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(54) Title: METHODS OF TREATING LIVER FIBROSIS

(57) Abstract: The present invention provides methods of reducing liver fibrosis; methods of increasing liver function in an individual suffering from liver fibrosis; and methods of reducing the incidence of complications associated with cirrhosis of the liver. The methods generally involve administering a therapeutically effective amount of IFN-γ.

# METHODS OF TREATING LIVER FIBROSIS

## FIELD OF THE INVENTION

This invention is in the field of therapy of liver fibrosis.

## BACKGROUND OF THE INVENTION

Fibrosis of the liver occurs due to a chronic toxic insult to the liver such as hepatitis C virus (HCV) or hepatitis B virus (HBV) 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.

Over the last decade, significant progress has been made in dissecting the cellular and molecular mechanisms involved in hepatic fibrogenesis. The constituents of the hepatic scar are similar whether the injury is viral, toxic, immune or metabolic. There is an overall increase in extracellular matrix, which includes collagens, proteoglycans, and glycoproteins such as fibronectin, laminin, and others. Cytokines play major roles in all stages in the development of fibrosis, including hepatocyte injury, inflammatory response, altered function of sinusoidal cells (particularly hepatic stellate cells), extracellular matrix accumulation, and matrix degradation.

The current concept is that fibrosis is not a static process; extracellular matrix is constantly being laid down and resorbed and the progressive accumulation of fibrous tissue is thought to represent a relative imbalance between pro-fibrotic processes and anti-fibrotic processes. The central cell involved in the pathogenesis of hepatic fibrosis is the hepatic stellate cell (HSC), also known as lipocytes, fat-storing cells, Ito cells, or myofibroblasts (Li and Friedman 1999). These cells are the primary source of extracellular matrix production during liver injury. HSCs can convert from a resting vitamin A-rich perisinusoidal cell to one that is proliferative, fibrogenic, and contractile. HSCs are thought to have counterparts in other organs that demonstrate a fibrogenic response to chronic injury, such as fibroblasts found in the kidney and lungs. During fibrogenesis, HSC undergo a process of activation

by acquiring a myofibroblast-like phenotype characterized by increased proliferation and extracellular matrix component synthesis. The process of HSC activation is the result of a complex interplay in which different cell types, oxidative stress, and growth factors play important roles. Cytokines play an especially important role in perpetuating and modulating the effects of activated HSCs.

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.

HCV infection is the most common chronic blood borne infection in the United States. Although the numbers of new infections has declined, the burden of chronic infection is substantial, with CDC 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.

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 methods of reducing liver fibrosis. 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.

## SUMMARY OF THE INVENTION

The present invention provides methods of reducing liver fibrosis; methods of increasing liver function in an individual suffering from liver fibrosis; and methods of reducing the incidence of complications associated with cirrhosis of the liver. The methods generally involve administering a therapeutically effective amount of IFN-γ.

In one aspect, the invention features use of IFN-γ in the manufacture of a medicament for reducing liver fibrosis in an individual.

In another aspect, the invention features use of IFN-γ in the manufacture of a medicament for increasing liver function in an individual suffering from liver fibrosis.

In another aspect, the invention features use of IFN-γ in the manufacture of a medicament for reducing the incidence of a complication of cirrhosis of the liver, in an individual suffering from liver fibrosis.

## FEATURES OF THE INVENTION

In some embodiments, the invention features a method of reducing liver fibrosis in an individual, generally involving administering IFN-γ in an amount effective to reduce liver fibrosis. Liver fibrosis may be due to any condition that is known to result in cirrhosis or fibrosis, e.g., a condition selected from the group consisting of chronic alcohol exposure, hepatitis B virus infection, non-alcoholic steatohepatitis, hepatitis C virus infection, Wilson's disease, alpha-1-antitrypsin deficiency, hemochromatosis, primary biliary cirrhosis, primary sclerosing cholangitis, and autoimmune hepatitis. In many 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, comprising administering 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, generally involving administering to an individual suffering from liver fibrosis 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, in many embodiments, IFN-γ is administered subcutaneously in an amount of from about 25 μg to about 300 μg per dose, and IFN-γ is administered in multiple doses. In many embodiments, IFN-γ is administered for a period of at least three months, and may be administered over longer periods of time.

## DEFINITIONS

As used herein, the term "hepatic fibrosis," used interchangeably herein with "liver fibrosis," refers to the growth of scar tissue in the liver due to any of a variety of chronic toxic insults, including, but not limited to, chronic alcohol abuse; chronic exposure to drugs, including, but not limited to acetominophen, amiodarone, aspirin, azathioprine, isoniazid, methyldopa, methotrexate, mitrfurantoin, propylthiouracil, and sulfonamides; chronic exposure to certain chemical agents, including, but not limited to, carbon tetrachloride, dimethyl nitrosamine, vinyl chloride, polychlorinated biphenyls, aflatoxins, and pesticides; infection with *Schistosoma mansoni*; diabetes; autoimmune disorders, including, but not limited to, primary sclerosing cholangitis, primary biliary cirrhosis, autoimmune hepatitis, lupoid hepatitis, and inflammatory bowel disease; hemochromatosis; alpha-1-antitrysin deficiency; chronic cholestatic hepatitis; non-alcoholic steatohepatitis; chronic biliary obstruction; Wilson's disease; and other conditions known to cause cirrhosis.

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 "dosing event" as used herein refers to administration of an antiviral agent to a patient in need thereof, which event may encompass one or more releases of an antiviral agent from a drug-dispensing device. Thus, the term "dosing event," as used herein, includes, but is not limited to, installation of a depot comprising an antiviral agent; installation of a continuous delivery device (e.g., a pump or other controlled release injectible system); and a single subcutaneous injection followed by installation of a continuous delivery system.

The term "depot" refers to any of a number of implantable, biodegradable or non-biodegradable, controlled release systems that are generally non-containerized and that act as a reservoir for a drug, and from which drug is released. Depots include polymeric non-polymeric biodegradable materials, and may be solid, semi-solid, or liquid in form.

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," used interchangeably herein, 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 "an IFN-γ 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 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-γ to an individual in need thereof. 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 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-γ. As used herein, a "therapeutically effective amount" of IFN-γ is an

amount of IFN-γ that is effective 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.

Whether treatment with 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. Whether liver fibrosis is reduced 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-γ is an amount of 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-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-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-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-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-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-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-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-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 range of alanine transaminase is from about 7 to about 56 units per liter 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-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 IFNγ 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 IFNγ 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-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 compositions of the present invention may be any of natural IFN-γs, recombinant IFN-γs and the derivatives thereof so far as they have a 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 which, 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 carboxy terminal end 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, herein incorporated by reference. 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-γ is 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.

In the subject methods, the active agent(s) may be administered to the host using any convenient means capable of resulting in the desired therapeutic effect. Thus, the agent 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., administration.

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.

Effective dosages of IFN-γ can 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.

Those of skill 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. A preferred means is to measure the physiological potency of a given compound.

In specific embodiments of interest, IFN-γ is administered to an individual in a unit dosage form of from about 25 μg to about 500 μg, from about 50 μg to about 400 μg, or from about 100 μg to about 300 μg. In particular embodiments of interest, the dose is about 200 μg IFN-γ. In many embodiments of interest, IFN-γ1b is administered.

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.

Where the agent is a polypeptide, polynucleotide (e.g., a polynucleotide encoding 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-γ coding sequence preferentially in liver cells.

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.

In particular embodiments of interest, IFN-γ is administered as a solution suitable for subcutaneous injection. For example, IFN-γ is in a formulation containing 40 mg mannitol/mL, 0.72 mg sodium succinate/mL, 0.10 mg polysorbate 20/mL. In particular embodiments of interest, IFN-γ is administered in single-dose forms of 200 μg/dose subcutaneously.

Multiple doses of IFN-γ can be administered. Where multiple doses of INF-γ are administered, the frequency of administration is 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.

Where multiple doses of IFN-γ are administered, the multiple doses are administered 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-γ is administered three times per week over a period of about 48 weeks.

In some embodiments, IFN-γ is administered by continuous infusion, or with a device or system that provides for sustained release or controlled release. In these

embodiments, IFN-γ is administered 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.

Drug delivery devices that are suitable for use in the subject methods include, but are not limited to, injection devices; an implantable device, e.g., pumps, such as an osmotic pump, that may or may not be connected to a catheter; biodegradable implants; liposomes; depots; and microspheres. Any known delivery system can be used in the present invention. In addition, a combination of any known delivery system can be used.

The drug delivery system can be any device, including an implantable device, which device can be based on, for example, mechanical infusion pumps, electromechanical infusion pumps, depots, microspheres. Essentially, any drug delivery system that provides for controlled release as described above (at least biphasic release) is suitable for use in the instant invention. In some embodiments, the drug delivery system is a depot. In other embodiments, the drug delivery system is a continuous delivery device (e.g., an injectable system, a pump, etc.). In still other embodiments, the drug delivery system is a combination of a injection device (e.g., a syringe and needle) and a continuous delivery system. The term "continuous delivery system" is used interchangeably herein with "controlled delivery system" and encompasses continuous (e.g., controlled) delivery devices (e.g., pumps) in combination with catheters, injection devices, and the like, a wide variety of which are known in the art, including, but not limited to, injection devices; an implantable device, e.g., pumps, such as an osmotic pump, that may or may not be connected to a catheter, biodegradable implants; liposomes; depots; and microspheres.

In some embodiments, the drug delivery system is a pump, e.g., an implantable pump, particularly an adjustable implantable pump. Of particular interest is the use of an adjustable pump, particularly a pump that is adjustable while in position for delivery (e.g., externally adjustable from outside the patient's body. Such pumps include programmable pumps that are capable of providing high concentrations of IFN-α or other antiviral agent over extended periods of time, e.g., 24-72 hours, and to achieve AUC serum IFN-γ concentrations to be therapeutically effective.

Mechanical or electromechanical infusion pumps can also be suitable for use with the present invention. Examples of such devices include those described in, for example, U.S. Pat. Nos. 4,692,147; 4,360,019; 4,487,603; 4,360,019; 4,725,852, and the like. In general,

the present methods of drug delivery can be accomplished using any of a variety of refillable, pump systems. Pumps provide consistent, controlled release over time.

In one embodiment, the drug delivery system is an at least partially implantable device. The implantable device can be implanted at any suitable implantation site using methods and devices well known in the art. An implantation site is a site within the body of a subject at which a drug delivery device is introduced and positioned. Implantation sites include, but are not necessarily limited to a subdermal, subcutaneous, intramuscular, or other suitable site within a subject's body. Subcutaneous implantation sites are generally preferred because of convenience in implantation and removal of the drug delivery device.

DISORDERS AMENABLE TO TREATMENT

The present invention provides methods of treating liver fibrosis by administering 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, 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, but are not limited to, individuals who are infected with HCV; individuals who are infected with HBV; individuals who are infected with *Schistosoma mansoni*; individuals who have been exposed to chemical agents known to result in liver fibrosis; individuals who have been diagnosed with Wilson's disease; individuals diagnosed with hemochromatosis; and individuals with alcoholic liver disease; individuals with non-alcoholic steatohepatitis; individuals with autoimmune hepatitis; individuals with primary sclerosing cholangitis, primary biliary cirrhosis, or alpha-1-antitrysin deficiency.

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. In many embodiments, individuals of interest include those 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. Use of IFN-γ in the manufacture of a medicament for reducing liver fibrosis in an individual.

2. The use according to claim 1, wherein the individual has a condition selected from the group consisting of chronic alcohol exposure, hepatitis B virus infection, non-alcoholic steatohepatitis, hepatitis C virus infection, Wilson's disease, alpha-1-antitrypsin deficiency, hemochromatosis, primary biliary cirrhosis, primary sclerosing cholangitis, and autoimmune hepatitis.

3. The use of claim 1, wherein liver fibrosis is reduced in severity, as measured by a standard scoring system.

4. Use of IFN-γ in the manufacture of a medicament for increasing liver function in an individual suffering from liver fibrosis.

5. The use of claim 4, wherein the liver function is determined 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.

6. Use of IFN-γ in the manufacture of a medicament for reducing the incidence of a complication of cirrhosis of the liver, in an individual suffering from liver fibrosis.

7. The use of claim 6, wherein the complication of cirrhosis of the liver is selected from the group consisting of portal hypertension, progressive liver insufficiency, and hepatocellular carcinoma.

8. The use of any one of claims 1-7, wherein IFN-γ is for subcutaneous administration in an amount of from about 25 μg to about 300 μg per dose.

9. The use of any one of claims 1-7, wherein IFN-γ is for administration in an amount of about 200 μg per dose.

10. The use of any one of claims 1-7, wherein IFN-γ is for administration for a period of at least about three months.

11. The use of any one of claims 1-7, wherein the IFN-γ is IFN-γ1b.

12. The use of any one of claims 1-7, wherein the IFN-γ is for subcutaneous administration.

13. The use of any one of claims 1-7, wherein IFN-γ is for administration in multiple doses.

14. The use of any one of claims 1-7, wherein IFN-γ is for administration at least twice per month.

15. The use of any one of claims 1-7, wherein the dosage regimen is once per week.

16. The use of any one of claims 1-7, wherein the dosage regimen is twice per week.

17. The use of any one of claims 1-7, wherein the dosage regimen is three times per week.

18. The use of any one of claims 1-7, wherein IFN-γ is for administration for a period of at least about one year.

19. The use of any one of claims 1-7, wherein the dosage regimen is once per week for at least about one year.

20. The use of any one of claims 1-7, wherein the dosage regimen is three times per week for at least about one year.