METHODS OF TREATING LIVER FIBROSIS AND HEPATITIS C VIRUS INFECTION

FIELD OF THE INVENTION

This invention is in the field of liver fibrosis and hepatitis C virus infection.

BACKGROUND OF THE INVENTION

Fibrosis occurs as a result of a chronic toxic insult to the liver, such as chronic hepatitis C virus (HCV) infection, autoimmune injury, and chronic exposure to toxins such as alcohol. Chronic toxic insult leads to repeated cycles of hepatocyte injury and repair accompanied by chronic inflammation. Over a variable period of time, abnormal extracellular matrix progressively accumulates as a consequence of the host's wound repair response. Left unchecked, this leads to increasing deposition of fibrous material until liver architecture becomes distorted and the liver's regenerative ability is compromised. The progressive accumulation of scar tissue within the liver finally results in the histopathologic picture of cirrhosis, defined as the formation of fibrous septae throughout the liver with the formation of micronodules.

Hepatitis C virus (HCV) infection is the most common chronic blood borne infection in the United States. Although the numbers of new infections have declined, the burden of chronic infection is substantial, with Centers for Disease Control estimates of 3.9 million (1.8%) infected persons in the United States. Chronic liver disease is the tenth leading cause of death among adults in the United States, and accounts for approximately 25,000 deaths annually, or approximately 1% of all deaths. Studies indicate that 40% of chronic liver disease is HCV-related, resulting in an estimated 8,000-10,000 deaths each year. HCV-associated end-stage liver disease is the most frequent indication for liver transplantation among adults.

Antiviral therapy of chronic hepatitis C has evolved rapidly over the last decade, with significant improvements seen in the efficacy of treatment. Nevertheless, even with combination therapy using pegylated IFN-α plus ribavirin, 40% to 50% of patients fail therapy, i.e., are nonresponders or relapsers. These patients currently have no effective therapeutic alternative. In particular, patients who have advanced fibrosis or cirrhosis on liver biopsy are at significant risk of developing complications of advanced liver disease, including ascites, jaundice, variceal bleeding, encephalopathy, and progressive liver failure, as well as a markedly increased risk of hepatocellular carcinoma.

The high prevalence of chronic HCV infection has important public health implications for the future burden of chronic liver disease in the United States. Data derived from the National Health and Nutrition Examination Survey (NHANES III) indicate that a large increase in the rate of new HCV infections occurred from the late 1960s to the early 1980s, particularly among persons between 20 to 40 years of age. It is estimated that the number of persons with long-standing HCV infection of 20 years or longer could more than quadruple from 1990 to 2015, from 750,000 to over 3 million. The proportional increase in persons infected for 30 or 40 years would be even greater. Since the risk of HCV-related chronic liver disease is related to the duration of infection, with the risk of cirrhosis progressively increasing for persons infected for longer than 20 years, this will result in a substantial increase in cirrhosis-related morbidity and mortality among patients infected between the years of 1965-1985.

There is a need in the art for improved methods for treating diseases of the liver such as fibrosis as well as for treating HCV infection. The present invention addresses this need, and provides related advantages.

Literature

METAVIR (1994) *Hepatology* **20**:15-20; Brunt (2000) *Hepatol.* **31**:241-246; Alpini (1997) *J. Hepatol.* **27**:371-380; Baroni et al. (1996) *Hepatol.* **23**:1189-1199; Czaja et al. (1989) *Hepatol.* **10**:795-800; Grossman et al. (1998) *J. Gastroenterol. Hepatol.* **13**:1058-1060; Rockey and Chung (1994) *J. Invest. Med.* **42**:660-670; Sakaida et al. (1998) *J. Hepatol.* **28**:471-479; Shi et al. (1997) *Proc. Natl. Acad. Sci. USA* **94**:10663-10668; Baroni et al. (1999) *Liver* **19**:212-219; Lortat-Jacob et al. (1997) *J. Hepatol.* **26**:894-903; Llorent et al. (1996) *J. Hepatol.* **24**:555-563; U.S. Patent No. 5,082,659; European Patent Application EP 294,160; U.S. Patent No. 4,806,347; Balish et al. (1992) *J. Infect. Diseases* 166:1401-1403; Katayama et al. (2001) *J. Viral Hepatitis* 8:180-185; U.S. Patent No. 5,082,659; U.S. Patent No. 5,190,751; U.S. Patent No. 4,806,347; Wandl et al. (1992) *Br. J. Haematol.* 81:516-519; European Patent Application No. 294,160; Canadian Patent No. 1,321,348; European Patent Application No. 276,120; Wandl et al. (1992) *Sem. Oncol.* 19:88-94; Balish et al. (1992) *J. Infectious Diseases* 166:1401-1403; Van Dijk et al. (1994) *Int. J. Cancer* 56:262-268; Sundmacher et al. (1987) *Current Eye Res.* 6:273-276.

SUMMARY OF THE INVENTION

The present invention provides methods of treating hepatitis C virus (HCV) infection; methods of reducing liver fibrosis; methods of increasing liver function in an

individual suffering from liver fibrosis; methods of reducing the incidence of complications associated with HCV and cirrhosis of the liver; methods of reducing viral load. The methods generally involve administering a therapeutically effective amount of IFN-α and IFN-γ concurrently.

FEATURES OF THE INVENTION

The invention features a method of treating hepatitis C virus (HCV) infection, generally involving administering to an individual IFN-γ and IFN-α concurrently and in an amount effective to achieve a sustained viral response.

The invention features a method of reducing liver fibrosis in an individual, generally involving administering IFN-α and IFN-γ concurrently and in an amount effective to reduce liver fibrosis. In some embodiments, the degree of liver fibrosis is determined by pre-treatment and post-treatment staging of a liver biopsy, wherein the stage of liver fibrosis, as measured by a standardized scoring system, is reduced by at least one unit when comparing pre-treatment with post-treatment liver biopsies.

The invention also features a method of increasing liver function in an individual suffering from liver fibrosis, generally involving administering IFN-α and IFN-γ in an amount effective to increase a liver function. Liver function may be indicated by measuring a parameter selected from the group consisting of serum transaminase level, prothrombin time, serum bilirubin level, blood platelet count, serum albumin level, improvement in portal wedge pressure, reduction in degree of ascites, reduction in a level of encephalopathy, and reduction in a degree of internal varices.

The invention also features a method of reducing the incidence of a complication of cirrhosis of the liver. The methods generally involve administering IFN-α and IFN-γ in an amount effective to reduce the incidence of a complication of cirrhosis of the liver. Examples of complications of cirrhosis of the liver are portal hypertension, progressive liver insufficiency, and hepatocellular carcinoma.

In carrying out the methods described above, IFN-α and IFN-γ are administered to the individual. In some embodiments, IFN-α and IFN-γ are administered in the same formulation. In other embodiments, IFN-α and IFN-γ are administered in separate formulations. When administered in separate formulations, IFN-α and IFN-γ can be administered substantially simultaneously, or can be administered within about 24 hours of one another. In many embodiments, IFN-α and IFN-γ are administered subcutaneously in multiple doses.

Dosages of IFN-γ range from about 25 μg/dose to about 300 μg/dose. Effective dosages of consensus IFN-α include about 3 μg, about 9 μg, about 15 μg, about 18 μg, or about 27 μg per dose. Effective dosages of IFN-α2a and IFN-α2b range from 3 million international units (MIU) to 10 MIU per dose. Effective dosages of PEGylated IFN-α2a range from 90 to 180 μg per dose. Effective dosages of PEGylated IFN-α2b range from 0.5 μg/kg body weight to 1.5 μg/kg body weight per dose. In many embodiments, IFN-α and IFN-γ is administered for a period of at least three months, and may be administered over longer periods of time.

In some embodiments, IFN-γ is administered during the entire course of IFN-α treatment. In other embodiments, IFN-γ is administered for a period of time that is overlapping with that of the IFN-α treatment, e.g., the IFN-γ treatment can begin before the IFN-α treatment begins and end before the IFN-α treatment ends; the IFN-γ treatment can begin after the IFN-α treatment begins and end after the IFN-γ treatment ends; the IFN-γ treatment can begin after the IFN-α treatment begins and end before the IFN-α treatment ends; or the IFN-γ treatment can begin before the IFN-α treatment begins and end after the IFN-α treatment ends.

In other embodiments, ribavirin is administered in addition to IFN-α and IFN-γ.

DEFINITIONS

As used herein, the term "hepatic fibrosis," used interchangeably herein with "liver fibrosis," refers to the growth of scar tissue in the liver that can occur in the context of a chronic hepatitis infection.

The terms "individual," "host," "subject," and "patient" are used interchangeably herein, and refer to a mammal, including, but not limited to, primates, including simians and humans.

As used herein, the term "liver function" refers to a normal function of the liver, including, but not limited to, a synthetic function, including, but not limited to, synthesis of proteins such as serum proteins (e.g., albumin, clotting factors, alkaline phosphatase, aminotransferases (e.g., alanine transaminase, aspartate transaminase), 5'-nucleosidase, γ-glutaminyltranspeptidase, etc.), synthesis of bilirubin, synthesis of cholesterol, and synthesis of bile acids; a liver metabolic function, including, but not limited to, carbohydrate metabolism, amino acid and ammonia metabolism, hormone metabolism, and lipid metabolism; detoxification of exogenous drugs; a hemodynamic function, including splanchnic and portal hemodynamics; and the like.

The term "sustained viral response" (SVR; also referred to as a "sustained response" or a "durable response"), as used herein, refers to the response of an individual to a treatment regimen for HCV infection, in terms of serum HCV titer. Generally, a "sustained viral response" refers to no detectable HCV RNA (e.g., less than about 500, less than about 200, or less than about 100 genome copies per milliliter serum) found in the patient's serum for a period of at least about one month, at least about two months, at least about three months, at least about four months, at least about five months, or at least about six months following cessation of treatment.

"Treatment failure patients" as used herein generally refers to HCV-infected patients who failed to respond to previous therapy for HCV (referred to as "non-responders") or who initially responded to previous therapy, but in whom the therapeutic response was not maintained (referred to as "relapsers"). The previous therapy generally can include treatment with IFN-α monotherapy or IFN-α combination therapy, where the combination therapy may include administration of IFN-α and an antiviral agent such as ribavirin.

As used herein, the terms "treatment," "treating," and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse affect attributable to the disease. "Treatment," as used herein, covers any treatment of a disease in a mammal, particularly in a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; and (c) relieving the disease, i.e., causing regression of the disease.

The terms "individual," "host," "subject," and "patient" are used interchangeably herein, and refer to a mammal, including, but not limited to, murines, simians, humans, mammalian farm animals, mammalian sport animals, and mammalian pets.

Before the present invention is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either both of those included limits are also included in the invention.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

It must be noted that as used herein and in the appended claims, the singular forms "a," "and," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a method" includes a plurality of such methods and reference to "a dose" includes reference to one or more doses and equivalents thereof known to those skilled in the art, and so forth.

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides methods of treating HCV infection, methods of treating liver fibrosis, including reducing clinical liver fibrosis, reducing the likelihood that liver fibrosis will occur, and reducing a parameter associated with liver fibrosis. The methods generally involve administering an effective amount of IFN-α and IFN-γ to an individual in need thereof. The combination therapy has synergistic effects that are more effective than IFN-α or IFN-γ alone. Of particular interest in many embodiments is treatment of humans.

Liver fibrosis is a precursor to the complications associated with liver cirrhosis, such as portal hypertension, progressive liver insufficiency, and hepatocellular carcinoma. A reduction in liver fibrosis thus reduces the incidence of such future complications. Accordingly, the present invention further provides methods of reducing the likelihood that an individual will develop complications associated with cirrhosis of the liver.

The present methods generally involve administering a therapeutically effective amount of IFN-α and IFN-γ. As used herein, a "therapeutically effective amount" of the combination of IFN-α and IFN-γ is an amount of IFN-α and IFN-γ that is effective in treating an HCV infection, in achieving a sustained viral response, in reducing liver fibrosis; and/or that is effective in reducing the likelihood that an individual will develop liver fibrosis; and/or that is effective in reducing a parameter associated with liver fibrosis; and/or that is effective in reducing a disorder associated with cirrhosis of the liver.

Hepatitis C Virus

The present invention provides methods for treating HCV. The methods generally involve administering IFN-α and IFN-γ to an individual in an amount that is effective to decrease viral load in the individual, and to achieve a sustained viral response.

Whether a subject method is effective in treating an HCV infection can be determined by measuring viral load, or by measuring a parameter associated with HCV infection, including, but not limited to, liver fibrosis, elevations in serum transaminase levels, and necroinflammatory activity in the liver. Indicators of liver fibrosis are discussed in detail below.

Viral load can be measured by measuring the titer or level of virus in serum. These methods include, but are not limited to, a quantitative polymerase chain reaction (PCR) and a branched DNA (bDNA) test. Quantitative assays for measuring the viral load (titer) of HCV RNA have been developed. Many such assays are available commercially, including a quantitative reverse transcription PCR (RT-PCR) (Amplicor HCV Monitor™, Roche Molecular Systems, New Jersey); and a branched DNA (deoxyribonucleic acid) signal amplification assay (Quantiplex™ HCV RNA Assay (bDNA), Chiron Corp., Emeryville, California). See, e.g., Gretch et al. (1995) *Ann. Intern. Med.* 123:321-329.

In general, an effective amount of IFNα and IFNγ is an amount that is effective to reduce viral load to undetectable levels, e.g., to less than about 5000, less than about 1000, less than about 500, or less than about 200 genome copies/mL serum. In some embodiments, an effective amount of IFNα and IFNγ is an amount that is effective to reduce viral load to less than 100 genome copies/mL serum. In many embodiments, the methods of

the invention achieve a sustained viral response, e.g., the viral load is reduced to undetectable levels for a period of at least about one month, at least about two months, at least about three months, at least about four months, at least about five months, or at least about six months following cessation of treatment.

As noted above, whether a subject method is effective in treating an HCV infection can be determined by measuring a parameter associated with HCV infection, such as liver fibrosis. Methods of determining the extent of liver fibrosis are discussed in detail below. In some embodiments, the level of a serum marker of liver fibrosis indicates the degree of liver fibrosis.

As one non-limiting example, levels of serum alanine aminotransferase (ALT) are measured, using standard assays. In general, an ALT level of less than about 45 international units is considered normal. In some embodiments, an effective amount of IFNα and IFNγ is an amount effective to reduce ALT levels to less than about 45 IU/ml serum.

A therapeutically effective amount of IFN-α and IFN-γ is an amount that is effective to reduce a serum level of a marker of liver fibrosis by at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, or at least about 80%, or more, compared to the level of the marker in an untreated individual, or to a placebo-treated individual. Methods of measuring serum markers include immunological-based methods, e.g., enzyme-linked immunosorbent assays (ELISA), radioimmunoassays, and the like, using antibody specific for a given serum marker.

Fibrosis

Whether treatment with IFN-α and IFN-γ is effective in reducing liver fibrosis is determined by any of a number of well-established techniques for measuring liver fibrosis and liver function. Liver fibrosis reduction is determined by analyzing a liver biopsy sample. An analysis of a liver biopsy comprises assessments of two major components: necroinflammation assessed by "grade" as a measure of the severity and ongoing disease activity, and the lesions of fibrosis and parenchymal or vascular remodeling as assessed by "stage" as being reflective of long-term disease progression. See, e.g., Brunt (2000) *Hepatol.* **31**:241-246; and METAVIR (1994) *Hepatology* **20**:15-20. Based on analysis of the liver biopsy, a score is assigned. A number of standardized scoring systems exist which provide a quantitative assessment of the degree and severity of fibrosis. These include the METAVIR, Knodell, Scheuer, Ludwig, and Ishak scoring systems.

The METAVIR scoring system is based on an analysis of various features of a liver biopsy, including fibrosis (portal fibrosis, centrilobular fibrosis, and cirrhosis); necrosis (piecemeal and lobular necrosis, acidophilic retraction, and ballooning degeneration); inflammation (portal tract inflammation, portal lymphoid aggregates, and distribution of portal inflammation); bile duct changes; and the Knodell index (scores of periportal necrosis, lobular necrosis, portal inflammation, fibrosis, and overall disease activity). The definitions of each stage in the METAVIR system are as follows: score: 0, no fibrosis; score: 1, stellate enlargement of portal tract but without septa formation; score: 2, enlargement of portal tract with rare septa formation; score: 3, numerous septa without cirrhosis; and score: 4, cirrhosis.

Knodell's scoring system, also called the Hepatitis Activity Index, classifies specimens based on scores in four categories of histologic features: I. Periportal and/or bridging necrosis; II. Intralobular degeneration and focal necrosis; III. Portal inflammation; and IV. Fibrosis. In the Knodell staging system, scores are as follows: score: 0, no fibrosis; score: 1, mild fibrosis (fibrous portal expansion); score: 2, moderate fibrosis; score: 3, severe fibrosis (bridging fibrosis); and score: 4, cirrhosis. The higher the score, the more severe the liver tissue damage. Knodell (1981) *Hepatol.* **1**:431.

In the Scheuer scoring system scores are as follows: score: 0, no fibrosis; score: 1, enlarged, fibrotic portal tracts; score: 2, periportal or portal-portal septa, but intact architecture; score: 3, fibrosis with architectural distortion, but no obvious cirrhosis; score: 4, probable or definite cirrhosis. Scheuer (1991) *J. Hepatol.* **13**:372.

The Ishak scoring system is described in Ishak (1995) *J. Hepatol.* **22**:696-699. Stage 0, No fibrosis; Stage 1, Fibrous expansion of some portal areas, with or without short fibrous septa; stage 2, Fibrous expansion of most portal areas, with or without short fibrous septa; stage 3, Fibrous expansion of most portal areas with occasional portal to portal (P-P) bridging; stage 4, Fibrous expansion of portal areas with marked bridging (P-P) as well as portal-central (P-C); stage 5, Marked bridging (P-P and/or P-C) with occasional nodules (incomplete cirrhosis); stage 6, Cirrhosis, probable or definite.The benefit of anti-fibrotic therapy can also be measured and assessed by using the Child-Pugh scoring system which comprises a multicomponent point system based upon abnormalities in serum bilirubin level, serum albumin level, prothrombin time, the presence and severity of ascites, and the presence and severity of encephalopathy. Based upon the presence and severity of abnormality of these parameters, patients may be placed in one of three categories of increasing severity of clinical disease: A, B, or C.

In some embodiments, a therapeutically effective amount of IFN-α and IFN-γ is an amount of IFN-α and IFN-γ that effects a change of one unit or more in the fibrosis stage based on pre- and post-therapy liver biopsies. In particular embodiments, a therapeutically effective amount of IFN-α and IFN-γ reduces liver fibrosis by at least one unit in the METAVIR, the Knodell, the Scheuer, the Ludwig, or the Ishak scoring system.

Secondary, or indirect, indices of liver function can also be used to evaluate the efficacy of IFN-α and IFN-γ treatment. Morphometric computerized semi-automated assessment of the quantitative degree of liver fibrosis based upon specific staining of collagen and/or serum markers of liver fibrosis can also be measured as an indication of the efficacy of a subject treatment method. Secondary indices of liver function include, but are not limited to, serum transaminase levels, prothrombin time, bilirubin, platelet count, portal pressure, albumin level, and assessment of the Child-Pugh score. An effective amount of IFN-α and IFN-γ is an amount that is effective to increase an index of liver function by at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, or at least about 80%, or more, compared to the index of liver function in an untreated individual, or to a placebo-treated individual. Those skilled in the art can readily measure such indices of liver function, using standard assay methods, many of which are commercially available, and are used routinely in clinical settings.

Serum markers of liver fibrosis can also be measured as an indication of the efficacy of a subject treatment method. Serum markers of liver fibrosis include, but are not limited to, hyaluronate, N-terminal procollagen III peptide, 7S domain of type IV collagen, C-terminal procollagen I peptide, and laminin. Additional biochemical markers of liver fibrosis include α-2-macroglobulin, haptoglobin, gamma globulin, apolipoprotein A, and gamma glutamyl transpeptidase.

A therapeutically effective amount of IFN-α and IFN-γ is an amount that is effective to reduce a serum level of a marker of liver fibrosis by at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, or at least about 80%, or more, compared to the level of the marker in an untreated individual, or to a placebo-treated individual. Those skilled in the art can readily measure such serum markers of liver fibrosis, using standard assay methods, many of which are commercially available, and are used routinely in clinical settings.

Methods of measuring serum markers include immunological-based methods, e.g., enzyme-linked immunosorbent assays (ELISA), radioimmunoassays, and the like, using antibody specific for a given serum marker.

Quantitative tests of functional liver reserve can also be used to assess the efficacy of treatment with IFN-α and IFN-γ. These include: indocyanine green clearance (ICG), galactose elimination capacity (GEC), aminopyrine breath test (ABT), antipyrine clearance, monoethylglycine-xylidide (MEG-X) clearance, and caffeine clearance.

As used herein, a "complication associated with cirrhosis of the liver" refers to a disorder that is a sequellae of decompensated liver disease, i.e., or occurs subsequently to and as a result of development of liver fibrosis, and includes, but it not limited to, development of ascites, variceal bleeding, portal hypertension, jaundice, progressive liver insufficiency, encephalopathy, hepatocellular carcinoma, liver failure requiring liver transplantation, and liver-related mortality.

A therapeutically effective amount of IFN-α and IFN-γ is an amount that is effective in reducing the incidence (e.g., the likelihood that an individual will develop) of a disorder associated with cirrhosis of the liver by at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, or at least about 80%, or more, compared to an untreated individual, or to a placebo-treated individual.

Whether treatment with IFN-α and IFN-γ is effective in reducing the incidence of a disorder associated with cirrhosis of the liver can readily be determined by those skilled in the art.

Reduction in liver fibrosis increases liver function. Thus, the invention provides methods for increasing liver function, generally involving administering a therapeutically effective amount of IFN-α and IFN-γ. Liver functions include, but are not limited to, synthesis of proteins such as serum proteins (e.g., albumin, clotting factors, alkaline phosphatase, aminotransferases (e.g., alanine transaminase, aspartate transaminase), 5'-nucleosidase, γ-glutaminyltranspeptidase, etc.), synthesis of bilirubin, synthesis of cholesterol, and synthesis of bile acids; a liver metabolic function, including, but not limited to, carbohydrate metabolism, amino acid and ammonia metabolism, hormone metabolism, and lipid metabolism; detoxification of exogenous drugs; a hemodynamic function, including splanchnic and portal hemodynamics; and the like.

Whether a liver function is increased is readily ascertainable by those skilled in the art, using well-established tests of liver function. Thus, synthesis of markers of liver function such as albumin, alkaline phosphatase, alanine transaminase, aspartate transaminase, bilirubin, and the like, can be assessed by measuring the level of these markers in the serum, using standard immunological and enzymatic assays. Splanchnic circulation and portal hemodynamics can be measured by portal wedge pressure and/or resistance using standard methods. Metabolic functions can be measured by measuring the level of ammonia in the serum.

Whether serum proteins normally secreted by the liver are in the normal range can be determined by measuring the levels of such proteins, using standard immunological and enzymatic assays. Those skilled in the art know the normal ranges for such serum proteins. The following are non-limiting examples. The normal level of alanine transaminase is about 45 IU per milliliter of serum. The normal range of aspartate transaminase is from about 5 to about 40 units per liter of serum. Bilirubin is measured using standard assays. Normal bilirubin levels are usually less than about 1.2 mg/dL. Serum albumin levels are measured using standard assays. Normal levels of serum albumin are in the range of from about 35 to about 55 g/L. Prolongation of prothrombin time is measured using standard assays. Normal prothrombin time is less than about 4 seconds longer than control.

A therapeutically effective amount of IFN-α and IFN-γ is one that is effective to increase liver function by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or more. For example, a therapeutically effective amount of IFN-α and IFN-γ is an amount effective to reduce an elevated level of a serum marker of liver function by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or more, or to reduce the level of the serum marker of liver function to within a normal range. A therapeutically effective amount of IFN-γ is also an amount effective to increase a reduced level of a serum marker of liver function by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or more, or to increase the level of the serum marker of liver function to within a normal range.

Interferon-Alpha

Any known IFN-α can be used in the instant invention. The term "interferon-alpha" as used herein refers to a family of related polypeptides that inhibit viral replication and cellular proliferation and modulate immune response. The term "IFN-α" includes naturally

occurring IFN-α; synthetic IFN-α; derivatized IFN-α (e.g., PEGylated IFN-α, glycosylated IFN-α, and the like); and analogs of naturally occurring or synthetic IFN-α; essentially any IFN-α that has antiviral properties, as described for naturally occurring IFN-α.

Suitable alpha interferons include, but are not limited to, naturally-occurring IFN-α (including, but not limited to, naturally occurring IFN-α2a, IFN-α2b); recombinant interferon alpha-2b such as Intron-A interferon available from Schering Corporation, Kenilworth, N.J.; recombinant interferon alpha-2a such as Roferon interferon available from Hoffmann-La Roche, Nutley, N. J.; recombinant interferon alpha-2C such as Berofor alpha 2 interferon available from Boehringer Ingelheim Pharmaceutical, Inc., Ridgefield, Conn.; interferon alpha-n1, a purified blend of natural alpha interferons such as Sumiferon available from Sumitomo, Japan or as Wellferon interferon alpha-n1 (INS) available from the Glaxo-Wellcome Ltd., London, Great Britain; and interferon alpha-n3 a mixture of natural alpha interferons made by Interferon Sciences and available from the Purdue Frederick Co., Norwalk, Conn., under the Alferon Tradename.

The term "IFN-α" also encompasses consensus IFN-α. Consensus IFN-α (also referred to as "CIFN" and "IFN-con") encompasses but is not limited to the amino acid sequences designated IFN-con_1, IFN-con_2 and IFN-con_3 which are disclosed in U.S. Pat. Nos. 4,695,623 and 4,897,471; and consensus interferon as defined by determination of a consensus sequence of naturally occurring interferon alphas (e.g., Infergen®, Amgen, Thousand Oaks, Calif.). DNA sequences encoding IFN-con may be synthesized as described in the aforementioned patents or other standard methods. Use of CIFN is of particular interest.

The term "IFN-α" also encompasses derivatives of IFN-α that are derivatized (e.g., are chemically modified) to alter certain properties such as serum half-life. As such, the term "IFN-α" includes glycosylated IFN-α; IFN-α derivatized with polyethylene glycol ("PEGylated IFN-α"); and the like. PEGylated IFN-α, and methods for making same, is discussed in, e.g., U.S. Patent Nos. 5,382,657; 5,981,709; and 5,951,974. PEGylated IFN-α encompasses conjugates of PEG and any of the above-described IFN-α molecules, including, but not limited to, PEG conjugated to interferon alpha-2a (Roferon, Hoffman La-Roche, Nutley, N.J.), interferon alpha 2b (Intron, Schering-Plough, Madison, N.J.), interferon alpha-2c (Berofor Alpha, Boehringer Ingelheim, Ingelheim, Germany); and consensus interferon as defined by determination of a consensus sequence of naturally occurring interferon alphas (Infergen, Amgen, Thousand Oaks, Calif.).

Interferon-Gamma

The nucleic acid sequences encoding IFN-γ polypeptides may be accessed from public databases, e.g., Genbank, journal publications, etc. While various mammalian IFN-γ polypeptides are of interest, for the treatment of human disease, generally the human protein will be used. Human IFN-γ coding sequence may be found in Genbank, accession numbers X13274; V00543; and NM_000619. The corresponding genomic sequence may be found in Genbank, accession numbers J00219; M37265; and V00536. See, for example. Gray *et al.* (1982) *Nature* **295**:501 (Genbank X13274); and Rinderknecht *et al.* (1984) *J.B.C.* **259**:6790.

IFN-γ1b (Actimmune®; human interferon) is a single-chain polypeptide of 140 amino acids. It is made recombinantly in *E.coli* and is unglycosylated. Rinderknecht et al. (1984) *J. Biol. Chem.* **259**:6790-6797.

The IFN-γ to be used in the methods of the present invention may be any of natural IFN-γs, recombinant IFN-γs and the derivatives thereof so far as they have an IFN-γ activity, particularly human IFN-γ activity. Human IFN-γ exhibits the antiviral and anti-proliferative properties characteristic of the interferons, as well as a number of other immunomodulatory activities, as is known in the art. Although IFN-γ is based on the sequences as provided above, the production of the protein and proteolytic processing can result in processing variants thereof. The unprocessed sequence provided by Gray et al., *supra*, consists of 166 amino acids (aa). Although the recombinant IFN-γ produced in *E. coli* was originally believed to be 146 amino acids, (commencing at amino acid 20) it was subsequently found that native human IFN-γ is cleaved after residue 23, to produce a 143 aa protein, or 144 aa if the terminal methionine is present, as required for expression in bacteria. During purification, the mature protein can additionally be cleaved at the C terminus after reside 162 (referring to the Gray *et al.* sequence), resulting in a protein of 139 amino acids, or 140 amino acids if the initial methionine is present, *e.g.* if required for bacterial expression. The N-terminal methionine is an artifact encoded by the mRNA translational "start" signal AUG that, in the particular case of *E. coli* expression is not processed away. In other microbial systems or eukaryotic expression systems, methionine may be removed.

For use in the subject methods, any of the native IFN-γ peptides, modifications and variants thereof, or a combination of one or more peptides may be used. IFN-γ peptides of interest include fragments, and can be variously truncated at the carboxyl terminus relative to the full sequence. Such fragments continue to exhibit the characteristic properties of human gamma interferon, so long as amino acids 24 to about 149 (numbering from the residues of

the unprocessed polypeptide) are present. Extraneous sequences can be substituted for the amino acid sequence following amino acid 155 without loss of activity. See, for example, U.S. Patent No. 5,690,925. Native IFN-γ moieties include molecules variously extending from amino acid residues 24-150; 24-151, 24-152; 24- 153, 24-155; and 24-157. Any of these variants, and other variants known in the art and having IFN-γ activity, may be used in the present methods.

The sequence of the IFN-γ polypeptide may be altered in various ways known in the art to generate targeted changes in sequence. A variant polypeptide will usually be substantially similar to the sequences provided herein, i.e., will differ by at least one amino acid, and may differ by at least two but not more than about ten amino acids. The sequence changes may be substitutions, insertions or deletions. Scanning mutations that systematically introduce alanine, or other residues, may be used to determine key amino acids. Specific amino acid substitutions of interest include conservative and non-conservative changes. Conservative amino acid substitutions typically include substitutions within the following groups: (glycine, alanine); (valine, isoleucine, leucine); (aspartic acid, glutamic acid); (asparagine, glutamine); (serine, threonine); (lysine, arginine); or (phenylalanine, tyrosine).

Modifications of interest that may or may not alter the primary amino acid sequence include chemical derivatization of polypeptides, e.g., acetylation, or carboxylation; changes in amino acid sequence that introduce or remove a glycosylation site; changes in amino acid sequence that make the protein susceptible to PEGylation; and the like. Also included are modifications of glycosylation, e.g., those made by modifying the glycosylation patterns of a polypeptide during its synthesis and processing or in further processing steps; e.g., by exposing the polypeptide to enzymes that affect glycosylation, such as mammalian glycosylating or deglycosylating enzymes. Also embraced are sequences that have phosphorylated amino acid residues, e.g., phosphotyrosine, phosphoserine, or phosphothreonine.

Included in the subject invention are polypeptides that have been modified using ordinary chemical techniques so as to improve their resistance to proteolytic degradation, to optimize solubility properties, or to render them more suitable as a therapeutic agent. For examples, the backbone of the peptide may be cyclized to enhance stability (see Friedler *et al.* (2000) *J. Biol. Chem.* **275**:23783-23789). Analogs may be used that include residues

other than naturally occurring L-amino acids, e.g., D-amino acids or non-naturally occurring synthetic amino acids. The protein may be pegylated to enhance stability.

The polypeptides may be prepared by *in vitro* synthesis, using conventional methods as known in the art, by recombinant methods, or may be isolated from cells induced or naturally producing the protein. The particular sequence and the manner of preparation will be determined by convenience, economics, purity required, and the like. If desired, various groups may be introduced into the polypeptide during synthesis or during expression, which allow for linking to other molecules or to a surface. Thus cysteines can be used to make thioethers, histidines for linking to a metal ion complex, carboxyl groups for forming amides or esters, amino groups for forming amides, and the like.

The polypeptides may also be isolated and purified in accordance with conventional methods of recombinant synthesis. A lysate may be prepared of the expression host and the lysate purified using HPLC, exclusion chromatography, gel electrophoresis, affinity chromatography, or other purification technique. For the most part, the compositions which are used will comprise at least 20% by weight of the desired product, more usually at least about 75% by weight, preferably at least about 95% by weight, and for therapeutic purposes, usually at least about 99.5% by weight, in relation to contaminants related to the method of preparation of the product and its purification. Usually, the percentages will be based upon total protein.

DOSAGES, FORMULATIONS, AND ROUTES OF ADMINISTRATION

IFN-α and IFN-γ are administered to individuals in a formulation with a pharmaceutically acceptable excipient(s). A wide variety of pharmaceutically acceptable excipients are known in the art and need not be discussed in detail herein. Pharmaceutically acceptable excipients have been amply described in a variety of publications, including, for example, A. Gennaro (2000) "Remington: The Science and Practice of Pharmacy," 20th edition, Lippincott, Williams, & Wilkins; Pharmaceutical Dosage Forms and Drug Delivery Systems (1999) H.C. Ansel et al., eds., 7th ed., Lippincott, Williams, & Wilkins; and Handbook of Pharmaceutical Excipients (2000) A.H. Kibbe et al., eds., 3rd ed. Amer. Pharmaceutical Assoc.

The pharmaceutically acceptable excipients, such as vehicles, adjuvants, carriers or diluents, are readily available to the public. Moreover, pharmaceutically acceptable auxiliary substances, such as pH adjusting and buffering agents, tonicity adjusting agents, stabilizers, wetting agents and the like, are readily available to the public.

In the subject methods, the active agents may be administered to the host using any convenient means capable of resulting in the desired therapeutic effect. Thus, the agents can be incorporated into a variety of formulations for therapeutic administration. More particularly, the agents of the present invention can be formulated into pharmaceutical compositions by combination with appropriate, pharmaceutically acceptable carriers or diluents, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants and aerosols.

As such, administration of the agents can be achieved in various ways, including oral, buccal, rectal, parenteral, intraperitoneal, intradermal, transdermal, intracheal, etc.

In pharmaceutical dosage forms, the agents may be administered in the form of their pharmaceutically acceptable salts, or they may also be used alone or in appropriate association, as well as in combination, with other pharmaceutically active compounds. The following methods and excipients are merely exemplary and are in no way limiting.

For oral preparations, the agents can be used alone or in combination with appropriate additives to make tablets, powders, granules or capsules, for example, with conventional additives, such as lactose, mannitol, corn starch or potato starch; with binders, such as crystalline cellulose, cellulose derivatives, acacia, corn starch or gelatins; with disintegrators, such as corn starch, potato starch or sodium carboxymethylcellulose; with lubricants, such as talc or magnesium stearate; and if desired, with diluents, buffering agents, moistening agents, preservatives and flavoring agents.

The agents can be formulated into preparations for injection by dissolving, suspending or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives.

Furthermore, the agents can be made into suppositories by mixing with a variety of bases such as emulsifying bases or water-soluble bases. The compounds of the present invention can be administered rectally via a suppository. The suppository can include vehicles such as cocoa butter, carbowaxes and polyethylene glycols, which melt at body temperature, yet are solidified at room temperature.

Unit dosage forms for oral or rectal administration such as syrups, elixirs, and suspensions may be provided wherein each dosage unit, for example, teaspoonful, tablespoonful, tablet or suppository, contains a predetermined amount of the composition

containing one or more inhibitors. Similarly, unit dosage forms for injection or intravenous administration may comprise the inhibitor(s) in a composition as a solution in sterile water, normal saline or another pharmaceutically acceptable carrier.

The term "unit dosage form," as used herein, refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of compounds of the present invention calculated in an amount sufficient to produce the desired effect in association with a pharmaceutically acceptable diluent, carrier or vehicle. The specifications for the novel unit dosage forms of the present invention depend on the particular compound employed and the effect to be achieved, and the pharmacodynamics associated with each compound in the host.

In all embodiments, at least one dose of IFN-γ is administered concurrently with at least one dose of IFN-α. As used herein, the term "concurrently" indicates that IFN-α and IFN-γ are administered separately and are administered within about 5 seconds to about 15 seconds, within about 15 seconds to about 30 seconds, within about 30 seconds to about 60 seconds, within about 1 minute to about 5 minutes, within about 5 minutes to about 15 minutes, within about 15 minutes to about 30 minutes, within about 30 minutes to about 60 minutes, within about 1 hour to about 2 hours, within about 2 hours to about 6 hours, within about 6 hours to about 12 hours, within about 12 hours to about 24 hours, or within about 24 hours to about 48 hours of one another.

In some embodiments, IFN-γ is administered during the entire course of IFN-α treatment. In other embodiments, IFN-γ is administered for a period of time that is overlapping with that of the IFN-α treatment, e.g., the IFN-γ treatment can begin before the IFN-α treatment begins and end before the IFN-α treatment ends; the IFN-γ treatment can begin after the IFN-α treatment begins and end after the IFN-γ treatment ends; the IFN-γ treatment can begin after the IFN-α treatment begins and end before the IFN-α treatment ends; or the IFN-γ treatment can begin before the IFN-α treatment begins and end after the IFN-α treatment ends.

Effective dosages of IFN-γ range from about 0.5 $\mu g/m^2$ to about 500 $\mu g/m^2$, usually from about 1.5 $\mu g/m^2$ to 200 $\mu g/m^2$, depending on the size of the patient. This activity is based on 10^6 international units (IU) per 50 μg of protein. IFN-γ can be administered daily, every other day, three times a week, or substantially continuously.

Effective dosages of consensus IFN-α include about 3 μg, about 9 μg, about 15 μg, about 18 μg, or about 27 μg per dose. Effective dosages of IFN-α2a and IFN-α2b range

from 3 million international units (MIU) to 10 MIU per dose. Effective dosages of PEGylated IFN-α2a range from 90 to 180 μg per dose. Effective dosages of PEGylated IFN-α2b range from 0.5 μg/kg body weight to 1.5 μg/kg body weight per dose. IFN-α can be administered daily, every other day, once a week, three times a week, or substantially continuously.

In some embodiments, IFN-α is administered in a first dosing regimen, followed by a second dosing regimen. The first dosing regimen of IFN-α (also referred to as "the induction regimen ") generally involves administration of a higher dosage of IFN-α. For example, in the case of consensus IFN-α (CIFN), the first dosing regimen comprises administering CIFN at about 9 μg, about 15 μg, about 18 μg, or about 27 μg. The first dosing regimen can encompass a single dosing event, or at least two or more dosing events. The first dosing regimen of IFN-α can be administered daily, every other day, three times a week, or substantially continuously so as to achieve a desired average daily serum concentration of IFN-α.

The first dosing regimen of IFN-α is administered for a first period of time, which time period can be at least about 4 weeks, at least about 8 weeks, or at least about 12 weeks.

The second dosing regimen of IFN-α (also referred to as "the maintenance dose") generally involves administration of a lower amount of IFN-α. For example, in the case of CIFN, the second dosing regimen comprises administering CIFN at least about 3 μg, at least about 9 μg, at least about 15 μg, or at least about 18 μg. The second dosing regimen can encompass a single dosing event, or at least two or more dosing events.

The second dosing regimen of IFN-α can be administered daily, every other day, three times a week, or substantially continuously so as to achieve a desired average daily serum concentration of IFN-α.

In some embodiments, where an "induction"/"maintenance" dosing regimen of INF-α is administered, a "priming" dose of IFN-γ is included. In these embodiments, IFN-γ is administered for a period of time from about 1 day to about 14 days, from about 2 days to about 10 days, or from about 3 days to about 7 days, before the beginning of treatment with IFN-α. This period of time is referred to as the "priming" phase. In some of these embodiments, IFN-γ treatment is continued throughout the entire period of treatment with IFN-α. In other embodiments, IFN-γ treatment is discontinued before the end of treatment with IFN-α. In these embodiments, the total time of treatment with IFN-γ (including the "priming" phase) is from about 2 days to about 30 days, from about 4 days to about 25 days, from about 8 days to about 20 days, from about 10 days to about 18 days, or from about 12

days to about 16 days. In still other embodiments, IFN-γ treatment is discontinued once IFN-α treatment begins.

In other embodiments, IFN-α is administered in single dosing regimen. In these embodiments, the dose of IFN-α is generally in a range of from about 3 μg to about 15 μg, or from about 9 μg to about 15 μg. The dose of IFN-α is generally administered daily, every other day, three times a week, or substantially continuously. The dose of IFN-α is administered for a period of time, which period can be, for example, from at least about 24 weeks to at least about 48 weeks, or longer.

In some embodiments, where a single dosing regimen of INF-α is administered, a "priming" dose of IFN-γ is included. In these embodiments, IFN-γ is administered for a period of time from about 1 day to about 14 days, from about 2 days to about 10 days, or from about 3 days to about 7 days, before the beginning of treatment with IFN-α. This period of time is referred to as the "priming" phase. In some of these embodiments, IFN-γ treatment is continued throughout the entire period of treatment with IFN-α. In other embodiments, IFN-γ treatment is discontinued before the end of treatment with IFN-α. In these embodiments, the total time of treatment with IFN-γ (including the "priming" phase) is from about 2 days to about 30 days, from about 4 days to about 25 days, from about 8 days to about 20 days, from about 10 days to about 18 days, or from about 12 days to about 16 days. In still other embodiments, IFN-γ treatment is discontinued once IFN-α treatment begins.

Those of skill in the art will readily appreciate that dose levels can vary as a function of the specific compound, the severity of the symptoms and the susceptibility of the subject to side effects. Preferred dosages for a given compound are readily determinable by those of skill in the art by a variety of means.

Those of skill in the art will readily appreciate that dose levels can vary as a function of the specific compounds, the severity of the symptoms and the susceptibility of the subject to side effects. Preferred dosages for a given compound are readily determinable by those of skill in the art by a variety of means. A preferred means is to measure the physiological potency of a given compound.

In some embodiments, IFN-α and IFN-γ are administered in the same formulation, and are administered simultaneously. In other embodiments, IFN-α and IFN-γ are administered separately, e.g., in separate formulations. In some of these embodiments, IFN-α and IFN-γ are administered separately, and are administered simultaneously. In other embodiments, IFN-α and IFN-γ are administered separately and are administered within

about 5 seconds to about 15 seconds, within about 15 seconds to about 30 seconds, within about 30 seconds to about 60 seconds, within about 1 minute to about 5 minutes, within about 5 minutes to about 15 minutes, within about 15 minutes to about 30 minutes, within about 30 minutes to about 60 minutes, within about 1 hour to about 2 hours, within about 2 hours to about 6 hours, within about 6 hours to about 12 hours, within about 12 hours to about 24 hours, or within about 24 hours to about 48 hours of one another.

Multiple doses of IFN-α and IFN-γ can be administered, e.g., IFN-α and IFN-γ can be administered once per month, twice per month, three times per month, once per week, twice per week, three times per week, four times per week, five times per week, six times per week, or daily, over a period of time ranging from about one day to about one week, from about two weeks to about four weeks, from about one month to about two months, from about two months to about four months, from about four months to about six months, from about six months to about eight months, from about eight months to about 1 year, from about 1 year to about 2 years, or from about 2 years to about 4 years, or more. In particular embodiments of interest, IFN-α and IFN-γ is administered three times per week over a period of about 48 weeks.

Where the agent is a polypeptide, polynucleotide (e.g., a polynucleotide encoding IFN-α or IFN-γ), it may be introduced into tissues or host cells by any number of routes, including viral infection, microinjection, or fusion of vesicles. Jet injection may also be used for intramuscular administration, as described by Furth *et al.* (1992) *Anal. Biochem.* **205**:365-368. The DNA may be coated onto gold microparticles, and delivered intradermally by a particle bombardment device, or "gene gun" as described in the literature (see, for example, Tang *et al.* (1992) *Nature* **356**:152-154), where gold microprojectiles are coated with the therapeutic DNA, then bombarded into skin cells. Of particular interest in these embodiments is use of a liver-specific promoter to drive transcription of an operably linked IFN-α and IFN-γ coding sequences preferentially in liver cells.

Additional therapeutic agents

In some embodiments, the method further includes administration of ribavirin. Ribavirin, 1-β-D-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide, available from ICN Pharmaceuticals, Inc., Costa Mesa, Calif., is described in the Merck Index, compound No. 8199, Eleventh Edition. Its manufacture and formulation is described in U.S. Pat. No. 4,211,771. The invention also contemplates use of derivatives of ribavirin (see, *e.g.*, U.S. Pat. No. 6,277,830). The ribavirin may be administered orally in capsule or tablet form, or in the same or different administration form and in the same or different route as the IFN-α.

Of course, other types of administration of both medicaments, as they become available are contemplated, such as by nasal spray, transdermally, intravenously, by suppository, by sustained release dosage form, etc. Any form of administration will work so long as the proper dosages are delivered without destroying the active ingredient.

Ribavirin is generally administered in an amount ranging from about 30 mg to about 60 mg, from about 60 mg to about 125 mg, from about 125 mg to about 200 mg, from about 200 mg to about 300 gm, from about 300 mg to about 400 mg, from about 400 mg to about 1200 mg, from about 600 mg to about 1000 mg, or from about 700 to about 900 mg per day.

In some embodiments, ribavirin is administered throughout the entire course of IFN-α treatment. In other embodiments, ribavirin is administered less than the entire course of IFN-α treatment, e.g., only during the first phase of IFN-α treatment, only during the second phase of IFN-α treatment, or some other portion of the IFN-α treatment regimen.

DISORDERS AMENABLE TO TREATMENT

The present invention provides methods of treating HCV infection, and methods of treating liver fibrosis, by administering a combination of IFN-α and IFN-γ in a therapeutically effective amount to an individual in need thereof. Individuals who are to be treated according to the methods of the invention include individuals who have been clinically diagnosed with liver fibrosis, individuals diagnosed with HCV, as well as individuals who have not yet developed clinical liver fibrosis but who are considered at risk of developing liver fibrosis. Such individuals include individuals who are infected with HCV.

Individuals who have been clinically diagnosed as infected with HCV are of particular interest in many embodiments. Individuals who are infected with HCV are identified as having HCV RNA in their blood, and/or having anti-HCV antibody in their serum. Individuals who are clinically diagnosed as infected with HCV include naïve individuals (e.g., individuals not previously treated for HCV) and individuals who have failed prior treatment for HCV ("treatment failure" patients). Treatment failure patients include non-responders (e.g., individuals in whom the HCV titer was not significantly or sufficiently reduced by a previous treatment for HCV); and relapsers (e.g., individuals who were previously treated for HCV, whose HCV titer decreased, and subsequently increased).

Also of interest are HCV-positive individuals (as described above) who exhibit severe fibrosis or early cirrhosis (non-decompensated, Child's-Pugh class A or less), or more advanced cirrhosis (decompensated, Child's-Pugh class B or C) due to chronic HCV infection and who are viremic despite prior anti-viral treatment with IFN-α-based therapies

or who cannot tolerate IFN-α-based therapies, or who have a contraindication to such therapies. In particular embodiments of interest, HCV-positive individuals with stage 3 or 4 liver fibrosis according to the METAVIR scoring system are suitable for treatment with the methods of the present invention. In other embodiments, individuals suitable for treatment with the methods of the instant invention are patients with decompensated cirrhosis with clinical manifestations, including patients with far-advanced liver cirrhosis, including those awaiting liver transplantation. In still other embodiments, individuals suitable for treatment with the methods of the instant invention include patients with milder degrees of fibrosis including those with early fibrosis (stages 1 and 2 in the METAVIR, Ludwig, and Scheuer scoring systems; or stages 1, 2, or 3 in the Ishak scoring system.).

While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

CLAIMS

What is claimed is:

1. A method of reducing liver fibrosis in an individual, comprising administering IFN-α and IFN-γ in an amount effective to reduce liver fibrosis.

2. A method of treating a hepatitis C virus infection in an individual, comprising administering IFN-α and IFN-γ in an amount effective to achieve a sustained viral response.

3. The method of claim 1, wherein a degree of liver fibrosis is determined by staging, wherein the stage of liver fibrosis, as measured by a standardized scoring system, is reduced by at least one unit.

4. The method according to claim 1 or claim 2, wherein IFN-γ are administered subcutaneously in an amount of from about 25 μg to about 300 μg per dose, wherein IFN-α is administered in an amount of from about 3 μg to about 27 μg, and wherein the IFN-α and IFN-γ are administered simultaneously.

5. The method according to claim 1 or claim 2, wherein IFN-γ are administered subcutaneously in an amount of from about 25 μg to about 300 μg per dose, wherein IFN-α is administered in an amount of from about 3 μg to about 27 μg, and wherein the IFN-α and IFN-γ are administered within about 24 hours of one another.

6. The method according to claim 1 or claim 2, wherein IFN-γ are administered subcutaneously in an amount of from about 25 μg to about 300 μg per dose, wherein IFN-α is administered in an amount of from about 3 μg to about 27 μg, and wherein the IFN-α and IFN-γ is administered in multiple doses.

7. A method of increasing liver function in an individual suffering from liver fibrosis, comprising administering IFN-α and IFN-γ in an amount effective to increase a liver function.

8. The method according to claim 7, wherein the liver function is indicated by measuring a parameter selected from the group consisting of serum transaminase level,

prothrombin time, serum bilirubin level, blood platelet count, viral load and serum albumin level.

9. The method according to claim 7, wherein IFN-γ are administered subcutaneously in an amount of from about 25 μg to about 300 μg per dose, wherein IFN-α is administered in an amount of from about 3 μg to about 27 μg, and wherein IFN-α and IFN-γ are administered in serial doses.

10. A method of reducing the incidence of a complication of cirrhosis of the liver, comprising administering to an individual suffering from liver fibrosis a combination of IFN-α and IFN-γ in an amount effective to reduce the incidence of a complication of cirrhosis of the liver.

11. The method according to claim 10, wherein IFN-γ are administered subcutaneously in an amount of from about 25 μg to about 300 μg per dose, wherein IFN-α is administered in an amount of from about 3 μg to about 27 μg, and wherein IFN-α and IFN-γ are administered in multiple doses.

12. A method of treating hepatitis C virus in an individual, comprising administering a therapeutically effective amount of IFN-α and IFN-γ.

13. The method of claim 12, wherein the level of alanine aminotransferase is reduced to below about 45 international units per milliliter serum.

14. The method of claim 12, wherein the viral load of the individual is reduced to below about 500 genome copies per milliliter serum.

15. The method according to claim 12, wherein IFN-γ are administered subcutaneously in an amount of from about 25 μg to about 300 μg per dose, wherein IFN-α is administered in an amount of from about 3 μg to about 27 μg, and wherein IFN-α and IFN-γ are administered simultaneously.

16. The method according to claim 12, wherein IFN-γ are administered subcutaneously in an amount of from about 25 μg to about 300 μg per dose, wherein IFN-α

is administered in an amount of from about 3 μg to about 27 μg, and wherein IFN-α and IFN-γ are administered separately.

17. The method according to claim 12, wherein IFN-γ are administered subcutaneously in an amount of from about 25 μg to about 300 μg per dose, wherein IFN-α is administered in an amount of from about 3 μg to about 27 μg, and wherein IFN-α and IFN-γ are administered in serial doses.

18. A method of decreasing viral load in an individual suffering from hepatitis C virus, comprising administering IFN-α and IFN-γ in an amount effective to decrease viral load.