipient and donor IL28b variant is associated with better response to interferon-based therapy in liver transplant recipients [30].

Two consistent and well-established predictors of response to antiviral therapy are the ability to maintain target doses of antiviral medications and achieve an EVR [11, 18, 41, 53]. The ability to maintain goal levels of pegylated interferon and Ribavirin is difficult in liver transplant recipients, as only approximately 30% of treated patients are able to achieve target doses and duration of therapy [1]. The importance of maintaining target doses is that patients who are able to better tolerate antiviral therapy have a higher likelihood of achieving an SVR [53, 54]. A common reason for intolerability is cytopenia, particularly anemia and leukocytopenia [51, 55]. The immunosuppressive medications used in the post-transplant population exacerbate the bone marrow suppressive effects of pegylated interferon and Ribavirin. The use of granulocyte colony-stimulating factor (G-CSF) and epogen (Epo) analogs has been studied as a means of resolving the various cytopenias in order to maintain target doses of anti-viral therapy [51, 55]. The use of these growth factors is important to

maintain target doses and durations of anti-viral therapy; however, there is no data to date to indicate that they increase the likelihood of achieving a SVR [56].

Milestones defined in predicting the likelihood of achieving a SVR during antiviral therapy are similar in both liver transplant recipients and non-transplant patients. For instance, as in the general population, EVR, as defined by a >2 log decrease in HCV viral load by week 12 of therapy, has correlated very well with an SVR [1, 11, 18, 19]. It has been demonstrated that if patients being treated with combination pegylated interferon and ribavirin who do not demonstrate an EVR were unlikely to achieve SVR [54, 57]. It should be noted that the absence of EVR does not carry a negative predictive value of 100% as initially reported [57]. One reason for the discrepancy in the implication of EVR between the general population and the transplant recipients is the time required to achieve target doses.

Outcomes

The primary reason to treat recurrent HCV is to increase patient survival. It is well documented that in non-transplant patients, a SVR leads to decrease liver fibrosis and improved survival [58–60]. The improvement in survival among those treated for recurrent HCV after transplant remains less certain.

Achieving an SVR leads to histological improvement on subsequent biopsy among the majority of patients, which is sustained long term [61, 62]. Also the development of fibrosis and cirrhosis (HAI grade 4 fibrosis) has shown to be less among those achieving an SVR. The progression of fibrosis is either halted or possibly reversed among those who achieve an SVR, up to 5 years after antiviral treatment and possibly longer [61–63]. In those patients who achieve an SVR, progression to cirrhosis is also improved when compared with non-responders [63].

In the liver transplant population it has been shown that there is likely an overall survival benefit with anti-viral therapy among those who achieve an SVR [53, 63, 64]. However, only 25–45% of treated liver transplant recipients are able to attain an SVR [53, 55, 65]. Among patients who are able to achieve an SVR they can expect a 1-, 3-, and 5-year survival of 97, 92 and 92%, respectively. In non-responders the 1-, 3-, and 5-year survival falls to 96, 77, and 66%, respectively [63].

Graft survival rates are also improved after treatment for recurrent Hepatitis C [66–68]. In one large retrospective cohort study it was demonstrated that 5-year graft survival improved in the treated group vs. the untreated group; 92.5 and 66.1% respectively [66].

Cost Effectiveness

The treatment of recurrent HCV after liver transplant appears to be at least a modestly effective means of prolonging disease-free survival, particularly among those who achieve an SVR. HCV antiviral therapy after liver transplant was shown to be cost effective, particularly for those at higher risk of developing cirrhosis and for younger patients [69]. The cost effectiveness remained as long as SVR was greater than 10% and the cost of antiviral therapy was less than 200% of the cost at the time of the study [69]. This cost effectiveness has held up to repeat analysis, specifically among post-transplant patients with HCV genotype 1 [70].

Special Considerations

The HIV–HCV co-infected individuals who must undergo HCV anti-viral therapy after liver transplantation pose a particular clinical challenge due to multiple factors, including medication interactions, possible decreased tolerability to HCV anti-viral therapy, accelerated fibrosis, and worse overall survival when compared to matched HIV-negative, HCV-positive controls [71–74].

In multiple studies it has been demonstrated that HIV/HCV co-infection leads to decreased overall survival [71–74]. In one center's long-term

experience, the decreased overall survival trended with a 1-, 3-, and 5-year patient survival of 66.7, 55.6, and 33.3%, respectively, vs. 75.7, 71.6, and 71.6% in HIV-negative and HCV-positive controls. Graft survival was also adversely affected, with 1-, 3-, and 5-year graft survival of 63, 51.9, and 31.1%, respectively, compared to HIV-negative patients 68.2, 64.1, and 64.1% [74]. Other shorter-term studies have demonstrated worse outcomes [71, 72]. Also HCV-related death or progression to stage 4 fibrosis among HCV/HIV co-infected patients was found to be statistically significant, RR 2.6 ($p=0.03$).

The treatment regimen for HIV/HCV co-infected patients has not been well established. The HCV antiviral therapy should be dosed in the same manner as in those who are HIV negative and the highly active anti-retroviral therapy (HAART) should be continued during HCV treatment. However, it should be noted that increased hepatotoxicity has been demonstrated among patients treated with HAART and pegylated interferon and ribivirin, which may lead to the need to discontinue HAART [74]. The discontinuation of HAART should be done with extreme caution given that intolerance of HAART in post-liver transplant patient is associated with a very high mortality rate [75].

Retreatment After Remission

Although most patients treated with pegylated interferon and ribavirin do not achieve an SVR, there is no consensus on how to treat recipients who do not respond to currently available treatment. Options may include retreatment with pegylated interferon and ribavirin, use of consensus interferon, and/or waiting for upcoming experimental therapies.

In a small study of 27 patients who previously failed treatment with non-pegylated interferon and Ribavirin, retreatment with pegylated interferon and ribavirin led to an SVR of 30%, and improved fibrosis scores in 70% [76]. So if a patient has not yet been treated with the gold-standard pegylated interferon plus Ribavirin, retreatment should be strongly considered. However, among patients who fail initial therapy with pegylated interferon and ribavirin, there have been no large published studies analyzing retreatment with anti-viral therapy. In a small study of eight patients who had failed pegylated interferon and ribavirin, retreatment with consensus interferon and ribavirin led to an SVR in 6 of the 8 patients [77]. However, in a larger retrospective review of 34 liver transplant recipients who did not respond to pegylated interferon and ribavirin and were subsequently treated with consensus interferon and ribavirin, there was no significant increase in SVR [78].

Retransplantation

There is no universally accepted consensus on retransplantation for patients who have developed severe liver disease from recurrent HCV. The role of retransplantation in this setting is controversial since long-term survival is not well defined. Moreover, retransplantation for recurrent HCV was found to be associated with a worse prognosis than for other indications for retransplantation [79]. Retransplantation for recurrent HCV may carry up to a 30% increased mortality risk when compared to other causes for retransplantation [32, 79].

However, there are data among experienced centers which show no statistical difference among retransplantation for recurrent HCV [80, 81]. Factors associated with a more favorable outcome after retransplantation include MELD <21, portal HTN as sole manifestation of cirrhosis, shorted warm and cold ischemia times, previous SVR, and among patients who were retransplanted >30 days after initial liver transplantation [32, 79–81]. Factors associated with worse outcome include recipient age >55, renal failure, ventilator dependence, MELD >21, prolonged warm and cold ischemia times, early viral recurrence, and transplanted between 8 and 30 days after initial transplant [19, 32] (Table 25.2). Therefore patients who have graft failure and recurrent HCV, retransplantation remains a viable option and should be considered on a case-by-case basis.

Table 25.2 Factors affecting retransplantation outcomes

Factors favoring better outcomes for retransplantation	Factors associated with worse outcomes for retransplantation
MELD <21 [80]	MELD >21 [80]
Mild portal HTN [31, 77]	Recipient age >55 [80]
Shorter warm/cold ischemia time [79]	Longer warm/cold ischemia time [79]
Previous achievement of SVR [31]	Early viral recurrence [31]
Retransplantation >30 days after initial transplant [77]	Retransplant between 8 and 30 days after initial transplant [77]
	Ventilator dependence [79]
	Renal failure [79]

Future Therapies

Promising drugs against hepatitis C are those that directly target viral replication. For instance, protease inhibitors directly inhibit viral replication. Protease inhibitors are furthest along in study, and are likely to obtain approval by the Food and Drug Administration in 2011. When used alone, drugs in this class of medications are eventually associated with resistance [82–84]. However, when protease inhibitors are used in combination with pegylated interferon and/or ribavirin an SVR rate between 60 and 75% is obtained in naïve patients with chronic hepatitis C [85–88].

The protease inhibitors have signature adverse effects. For instance, telaprevir can be associated with a rash and boceprevir with anemia [89– 91]. There are no studies utilizing the combination of a protease inhibitor with pegylated interferon and ribavirin in liver transplant recipients. It remains to be determined what impact the protease inhibitors will have on immunosuppression levels and risk of acute cellular rejection. Tolerability may also be an issue in the pegylated interferon and ribavirin can already cause anemia in up to 40% of treated patients [11, 51].

Barriers to Advancement in Therapy

With the current advances being made in HCV antiviral therapy, there remain sizeable barriers to improvement among the post-liver transplant population. One must wonder if the new anti-viral therapies will be made available in a timely manner or will there be delays in applying these medications to the post-transplant population. Because of the decreased tolerability to medication, side effects, and early discontinuation of standard therapy in the post-transplant population, the benefits of treatment may be more difficult to establish. The drug–drug interactions among post-liver transplant patients become a major burden, specifically as a result of the intrinsic nephrotoxicity of many anti-rejection medications as well as hepatic dysfunction in post-liver transplant patients with recurrent HCV. Hence, pharmaceutical companies may be less likely to support early trials of new therapies in the post-transplant population due to the difficulties in establishing benefit in this difficult-to-treat population. In addition, as the post-transplant population is only a small proportion of all patients infected with HCV, studies using newer anti-virals will also face the obstacle of possibly being underpowered to show significant benefit of these newer treatments. Finally given the relatively short survival in the post-transplant population compared to the non-transplant population, overall mortality benefit will be equally difficult to prove.

Other barriers to newer therapies in the post-transplant population will be the increased cost of the new medications and whether cost effectiveness can be demonstrated, particularly if the increase in the rate of achieving SVR is only modest. If addressing the healthcare system as a whole, the question of resource allocation becomes a barrier as well, given the limited resources and healthcare dollars available.

Conclusion

In conclusion, recurrent hepatitis C continues to be a significant burden on patients following liver transplantation. Over the last few decades advances have been made in the treatment of recurrent hepatitis C, beginning with interferon mono-therapy up to the possibility of triple therapy with pegylated interferon, ribavirin, and a protease inhibitor with adjuvant therapy including Epo analogues and G-CSF. Past experience reveals that despite our advances in therapy we always have the need to improve outcomes and as the research presses forward we will one day be able to adequately manage this difficult-to-treat illness.

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