

Antifibrotics for Chronic Hepatitis C

Paul J. Pockros, MD*

KEYWORDS

- Hepatitis C virus • Interferon-gamma 1b • Antifibrotics
- Caspase inhibitors • Long-term pegylated interferons

Fibrosis is currently viewed as a dynamic rather than a static process; extracellular matrix is constantly being laid down and resorbed, and the progressive accumulation of fibrous tissue is believed to represent a relative imbalance between profibrotic and antifibrotic processes.¹ The central cells involved in the pathogenesis of hepatic fibrosis are hepatic stellate cells (HSCs), also known as lipocytes, fat-storing cells, Ito cells, or myofibroblasts.² Cytokines play major roles at all stages in the development of fibrosis, including hepatocyte injury, inflammatory response, altered function of sinusoidal cells (particularly HSCs), extracellular matrix accumulation, and matrix degradation. Cytokines play an especially important role in perpetuating and modulating the effects of activated HSCs. In experimental models of fibrosis, transforming growth factor- β (TGF- β) has been shown to play a key role in stimulating and maintaining the fibrogenic process. In the liver, TGF- β stimulates the expression of extracellular matrix proteins and collagen, inhibits collagenases, and promotes the activation of fat-storing HSCs toward a myofibroblast phenotype.³ In addition, inhibition of TGF- β is effective in preventing fibrosis and preserving organ function.¹

Antifibrotic agents developed or tested for treatment of chronic hepatitis C generally are targeted toward HSCs, TGF- β , the inflammatory response, or extracellular matrix accumulation. Although several agents have been or are being currently studied in this indication, to date none have proved to be effective. There is a clear need for drugs which inhibit or reverse hepatic fibrosis, as these would be immediately applicable to patients who have failed antiviral therapy or have contraindications to antiviral therapy such as those with decompensated liver disease or renal failure. These drugs would be important adjuncts in patients in whom rapid development of fibrosis threatens liver function before antiviral therapies may be effective, such as those who are coinfecting with hepatitis C virus (HCV) and HIV or those who have cholestatic fibrosing hepatitis due to recurrent HCV post-transplantation. A major impediment in the development of new drugs in this field has been the inability to identify reasonable

* Division of Gastroenterology and Hepatology, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037, USA.

E-mail address: pockros.paul@scrippshealth.org

histologic or clinical end points within a reasonable period of study. This factor has contributed to the failure of several the compounds discussed herein. **Box 1** lists several key agents studied or being studied as antifibrotics for HCV. There are only limited published data available for most of these compounds so only those best studied to date are discussed in this article.

INTERFERON-GAMMA

Interferon-gamma (IFN- γ) has been shown to profoundly affect the fibrotic process. IFN- γ has been found to be a key counterregulatory antifibrotic cytokine balancing

Box 1 Experimental antifibrotic agents
5-Lipoxygenase-activating protein inhibitors
Interferon- γ , - α
AA861 mycophenolate
Angiotensin-converting enzyme inhibitors
Octreotide
Anti- α 1 integrin
Pentoxifylline
Arginine-glycine-aspartate peptides
Caspase inhibitors
Peroxisome proliferator-activated receptor ligands
Estradiol
Prostaglandins, prostaglandin E2
Endothelin A receptor antagonists
Quercetin
Farnesoid X receptor agonists
Rapamycin
Glycyrrhizin/ <i>Salvia miltiorrhiza</i>
Retinoic acid
Halofuginone Sho-saiko-to (TJ-9)
Hepatocyte growth factor
Soluble platelet-derived growth factor antagonists
High-dose antioxidants (silymarin)
Soluble type II fusion molecule
HOE 77a
Transforming growth factor β -receptor blockade
Interleukin-1 antagonists
<i>Data from</i> McHutchison JG, Poynard T, Afdhal N, et al. Fibrosis as an end point for clinical trials in liver disease: a consensus report of the International Fibrosis Group. Clin Gastroenterol Hepatol 2006;4(10):1215.

the activity of TGF- β .⁴ IFN- γ inhibits the interaction of downstream proteins that are normally activated following the binding of TGF- β to its cellular receptor. As a result, transcriptional responses to TGF- β signaling are inhibited. The degree of inhibition is dependent in part on the relative amounts of TGF- β and IFN- γ , indicating that the extent of inhibition (or activation) of TGF- β responsive genes may be determined by the balance of TGF- β and IFN- γ signals. This provides a theoretical rationale for the use of pharmacologic doses of IFN- γ even in those settings in which there are elevated levels of intrinsic IFN- γ and TGF- β .⁵

Experimental data from in vitro studies, studies in animal models of liver fibrosis, and studies in humans with idiopathic pulmonary fibrosis (IPF) all support a potential therapeutic role for IFN- γ 1b in the inhibition of fibrosis in the liver and other organs.¹⁻⁷ Studies of the in vitro effects of IFN- γ on cultured murine and human HSC consistently demonstrate that IFN- γ inhibits the proliferation and culture-induced activation of these cells. These studies also demonstrate that IFN- γ inhibits the expression of mRNA encoding extracellular matrix proteins, leading to a significant reduction in the production of extracellular matrix proteins. Several studies have been published on the antifibrotic activity of IFN- γ in animal models of liver fibrosis. These studies consistently demonstrate that IFN- γ administered during the period of hepatic injury is capable of reducing the quantity of extracellular matrix and of reducing the degree of histologically evident fibrosis present in the liver.

Two studies have provided data relevant to assessing the potential antifibrotic effects of IFN- γ 1b in humans. The first was a randomized controlled study on the effects of IFN in IPF, a progressive fibrosing disease of unknown cause with a mean survival time of 2 to 4 years. Based on the antifibrotic effects of IFN- γ demonstrated in in vitro and in vivo animal models, Ziesche and colleagues⁶ conducted an open-label, randomized, controlled trial comparing the safety and efficacy of IFN- γ 1b plus low-dose prednisolone ($n = 9$) with that of prednisolone alone ($n = 9$) in patients with IPF confirmed by biopsy or by high-resolution computed tomography (HRCT). Over the course of the study, all patients treated with IFN- γ 1b showed improvement in pulmonary function. In contrast, patients treated with prednisolone alone showed deterioration in their condition. The differences between the 2 groups at 12 months were statistically significant for total lung capacity (TLC) ($P < .001$ for difference between groups) and for PaO₂ at rest and on maximal exertion (both $P < .001$ for difference in change from baseline).

The second study was a small pilot study comparing IFN- γ 1b with IFN- α in patients with chronic hepatitis C in which paired biopsies were obtained in a subset of patients. Saez-Royuela and colleagues⁷ conducted a randomized controlled pilot study of IFN- α 2c and IFN- γ 1b in 30 adults with chronic hepatitis C with persistently elevated serum alanine aminotransferase (ALT) levels. There was a trend toward a decrease in the fibrosis score in IFN- γ 1b recipients with a reduction in fibrosis from 1.2 ± 1.0 to 0.7 ± 1.0 on the Knodell fibrosis score. Combined with the findings from the trial in IPF, these studies provided a rationale to study hepatic fibrosis in a larger group of patients with HCV.

A large, multicenter, randomized controlled trial (AEGIS) using hepatic fibrosis as the primary end point in patients with advanced fibrosis or cirrhosis due to HCV enrolled a total of 502 patients with compensated liver disease and an Ishak fibrosis score of 4 to 6 and 488 of these patients received subcutaneous injections of IFN- γ 1b 100 μ g (group 1, $n = 169$), IFN- γ 1b 200 μ g (group 2, $n = 157$), or placebo (group 3, $n = 162$) 3 times a week for 48 weeks.⁸ Most patients (83.6%) had cirrhosis at baseline (Ishak score 5 or 6). Post-treatment liver biopsies were assessed in a blinded fashion for a reduction of 1 or more Ishak points (primary end point). Four hundred and twenty

patients with pretreatment and post-treatment liver biopsies were evaluable and showed no improvement in Ishak score between the 3 treatment groups (12.1%, 12.4%, and 16% of patients in groups 1, 2, and 3, respectively; $P > .05$). There were similar numbers of deaths in all 3 arms (5, 5, and 4, respectively), and most were related to complications of cirrhosis. The investigators concluded that IFN- γ 1b therapy was not able to reverse fibrosis in patients with advanced liver disease for 1 year, and that perhaps less advanced disease should be considered for future studies with IFN- γ 1b or other antifibrotic agents.

Two smaller studies using IFN- γ for HCV also failed to show any benefit in fibrosis progression or a sustained antiviral effect, suggesting that this compound will not likely be developed any further for the indication of HCV.^{9,10} IFN- γ had been studied earlier for chronic hepatitis B (HBV) and failed to show antiviral efficacy.^{11,12} A subsequent trial in HBV showed a mild antiviral effect and antifibrotic benefit, although the study was not powered to make definitive conclusions.¹³ Further development of IFN- γ for HBV or HCV is not currently underway.

Fibrosis progression in chronic liver disease has usually been evaluated by liver biopsy using insensitive semiquantitative numerical scores. An alternative to this is to measure fibrous tissue quantitatively using morphometric image analysis. Morphometry was used to quantify the amount of fibrous tissue in liver biopsies performed at baseline and after 48 weeks in 245 patients in the large IFN- γ 1b trial who had paired unfragmented, adequate-sized specimens.¹⁴ Because no effect of the drug on fibrosis was found in the trial, data from all 245 patients could be combined for analysis. At baseline, 78% had cirrhosis and 22% had bridging fibrosis. The mean morphometrically determined collagen content increased by 58% between baseline and 48 weeks. There were statistically significant but weak correlations of fibrosis with platelet count, albumin, bilirubin, international normalized ratio (INR), and hyaluronic acid, but changes in these did not correlate with or predict changes in fibrosis in the liver biopsy. The investigators concluded that in advanced chronic hepatitis C, fibrosis increases at a rapid pace, which can only be detected by morphometry (**Fig. 1**). A subsequent consensus report suggested that this technique be used in future therapeutic trials of agents to inhibit fibrosis progression.¹⁵

LONG-TERM PEGYLATED INTERFERON STUDIES ON LIVER FIBROSIS

A sensible approach to slow or reverse hepatic fibrosis in HCV would be to attempt to suppress viral replication and intrahepatic inflammation. The ideal study would use clinical end points such as death, transplantation, variceal hemorrhage, or hepatocellular carcinoma. Measurement of these end points requires large sample sizes and long follow-up periods, caveats that limit these studies to federal funding or major industry commitments. Three long-term studies of liver fibrosis in patients with advanced HCV using long-term IFN therapy have been completed and their data have been presented or published (**Table 1**). In 2008, Di Bisceglie published the long-awaited but disappointing results of the long-term Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) trial for nonresponder or relapse patients with advanced fibrosis or cirrhosis.¹⁶ In that study, 1050 patients were followed for 3.5 years on 90 μ g pegylated interferon (PegIFN)- α 2a per week or no therapy after a run-in phase during which patients were treated for 20 weeks and determined to be HCV RNA positive. At the end of follow-up, equal numbers of patients had died, decompensated, or developed hepatocellular carcinoma (HCC). The increase in fibrosis measured by liver biopsy before and after treatment was identical in both

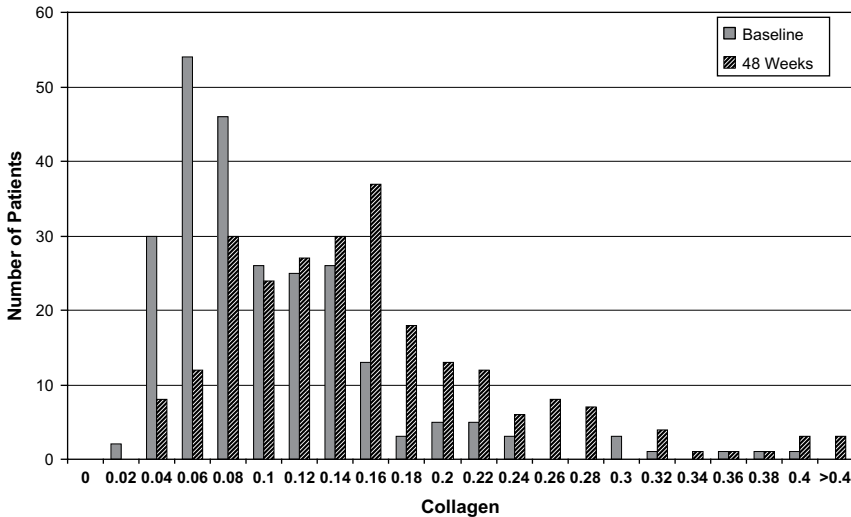


Fig. 1. Liver biopsy collagen content at baseline and after 48 weeks in 245 patients with advanced chronic hepatitis C. (From Goodman ZD, Becker RL, Pockros PJ, et al. Progression of fibrosis in advanced chronic hepatitis C: evaluation by morphometric image analysis. *Hepatology* 2007;45(4):890; with permission.)

groups. The author's conclusion was there was no proven benefit to long-term therapy with PegIFN in this population of nonresponder patients.

In the Evaluation of PegIntron in Control of hepatitis C cirrhosis (EPIC-3) study, patients with Metavir stage 2 to 4 fibrosis were randomized to receive either PegIFN- α 2b at 0.5 μ g/kg/wk or placebo for a 4-year period.¹⁷ The EPIC-3 study is essentially 3 studies in 1: (1) a trial in nonresponders to previous therapy with sustained virologic response (SVR) as the clinical end point for patients with F2-4 fibrosis; (2) a trial to improve liver histology with long-term therapy in patients with F2-3 fibrosis;

	HALT-C ¹⁶	EPIC-3 ¹⁷	COPILOT ¹⁸
Fibrosis stage	Ishak 3-6	Metavir 2-4	Ishak 3-6
n	1000	2200 (3 studies)	600
End point	Fibrosis/clinical	Fibrosis/clinical	Clinical
Arm 1	PegIFN- α 2a	PegIFN- α 2b	PegIFN- α 2b
—	90 μ g	0.5 μ g/kg	0.5 μ g/kg
Arm 2	Observation	Observation	Colchicine
Run-in phase	Yes	Yes	No
Duration (years)	3.5	4	4

Abbreviations: HALT-C, Hepatitis C Antiviral Long-term Treatment against Cirrhosis trial; EPIC-3, Evaluation of PegIntron in Control of hepatitis C cirrhosis study; COPILOT, Colchicine versus PegIntron Long Term trial; IFN, interferon; Peg, pegylated.

Data from McHutchison JG, Poynard T, Afdhal N, et al. Fibrosis as an end point for clinical trials in liver disease: a consensus report of the International Fibrosis Group. *Clin Gastroenterol Hepatol* 2006;4(10):1219.

and (3) a trial to improve clinical end points in cirrhotic patients (nonresponders and previously untreated patients). The final results of the EPIC-3 trial were recently published and no benefit in the treatment arm for the primary endpoints. However, an ad hoc sub analysis demonstrated a significant difference in emergence or enlargement of varices requiring therapy between the treatment arm and the control arm. Because this trial was not blinded and the appearance of varices is a subjective measure, it is unclear how important this effect may be.¹⁸

In the Colchicine versus Peg-Intron Long Term (COPILOT) trial, roughly half the number of patients (555) of the HALT-C trial were randomized to 0.5 µg/kg/wk of PegIFN-α2b versus colchicine 0.6 mg twice a day over 4 years of follow-up.¹⁹ All patients again had advanced fibrosis and had failed prior therapy as in the HALT-C study. The intention to treat (ITT) analysis of outcomes showed there was more HCC in the PegIFN ($P = \text{NS}$) treated arm and more complications of portal hypertension in the colchicine treated arm than the PegIFN arm, especially in the per protocol (PP) analysis ($P = .027$). There was a benefit on event-free survival only in patients with portal hypertension, as had been shown in the earlier PP analysis at year 2.²⁰ The investigators concluded that, "There still could be consideration for Peg-IFN alfa-2b as maintenance therapy in a subset of patients with cirrhosis and portal hypertension who failed eradication therapy. The mechanism of action we believe is mediated by the effects of portal hypertension and this seems to be more profound in the early phases of treatment."

Subsequent data from HALT-C presented by Shiffman and colleagues²¹ were disappointing regarding maintenance therapy and continued HCV viral suppression. Many hoped this subgroup would benefit from long-term therapy. However, Shiffman found that a significant improvement in clinical outcomes was observed in those who achieved a profound decline in HCV RNA in the first 20 weeks of treatment (>4 log or undetectable with subsequent breakthrough or relapse) with full-dose PegIFN and ribavirin whether or not they remained on maintenance therapy. This supports the notion that viral suppression helps but only if it is attained in the first 20 weeks of treatment, again leaving slow responders or nonresponders without a benefit and showing no efficacy of long-term PegIFN. Clearly, better options are needed for these patients than maintenance therapy with PegIFN.

CASPASE INHIBITORS AS ANTIFIBROTICS

Programmed cell death or apoptosis is a tightly controlled process of cellular suicide that occurs during normal development, normal tissue turnover, and in numerous diseases.¹ In the case of inflammatory disease states such as HCV, persistent inflammation may lead to disordered hepatocyte apoptosis, which in turn contributes to the progressive fibrosis.²² Abnormally high levels of apoptotic cells are found in the liver of individuals with HCV infection and leads to stellate cell activation.²³ This in turn leads to stellate cell profibrogenic gene expression and ultimately results in increased hepatic collagen deposition.^{22,23} Although inflammation may cause apoptosis, it is also known that hepatocyte apoptosis promotes inflammation, thus creating a harmful synergy of inflammation and apoptosis and eventually leading to fibrosis.²⁴ One treatment strategy is to reduce the inflammation and resultant fibrosis induced or mediated by hepatocyte apoptosis. Caspases, intracellular aspartate-specific cysteine proteases that function as inducers and effectors of apoptotic cell death, play a critical role in liver injury in chronic HCV infection.^{25,26} Increased levels of apoptotic cells have been observed in the liver of patients with chronic HCV infection²⁶ and the number of apoptotic cells present in liver biopsies has been shown to correlate with

the grade of inflammation.²⁵ Accordingly, novel agents inhibiting caspases²⁵ have been tested in this clinical arena.

IDN-6556 (now known as PF-03491390) is a specific and irreversible caspase inhibitor that shows no inhibition of other classes of proteases or other enzymes or receptors.²⁷ IDN-6556 has been shown to have activity in animal models of liver disease in which apoptosis is believed to contribute to the pathogenesis. For instance, apoptosis has been described in a model of liver fibrosis occurring after ligation of the mouse bile duct. In this model, application of IDN-6556 suppresses apoptosis and inflammation and prevents liver fibrosis.²⁸ For this reason, a human study was performed to explore the effect of IDN-6556 in patients with HCV.

In a multicenter, double-blind, placebo-controlled, dose-ranging study with a 14-day dosing period, 105 patients were enrolled and 79 received active drug.²⁹ Eighty (80) patients had chronic hepatitis C and 25 had other liver diseases including non-alcoholic steatohepatitis (NASH), hepatitis B, primary biliary cirrhosis (PBC), and primary sclerosing cholangitis (PSC). IDN-6556 doses ranged from 5 mg to 400 mg daily given from 1 to 3 times daily. In the HCV patients all doses of IDN-6556 significantly lowered ALT and aspartate aminotransferase (AST) ($P = .0041$ to $P < .0001$ for various dosing groups in Wilcoxon tests comparing IDN-6556 with placebo) with the exception of the lowest dose (Fig. 2). Adverse experiences were not different between those on IDN-6556 and placebo. Mean HCV RNA levels did not show significant changes. Longer studies to assess the potential effects of IDN-6556 on liver inflammation and fibrosis were planned but drug development was halted. An area of concern for any caspase inhibitor studied in HCV is the risk of development of cancer. Because of this concern, patients were excluded from enrollment in this study if they had cirrhosis or elevation of α -fetoprotein levels, and the duration of treatment was limited to a 14 days. However, this risk clearly may cause drug developers to pause before investing large sums of money into caspase inhibitors for this indication. To our knowledge only 1 other compound (Gilead Sciences, Inc, GS-9450) is currently in development for chronic hepatitis C.³⁰

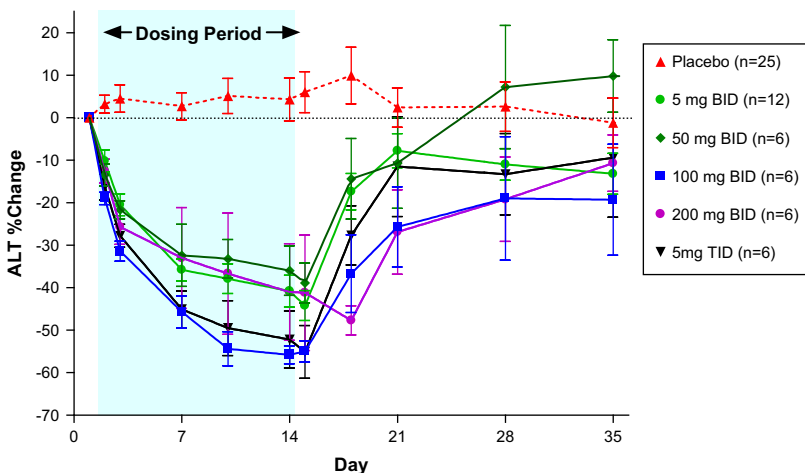


Fig. 2. ALT % change from baseline with IDN-6556, at doses twice and three times per day (means \pm SEM shown). (From Pockros PJ, Schiff ER, Shiffman ML, et al. Oral IDN-6556, an antiapoptotic caspase inhibitor, lowers aminotransferases in patients with chronic hepatitis C. *Hepatology* 2007;46(2):326; with permission.)

SUMMARY

Development and testing of antifibrotic agents for the treatment of chronic hepatitis C have generally been targeted toward HSCs, TGF- β , the inflammatory response, or extracellular matrix accumulation. Although several agents such as IFN- γ , long-term PegIFN and caspase inhibitors have been studied in this indication, none have been proved to be effective to date. There is a clear need for drugs that inhibit or reverse hepatic fibrosis as these would be immediately applicable to patients who have failed antiviral therapy or have contraindications to antiviral therapy such as those with decompensated liver disease or renal failure. These drugs would be important adjuncts for patients in whom rapid development of fibrosis threatens liver function before antiviral therapies may be effective, such as those who are coinfecting with HCV and HIV or those who have cholestatic fibrosing hepatitis due to recurrent HCV post-transplantation. A major impediment in the development of new drugs in this field has been the inability to identify histologic or clinical end points within a reasonable period of study. Progress will need to be made in providing suitable end points to therapy, which should then promote the development of newer agents.

REFERENCES

1. Friedman SL. Evaluation of fibrosis and hepatitis C. *Am J Med* 1999;107(6B): 27S-30S.
2. Nakamura T, Sakata R, Ueno T, et al. Inhibition of transforming growth factor beta prevents progression of liver fibrosis and enhances hepatocyte regeneration in dimethylnitrosamine-treated rats. *Hepatology* 2000;32(2):247-55.
3. Border WA, Noble NA. Transforming growth factor beta in tissue fibrosis. *N Engl J Med* 1994;331(19):1286-92.
4. Ulloa L, Doody J, Massague J. Inhibition of transforming growth factor-beta/SMAD signalling by the interferon-gamma/STAT pathway. *Nature* 1999; 397(6721):710-3.
5. Friedman SL. Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. *J Biol Chem* 2000;275:2247-50.
6. Ziesche R, Hofbauer E, Wittmann K, et al. A preliminary study of long-term treatment with interferon gamma-1b and low-dose prednisolone in patients with idiopathic pulmonary fibrosis. *N Engl J Med* 1999;341(17):1264-9.
7. Saez-Royuela F, Porres JC, Moreno A, et al. High doses of recombinant alpha-interferon or gamma-interferon for chronic hepatitis C: a randomized, controlled trial. *Hepatology* 1991;13(2):327-31.
8. Pockros PJ, Jeffers L, Afdhal N, et al. Final results of a double-blind, placebo-controlled trial of the anti-fibrotic efficacy of interferon-gamma 1b in chronic hepatitis C with advanced fibrosis or cirrhosis. *Hepatology* 2007;45(3):569-78.
9. Muir AJ, Sylvestre PB, Rockey DC. Interferon gamma-1 for the treatment of chronic hepatitis C infection [abstract]. *Gastroenterology* 2003;124(Suppl 1):A 718.
10. Soza A, Heller T, Ghany M, et al. Pilot study of interferon gamma for chronic hepatitis C. *J Hepatol* 2005;43(1):67-71.
11. Di Bisceglie AM, Rustgi AK, Kassianides C, et al. Therapy of chronic hepatitis B with recombinant human alpha and gamma interferon. *Hepatology* 1990;11(2): 266-70.
12. Kakumu S, Ishikawa T, Mizokami M, et al. Treatment with human gamma interferon of chronic hepatitis B: comparative study with alpha interferon. *J Med Virol* 1991;35(1):32-7.

13. Weng HL, Wang BE, Jia JD, et al. Effect of interferon gamma on hepatic fibrosis in chronic hepatitis B virus infection: a randomized controlled study. *Clin Gastroenterol Hepatol* 2005;3(8):819–28.
14. Goodman ZD, Becker RL, Pockros PJ, et al. Progression of fibrosis in advanced chronic hepatitis C: evaluation by morphometric image analysis. *Hepatology* 2007;45(4):886–94.
15. McHutchison JG, Poynard T, Afdhal N, et al. Fibrosis as an end point for clinical trials in liver disease: a consensus report of the International Fibrosis Group. *Clin Gastroenterol Hepatol* 2006;4(10):1214–20.
16. Di Bisceglie AM, Shiffman ML, Everson GT, et al. Prolonged therapy of advanced chronic hepatitis C with low-dose peginterferon. *N Engl J Med* 2008;359(23):2429–41.
17. Poynard T, Schiff E, Terg R, et al. High early viral response (EVR) with PEG-Intron/Rebetol (PR) weight based dosing (WBD) in previous interferon/ribavirin HCV treatment failures; early results of the EPIC3 trial [abstract]. *Hepatology* 2004;40(Suppl 1):238A.
18. Poynard T, Colombo M, Bruix J, et al. Peginterferon alfa-2b and ribavirin: effective in patients with hepatitis C who failed interferon alfa/ribavirin therapy. *Gastroenterology* 2009;136(5):1618–28.
19. Afdhal NH, Levine R, Brown R Jr, et al. Colchicine versus peg-interferon alfa 2B long term therapy: results of the 4 year copilot trial [abstract]. *J Hepatol* 2008;48(Suppl 2):S4.
20. Afdhal N, Frelich B, Levine R, et al. Colchicine versus PEG-Intron long term (COPILOT) trial: interim analysis of clinical outcomes at year 2 [abstract]. *Hepatology* 2004;40:239A.
21. Shiffman ML, Morishima C, Lindsay KL, et al. Suppression of serum HCV RNA levels during maintenance peginterferon (PEG-IFN) alfa-2a therapy and clinical outcomes in the HALT-C trial [abstract]. *J Hepatol* 2008;48(Suppl 2):S62.
22. Guicciardi ME, Gores GJ. Apoptosis: a mechanism of acute and chronic liver injury. *Gut* 2005;54(7):1024–33.
23. Bataller R, Brenner DA. Liver fibrosis. *J Clin Invest* 2005;115(2):209–18.
24. Canbay A, Friedman S, Gores GJ. Apoptosis: the nexus of liver injury and fibrosis. *Hepatology* 2004;39(2):273–8.
25. Bantel H, Luger A, Poremba C, et al. Caspase activation correlates with the degree of inflammatory liver injury in chronic hepatitis C virus infection. *Hepatology* 2001;34(4 Pt 1):758–67.
26. Pianko S, Patella S, Ostapowicz G, et al. Fas-mediated hepatocyte apoptosis is increased by hepatitis C virus infection and alcohol consumption, and may be associated with hepatic fibrosis: mechanisms of liver cell injury in chronic hepatitis C virus infection. *J Viral Hepat* 2001;8(6):406–13.
27. Hoglen NC, Chen LS, Fisher CD, et al. Characterization of IDN-6556 (3-[2-(2-tert-butyl-phenylaminoxy)amino]-propionylamino]-4-oxo-5-(2,3,5,6-tetrafluorophenoxy)-pentanoic acid): a liver-targeted caspase inhibitor. *J Pharmacol Exp Ther* 2004;309(2):634–40.
28. Canbay A, Feldstein A, Baskin-Bey E, et al. The caspase inhibitor IDN-6556 attenuates hepatic injury and fibrosis in the bile duct ligated mouse. *J Pharmacol Exp Ther* 2004;308(3):1191–6.
29. Pockros PJ, Schiff ER, Shiffman ML, et al. Oral IDN-6556, an antiapoptotic caspase inhibitor, lowers aminotransferases in patients with chronic hepatitis C. *Hepatology* 2007;46(2):324–9.
30. Available at: ClinicalTrials.gov. Identifier: NCT00725803. Accessed March 6, 2009.