Peer reviewed article

Interferon therapy of hepatitis C: Molecular insights into success and failure

Magdalena Sarasin-Filipowicz

Department of Biomedicine, and Department of Gastroenterology and Hepatology, University Hospital Basel, Switzerland

Summary

20 years have passed since the discovery of the hepatitis C virus (HCV), and yet therapeutic options remain limited. Current standard treatment of chronic hepatitis C (CHC) consists of pegylated interferon alpha (pegIFNα) and ribavirin, and leads to a sustained virological response in approximately half of treated patients. Understanding non-responsiveness to pegIFNα, by analysing the molecular mechanisms underlying treatment failure, is important for future therapeutic improvements. In the following review the current status of knowledge on the crosstalk between HCV and IFNs, as well as on the molecular events occurring in liver tissue of HCV-infected patients in response to pegIFNα, is discussed. Furthermore, the review focuses on the prospect of developing a prognostic test that might direct treatment to those patients who will benefit from it. The outlook on novel therapeutics, including small molecule inhibitors of HCV proteins and immune modulators, is broadened by a glance at the exciting field of micro-RNAs that are likely to be implicated in viral replication and pathogenesis of CHC, thus representing a new therapeutic target.

Key words: Hepatitis C Virus; IFN-stimulated genes; STAT1 activation; liver biopsy; micro-RNA 122

Natural history and current treatment of HCV infection

Hepatitis C virus (HCV) infection, a major cause of liver cirrhosis and liver cancer, represents an enormous global health problem affecting 130–170 million people worldwide [1]. Since a protective vaccine against HCV does not yet exist and the therapeutic options remain limited, the number of patients presenting with long-term complications from this infection, and therefore also the number of patients in need for liver transplant, is expected to further increase in the next 20 years [2].

HCV was first identified in 1989 by screening serum from an infected chimpanzee with human serum from a non-A non-B post-transfusion hepatitis patient [3]. Before the development of an assay for HCV antibody detection as a screening measure [4], HCV was mainly transmitted through transfusions of blood products. Parenteral infec-

Abbreviations

CHC	chronic hepatitis C
EoTR	end of treatment response
(c)EVR	(complete) early virological response
FU	follow-up
GT	genotype
HCV	hepatitis C virus
(peg)IFNα	(pegylated) interferon alpha
IFNAR	IFNα receptor
IL	interleukin
ISG	interferon-stimulated gene
miR	micro-RNA
non-RR	non-rapid responder
OAS	oligoadenylate synthetase
PBMCs	peripheral blood mononuclear cells
PKR	protein kinase R
PNR	primary non-response
RR	rapid responder
RVR	rapid virological response
SAMe	S-adenosyl-methionine
(p-)STAT	(phosphorylated) signal transducer and activator of transcription
STAT-C	specifically targeted antiviral therapy for HCV
SVR	sustained virological response
TLR	toll-like receptor
USP18	ubiquitin-specific peptidase 18
VL	viral load

No conflict of interest in relation to this article.

tions continue to occur, mainly in risk populations that include injection drug users, haemodialysis patients and persons with high-risk sexual behaviour.

HCV inoculation leads to an acute hepatitis that is rarely diagnosed due to a lack of (specific) symptoms. It is assumed that 20–30% of patients clear the virus spontaneously within 6 months of infection. Progression to chronic hepatitis C (CHC), defined as an infection persisting for more than 6 months with the presence of HCV antibodies and HCV-RNA detectable in the serum, occurs in the remaining 70–80% of infected individuals. These patients are at risk to develop liver cirrhosis within 20–40 years. Once liver cirrhosis is established, the risk to develop hepato-cellular carcinoma is 1–4% per year [5].

Acute hepatitis C, if diagnosed, can be cured in most cases [6, 7], whereas CHC is resistant to treatment in almost half of the patients. Therapy of CHC has evolved from interferon alpha (IFNα) monotherapy to the use of a polyethylene glycol modified form of IFNα, called pegylated IFNα (pegIFNα), together with the nucleoside analogue ribavirin. This combination treatment represents the standard of care since 2001 and results in a sustained virological response (SVR) rate of 46–55% [8–10]. PegIFNα has an extended half-life compared to unmodified IFNα and can be therefore administered only once a week. There are currently two pegIFNα isoforms used for the treatment of CHC: pegIFNα-2a (administered subcutaneously in a dose of 180 µg/week) and pegIFNα-2b (given in a dose of 1.5 µg/kg body weight/week). Ribavirin is given orally twice a day (800–1200 mg/day, depending on body weight).

Treatment duration in CHC depends on the HCV genotype (GT) and the initial response to the antiviral drugs, as determined by measurements of serum viral load (VL) by PCR at baseline and after 4 and 12 weeks of therapy. The current definitions of treatment response are based on the VL (table 1). The goal of the treatment is to achieve an end of treatment response (EoTR) followed by an SVR. In some patients, however, the virus is detectable at the follow-up (FU) visit 6 months after treatment is stopped, and this is classified as relapse.

Knowledge of the HCV GT is important because it helps to predict the outcome of antiviral therapy and influences the choice of the therapeutic regimen [11]. Numerous clinical studies have demonstrated that 50–60% of patients infected with HCV GT 1, 20% of GT 2 or GT 3 patients and 30–40% of GT 4 patients are not cured by current standard treatment [8–10, 12, 13]. Treatment duration is 48 weeks for GTs 1 and 4, and 24 weeks for GTs 2 and 3. The therapy is, however, discontinued after 12 weeks in patients who do not achieve early virological response (EVR). For GTs 2 and 3, a 12-week treatment regimen was shown to be effective in patients who achieved a rapid virological response (RVR) [14]. The reason for these differential responses to treatment is currently unknown. Factors other than GT associated with non-response are high baseline HCV VL (>2×10^6 copies/ml or >800 000 IU/ml), high fibrosis stage in the liver, old age, male gender, African American race, obesity, alcohol intake, insulin resistance, liver steatosis and changes in the host immune response, such as high interleukin-8 (IL-8) and IL-10 serum levels [9, 15–17].

Risks and benefits of treatment have to be evaluated individually because of the typically slow course of natural infection and the frequent occurrence of side effects. Therapy is recommended for patients with persistently elevated transaminase levels (alanine aminotransferase ALAT and aspartate aminotransferase ASAT), documented viral replication (HCV-RNA detectable in the serum), and fibrosis documented by liver histology (Metavir fibrosis stage F ≥2 out of maximal 4). Patients with normal ALAT levels and none or little histological signs of inflammation and fibrosis have an excellent prognosis without therapy [18, 19]. The most frequent side effects of IFNα-based therapy consist of influenza-like symptoms, skin disorders, digestive dysfunction, depression, thyroid dysfunction, thrombocytopenia and neutropenia. The concomitant use of ribavirin can lead to haemolysis and consecutive anemia in 30% of patients. Interestingly, occurrence of anaemia during the early phase of treatment was linked to better treatment outcome in patients with GT 1 [20].

IFNα is crucial for the treatment of HCV infection and will continue to be indispensable also in the era of novel antiviral compounds that are currently evaluated in clinical studies (discussed in the chapter "The future of anti-HCV therapy").

Table 1

Definitions of treatment response.

Rapid virological response (RVR):	Negative HCV-RNA at week 4 of treatment
Early virological response (EVR):	HCV-RNA reduction >2 $\log_{10}$ after 12 weeks
Complete EVR (cEVR):	Negative HCV-RNA at week 12 of treatment
Primary non-response (PNR):	Less than 2 $\log_{10}$ decrease in viral titer after 12 weeks
End of treatment response (EoTR):	No detectable serum HCV-RNA at the end of treatment
End of treatment non-response:	Detectable serum HCV-RNA at the end of treatment
Sustained virological response (SVR):	Undetectable HCV-RNA 6 months after EoTR
Relapse:	Detectable HCV-RNA after having achieved EoTR

Antiviral effects of type I interferons and how HCV counteracts them

The IFN family of cytokines exerts antiviral, immunoregulatory and antiproliferative effects on target cells. 13 subtypes of IFNα and a single IFNβ isoform belong to type I IFNs in humans [21]. Type I IFNs are produced in response to pathogens as part of the innate immune system. RNA viruses such as HCV, are recognised in the infected cell by two pathways, one involving Toll-like receptor 3 (TLR3) and the other involving cytosolic RIG-I like helicases [22–24]. Activation of these pathways results in the induction of type I IFNs. Type II and III IFNs will not be discussed in this review. All type I IFNs bind to the same cell surface receptor, the IFNα receptor (IFNAR) consisting of two chains, and induce intracellular signalling through the Jak-STAT pathway [25] (fig. 1).

IFNs inhibit replication of numerous viruses, and are beneficial in the treatment of multiple sclerosis and malignancies including cutaneous melanoma, hairy cell leukaemia and renal-cell carcinoma [21]. The important role of IFNα in antiviral responses is based on direct antiviral actions through transcriptional activation of hundreds of IFN-stimulated genes (ISGs). Various ISGs with potent antiviral efficacies have been identified to date. Induction of these ISG-encoded proteins and their related pathways can lead to a block in viral transcription, degradation of viral RNA, inhibition of translation or interference with various steps of viral replication [26]. Protein kinase R (PKR), 2'–5' oligoadenylate synthetase (OAS) and Mx proteins are among the best-characterised ISGs. By phosphorylating the α subunit of eukaryotic initiation factor, eIF2α, PKR inhibits translation of both viral and cellular proteins, explaining the antiviral and antiproliferative effect of IFN. OAS activates a pathway leading to the cleavage of viral and cellular RNA by RNase L [27] and Mx proteins interfere with replication of negative-stranded RNA viruses such as influenza [28]. Important immuno-modulatory effects of IFNα are mediated through activation of natural killer cells and cytotoxic T lymphocytes, which recognise infected cells and attempt to clear them [29].

The exact mechanisms by which endogenous or therapeutically applied IFNα exerts its effects against HCV remain poorly understood. Long-standing efforts have been made to identify the IFN-induced proteins that are capable of mounting a response against HCV infection; research towards this aim has been hampered, however, by the lack of HCV cell culture and small animal models. The chimpanzee remains the only suitable animal model for studying HCV infection *in vivo*, which is, however, not widely applicable due to ethical and economical concerns. Efficient HCV replication in cell culture has only been available since 1999, when an HCV subgenomic replicon system in human hepatoma-derived Huh7 cells was established [30]. In 2005, an *in vitro* model of HCV replication that reproduces the full viral life cycle [31, 32], including production of infectious virus, was developed. These models represent useful tools for investigating the efficacy of IFNα and identifying potential IFN-induced effector proteins against HCV. It is assumed that Viperin and PKR are important players in the battle against HCV [33, 34]. Interestingly, HCV proteins were shown to interact with some of those IFN-induced antiviral effectors. For example, the two HCV proteins NS5A and E2 are known to interact with PKR [35, 36]. These interactions lead to an inhibition of PKR activity and therefore to an HCV-mediated IFNα resistance.

HCV has a striking capability to establish chronic infection, as 70–80% of infected individuals fail to eliminate the virus. Inhibition of the Jak-STAT pathway, which is crucial for most of the known antiviral effects of IFNs, might therefore be a central target of HCV providing an important advantage for the virus early in the course of infection. Indeed, our laboratory has previously reported that HCV proteins inhibit IFN-induced signalling in cell lines, transgenic mice and patients with CHC [37–39]. This inhibition was associated with an HCV-induced upregulation of the protein phosphatase 2A, finally resulting in STAT1 hypomethylation [39]. Other research groups have identified further mechanisms including inhibition of STAT1 activation through upregulation of the negative regulator SOCS3 by HCV core protein [40] and proteasome-dependent degradation of STAT1 in Huh7 cells expressing HCV proteins [41]. Epidemiological studies show that the remaining 20–30% of patients overcome acute HCV infection, suggesting that innate and/or adaptive immune responses are indeed capable of controlling the outcome of HCV infection. Evidently, HCV cannot block IFN signalling

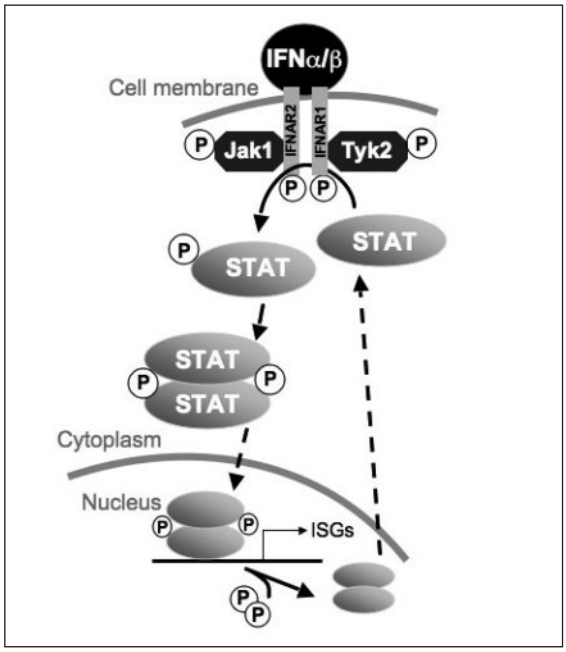


Figure 1

IFNα/β-induced Jak-STAT signalling. Binding of type I IFNs to the IFNα receptor (IFNAR), consisting of the two chains IFNAR1 and IFNAR2, activates the receptor-associated cytoplasmic Janus kinases Jak1 and Tyk2. This leads to phosphorylation and dimerisation of signal transducer and activation of transcription (STAT) proteins that transmit the signal into the nucleus and regulate gene transcription of IFN-stimulated genes (ISGs) by binding to response elements in the host's DNA.

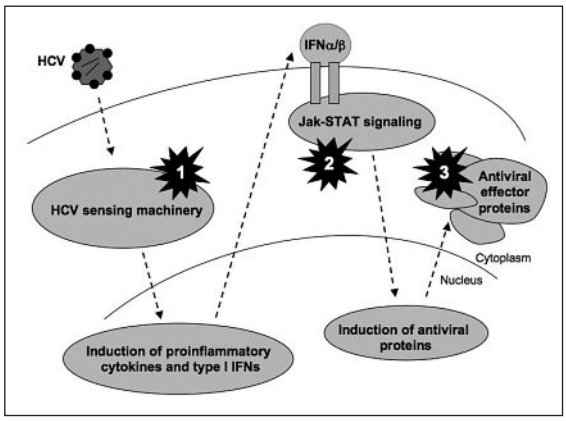


Figure 2

HCV interference with antiviral defence. Summary of the possible levels of HCV interference with the host antiviral defence machinery: 1. Disruption of the viral sensory pathways through HCV NS3-4A protease-mediated cleavage of the adaptor molecules MAVS and TRIF. 2. Inhibition of the type I IFN-induced Jak-STAT signalling pathway by HCV proteins. 3. Inhibition of antiviral effector proteins (e.g., protein kinase R) by viral proteins.

completely, as demonstrated by the success of therapies based on the application of recombinant IFNα in some of the patients with CHC.

Many viruses, including the influenza virus, have developed strategies to inhibit early signalling events that lead to IFN production in infected host cells [42]. Similarly, HCV has not only evolved mechanisms that interfere with the downstream signalling pathway of IFNα/β (see above), but also mechanisms that disrupt pathways leading to type I IFN production. The virus-encoded protease NS3-4A was shown to inactivate the important adaptor proteins TRIF and MAVS (also known as Cardif, VISA and IPS-1) in the virus-sensing TLR3 and RIG-I pathways [43, 44]. Figure 2 summarises all levels of viral interference with the hosts' defence machinery.

The other crucial component of CHC treatment is the nucleoside analogue ribavirin, which, if given without IFN, displays no antiviral effects and does not cure patients [45]. However, it largely enhances the efficacy of IFN treatment [11], even though its mode of action is not fully understood.

Analysis of interferon signalling in the liver as a basis to understand treatment non-responsiveness in patients with chronic hepatitis C

To learn more about possible mechanisms underlying differential response to IFNα-based therapy, we investigated IFN-induced signalling and ISG induction in paired liver biopsies collected from patients with CHC before (B-1) treatment and 4 hours after (B-2) the first pegIFNα injection. In addition to the liver samples, blood samples for isolation of peripheral blood mononuclear cells (PBMCs) were collected before (PBMC-1) and during (PBMC-2) therapy (see figure 3A for study outline) [46]. Virological response early in the course of treatment is an excellent predictor of later SVR. Early virological response (EVR; >2 $\log_{10}$ drop in 12 weeks) is associated with an SVR in 65% of cases [9]; a RVR leads to an even greater SVR rate of 80–90% [10, 47]. We therefore divided the 16 patients, from which paired liver biopsy samples were obtained, into rapid responders (RRs; n = 10) and non-RRs (n = 6) at week 4 of treatment. The aims of the study were: (i) to analyse IFN-induced effects in liver and PBMCs, (ii) to correlate IFN-signalling data with virological response to treatment, and (iii) to understand the molecular basis for the failure of IFN therapies in patients with CHC.

Besides infecting humans, HCV can infect chimpanzees. Interestingly, however, chimpanzees do not respond to IFN-based treatment [48] and are therefore not a suitable model for studying the molecular basis underlying treatment failure. Furthermore, chimpanzees chronically infected with HCV have numerous ISGs induced in the liver [49]. Injection of pegIFNα into these animals does not result in a further increase of ISG expression in liver, unlike to PBMCs which show a robust response to IFN [48]. These findings point to the importance of tissue-specific differences when studying the HCV infection in humans. Many studies have been performed using PBMCs from CHC patients, but it is unknown whether results obtained with PBMCs can be extrapolated to IFN-induced events occurring in the liver, the major site of HCV replication. Similarly to chimpanzees, in many CHC patients the endogenous IFN system is activated in liver tissue collected prior to treatment [46, 50–53]. Importantly, there is a large variation in the pre-treatment level of ISG expression among patients, and the degree of activation of the endogenous IFN system is linked to the patients' response to IFN therapy. Patients with high expression of ISGs measured in pre-treatment liver biopsies represent poor responders, whereas those lacking pre-activation of ISGs show a good response to therapy [46, 51]. These findings are in line with the chimpanzee data, though they are counterintuitive, as one would expect patients exhibiting elevated IFN response to clear the virus more rapidly.

Prior to our work, no data involving repeated liver biopsies from CHC patients undergoing treatment were available. By analysing the phosphorylated form of the transcription factor STAT1 (p-STAT1) in the paired liver samples (B-1 and B-2) from 16 patients [46], we observed that nuclear translocation of p-STAT1 in the RR patient group occurs only after treatment with pegIFNα, whereas in the non-RRs p-STAT1 is already present in hepatocyte nuclei in the B-1 liver and no further activation is observed in response to the pegIFNα injection (figure 3B). In contrast to hepatocytes, other cells in the liver (e.g., Kupffer cells and lymphocytes) respond normally to the pegIFNα treatment in both RR and non-RR patients. These results indicate that some CHC patients do not benefit from the pegIFNα administration, as their hepatocytes linger in a pre-acti-

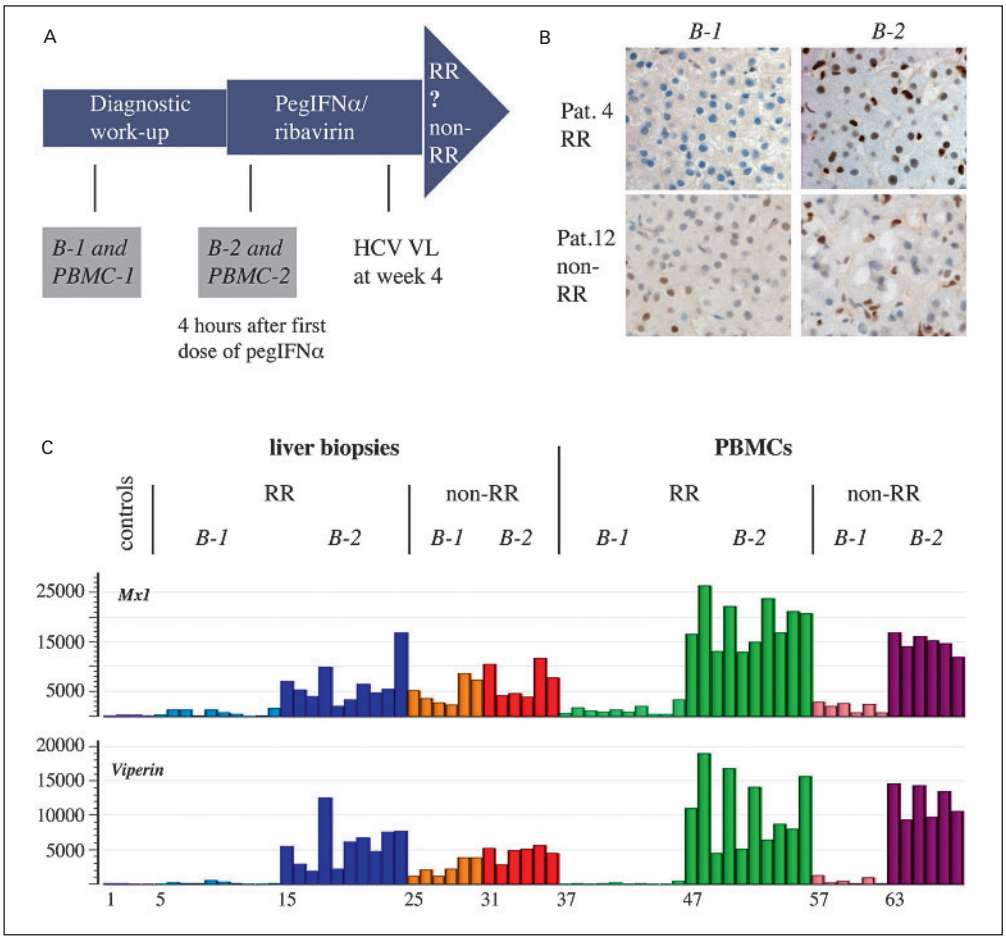
vated and likely "refractory" state. In the other group of patients (i.e., the future responders), infection with HCV seems not to be sensed by the liver in terms of activation of IFN signalling. In these patients, pegIFNα treatment succeeds to eliminate HCV possibly as a result of the antiviral defence system being rapidly mounted and confronting a virus that has not yet adapted to coping with an IFN response.

Microarray analysis of gene expression at the mRNA level revealed pronounced differences between the RR and non-RR patient groups in the number of genes responding to pegIFNα in the liver. We compared B-1 with B-2 liver samples of each individual patient, and found substantially more IFN-regulated mRNAs in the RR patient group. In PBMCs from the same patients, many more genes were regulated by pegIFNα than in liver, but, at the same time, differences between the patient response groups were far less pronounced. These observations argue for a strong local defect in the IFN-induced signalling pathways in the liver. Figure 3C depicts the typical ISG mRNA expression pattern, exemplified by Mx1 and Viperin genes, seen in the investigated patient samples; it underscores the differences between response groups and between analysed tissues.

We also analysed a large cohort of 112 pre-treatment liver biopsies and found that patients infected with "difficult-to-treat" HCV GTs 1 and 4 more often have a pre-activated endogenous IFN system prior to treatment, when compared to patients with HCV GTs 2 and 3. This observation might provide an explanation for the worse treatment prognosis of patients infected with GT 1 and 4. Why the endogenous IFN system is activated in a considerable group of patients but not in all of them is not clear. This could be due to the differences between viral GTs and quasispecies in their capacity to prevent the endogenous IFN induc-

Figure 3

IFN signalling and treatment outcomes in chronic hepatitis C.
A. An initial liver biopsy (B-1) and an initial PBMC sample (PBMC-1) were obtained during diagnostic examination of patients with CHC. The second biopsy (B-2) and a second sample of PBMCs (PBMC-2) were collected 4 hours after the first dose of pegIFNα. After 4 weeks of pegIFNα and ribavirin combination therapy, patients were defined as rapid responders (RRs) or non-RRs depending on the drop in HCV viral load (VL). B. We performed immunohistochemical staining of the activated transcription factor STAT1 in liver biopsy samples. Shown are representative examples of B-1 and B-2 of RR and non-RR patients. No nuclear staining is evident in pre-treatment biopsies of RR patients (Patient 4). The light blue colour of the nuclei originates from the counterstaining with hematoxylin. Four hours after pegIFNα, most hepatocytes show strong nuclear staining (in brown). In non-RR patients (Patient 12), weak nuclear staining is already present in pre-treatment biopsies, and pegIFNα induces little change in hepatocytes. The visible increased nuclear staining is confined to Kupffer cells. C. Gene expression pattern of two classical ISG mRNAs (Mx1 and Viperin). Expression in B-1 samples of RR patients (lanes 5–14) resembles healthy control tissue (lanes 1–4), whereas ISG levels in B-1 samples of non-RRs (25–30) are elevated. PegIFNα treatment increases the ISG mRNA level in B-2 samples in the RR (15–24), but not the non-RR (31–36) patient group. PBMC-1 and -2 samples do not differ substantially between RRs (37–56) and non-RRs (57–68). (Reprinted with permission from Sarasin-Filipowicz et al., Proc Natl Acad Sci U S A 2008;105:7034–9. Copyright 1993–2008 by the National Academy of Sciences, Washington, DC).



tion. As the HCV protease NS3-4A has been reported to cleave the adaptor protein MAVS *in vitro* [44], it is possible that HCV GTs differentially affect pathways that lead to the induction of type I IFNs. Remarkably, the success of the virus in preventing the induction of the endogenous IFN system would come at the cost of it being more susceptible to IFNα-based therapies. Why therapeutic pegIFNα is ineffective in patients having a pre-activated endogenous IFN system remains another unresolved issue. Negative regulators of the Jak-STAT pathway, amongst them – very importantly – the ubiquitin-specific peptidase USP18/UBP43, might be crucial players in preventing response to exogenous IFN [54, 55]. Notably, USP18 is highly elevated in the pre-activated livers of patients with CHC [51].

The elevated levels of ISG mRNAs in pre-treatment livers of patients with CHC can potentially be used to predict the treatment response. We have identified 29 genes that are of predictive value for treatment response at week 4. This set of genes, 76% of which represent ISGs, will now be used to develop a predictive test that will be validated in a prospective clinical trial. Hence, our results have potentially important implications for the treatment of patients with CHC. If the approach using a predictive test proves to be successful, the pegIFNα-based therapy could be specifically directed at those patients who will benefit from it.

Are microRNAs important players in HCV infection?

MicroRNAs (miRNAs) should also be considered when discussing HCV infection. MiRNAs are short (21–23 nucleotide-long) regulatory RNAs expressed in *metazoan* animals [56]. They regulate gene expression by base pairing to target mRNAs and inhibiting protein synthesis [57]. MiRNAs are involved in the control of nearly all cellular processes and over 50% of all human mRNAs are predicted to be miRNA targets [58].

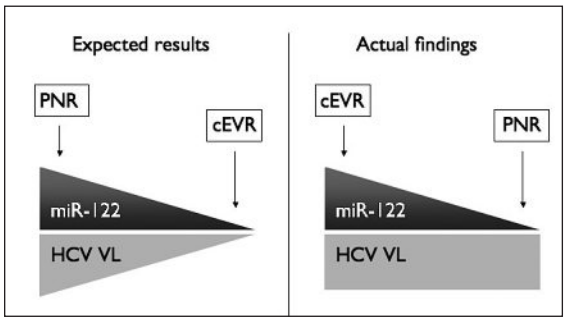
There is evidence that miRNAs play a role in viral infections and also in the innate immune response. Some viruses, such as CMV or human herpes virus-8, encode miRNAs in their own genomes, and these miRNAs may modify expression of the host's genes [59]. In contrast, some of the host-encoded miRNAs may have a profound effect on the life cycle of the infecting virus. MiR-122, a very abundant human miRNA, is expressed specifically in hepatocytes, and is the most spectacular example of the latter category. MiR-122 base pairs to genomic RNA of HCV and positively regulates replication of the virus in cell culture [60, 61]. This observation raised much interest in the role of miR-122 in HCV infection and as a potential therapeutic target. Recently, it was reported that the expression of miR-122 and several other miRNAs is regulated by IFN in Huh7 cells and primary mouse hepatocytes, and that these miRNAs might mediate at least some effects of IFN on HCV-RNA replication *in vitro* [62]. Therefore, we studied the status of miR-122 and other implicated miRNAs during the course of HCV infection and following IFNα therapy in CHC patients [63]. The availability of biopsy material collected for the studies discussed above [46] allowed us to study the proposed connection between miR-122 and HCV replication in a context of diseased tissue, and to test the effect of pegIFNα administration on the levels of miR-122 and other miRNAs in human liver.

Figure 4 summarises the expected results and our actual findings. If miR-122 were required for efficient HCV replication *in vivo* as it is in Huh7 cells [60], low hepatic miR-122 levels should lead to low HCV VL. Additionally, since low baseline VL is associated with a favourable response to therapy [8, 9], we were expecting to find low levels of miR-122 in good responders (fig. 4, left panel). Indeed, major differences were found in miR-122 levels between individual patients with CHC. However, rather unexpectedly, pre-treatment levels of miR-122 were markedly lower in patients who later showed no response to pegIFNα therapy (PNR patients). Moreover, we found no positive correlation between miR-122 abundance and intrahepatic or serum VL (fig. 4, right panel). It is possible that even the low miR-122 level found in PNRs is sufficient for robust HCV replication. Alternatively, the role of miR-122 in HCV replication may be less pronounced *in vivo* than *in vitro*.

It is currently unknown why PNRs have lower miR-122 levels than cEVR patients. We discussed earlier that PNR patients have a pre-activated IFN system in the liver already before treatment and that they show no significant changes in expression of IFN-regulated genes upon pegIFNα administration [46]. The decrease in miR-122 level in PNRs raised the possibility that miR-122 is a negatively regulated IFN target gene. Indeed, it has previously been reported that miR-122 is downregulated by treatment with IFNβ and that this downregulation contributes to the antiviral effect of IFN in Huh7 cells [62]. We compared the levels of miR-122 in paired liver biopsies collected before (B-1) and 4 hours after (B-2) administration of pegIFNα but found no decrease in the level of miR-122 in any of the 11 patients investigated. We can, however, not exclude that miR-122 is a late IFN-regulated gene in the human liver. The other miRNAs that we analysed were either not IFN regulated and not expressed at biologically relevant levels in human liver. Therefore, it is

Figure 4

Decreased levels of microRNA miR-122 in patients with CHC responding poorly to interferon therapy.
Levels of liver-specific microRNA miR-122 were measured in patients with CHC undergoing treatment with pegIFNα and ribavirin. The left panel depicts the expected results based on previously published *in vitro* findings. The right panel shows our actual findings of low miR-122 levels in the liver of PNR patients and a lack of correlation between the miR-122 abundance and HCV viral load. For details, see text.



very unlikely that miRNAs mediate antiviral IFN responses against HCV.

Is the role of miR-122 in HCV replication less important *in vivo* than it is *in vitro*? This question will be answered by ongoing trials in HCV-infected chimpanzees in which antisense oligonucleotides blocking miR-122 activity, that is miR-122-"antagomirs", are being evaluated as anti-HCV agents. If strategies aimed at reducing hepatic miR-122 levels prove to be successful, these "antagomirs" may be particularly promising in PNRs because of their low baseline miR-122 levels.

The future of anti-HCV therapy

Current standard treatment of CHC has known limitations given the high number of patients without response and the frequent occurrence of side effects. New prospects are created by introduction of the specifically targeted antiviral therapy for HCV (STAT-C), such as small molecule inhibitors of viral proteins. Currently, two HCV NS3-4A protease inhibitors are being evaluated in phase III clinical trials: Telaprevir (VX-950) and boceprevir (SCH 503034) [64, 65]. Inhibition of the HCV NS3-4A protease might not only decrease viral replication but also enhance antiviral innate immune pathways (as cleavage of MAVS is prevented, see above) [44]. Various nucleoside and non-nucleoside HCV RNA polymerase inhibitors are also being investigated. It is expected that small molecule inhibitors will soon be part of standard anti-HCV therapies. A major challenge will be the inevitable occurrence of viral resistance and frequent side effects, such as rashes and anemia [64]. Administration of a single protease or polymerase inhibitor leads to the development of resistant viral strains, as rapidly as 1–2 weeks after treatment initiation [66]. To overcome development of resistance, administration of "cocktails" of various inhibitors can be considered; the limiting factor will, however, be the high toxicity of such treatments. Also, when these novel molecules are combined with current standard treatment, resistance can be avoided. Early results from phase 2b trials show that SVR rates in GT1 patients increase from 40–50% to 67–75% when triple combinations are used [64, 65, 67]. The novel therapeutics may have potential to improve and shorten the duration of therapy; they will, however, not replace the use of pegIFNα and ribavirin. It is evident that non-responders to pegIFNα-based treatment will have an increased risk for the development of viral resistance because of persistently high HCV levels. Should it be possible to reverse pre-activation of the endogenous IFN system in the liver (by using immune modulators or IFN antibodies) non-responders might be turned into responders and many more patients could be cured. Research towards that aim is urgently needed.

Another strategy is to modify IFNs in order to increase efficacy or decrease toxicity. This approach has proven to be successful in the past with the introduction of pegIFNα. However, the newly developed albumin-modified IFNα is unlikely to improve treatment outcomes, although it can be less frequently administered thus elevating life quality of the patients [68]. IFN signalling can be enhanced by additional use of the methyl-group donor S-adenosyl-methionine (SAMe) [69]. HCV protein-induced hypomethylation of STAT1 can be reversed by SAMe in cell culture and ongoing clinical trials show promising results when retreating previous non-responders (ClinicalTrials.gov, Identifiers: NCT00310336 and NCT00475176).

Due to the limited response to treatment and the frequent side effects, prediction of response prior to treatment initiation would be very useful. Though factors like GT, baseline VL, and histological staging are well-characterised predictors of response, foreseeing treatment responses in individual patients remains difficult. So far, VL at week 4 of treatment is the most valuable criterion. It remains to be seen, whether a predictive test based on measurement of selected mRNAs in liver, as markers of pre-activation of the endogenous IFN system, will be of diagnostic use [46].

The results of studies with novel therapeutic compounds so far indicate that pegIFNα and ribavirin will remain the backbone of antiviral therapy of CHC even in the era of STAT-C. It can be anticipated that combination of STAT-C inhibitors with non-overlapping resistance profiles will further improve response to antiviral treatment.

The development and validation of tests for the prediction of response to the different combination therapies that will be used in the near future would allow physicians to tailor the treatment to the needs of individual patients.

Correspondence:
Dr. med. Magdalena Sarasin-Filipowicz, PhD
Hepatology Laboratory, Department of Biomedicine
University Hospital Basel
Hebelstrasse 20
CH-4031 Basel
Switzerland
E-Mail: magdalena.filipowicz@unibas.ch

References

1 Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis. 2005;5:558–67.
2 Williams R. Global challenges in liver disease. Hepatology. 2006;44:521–6.
3 Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science. 1989; 244:359–62.
4 Kuo G, Choo QL, Alter HJ, Gitnick GL, Redeker AG, Purcell RH, et al. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. Science. 1989;244:362–4.
5 Tsukuma H, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, Kitamura T, et al. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. N Engl J Med. 1993;328:1797–801.
6 Gerlach JT, Diepolder HM, Zachoval R, Gruener NH, Jung MC, Ulsenheimer A, et al. Acute hepatitis C: high rate of both spontaneous and treatment-induced viral clearance. Gastroenterology. 2003;125:80–8.
7 Jaeckel E, Cornberg M, Wedemeyer H, Santantonio T, Mayer J, Zankel M, et al. Treatment of acute hepatitis C with interferon alfa-2b. N Engl J Med. 2001;345:1452–7.
8 Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet. 2001;358:958–65.
9 Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL, Jr., et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med. 2002;347:975–82.
10 Hadziyannis SJ, Sette H, Jr., Morgan TR, Balan V, Diago M, Marcellin P, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med. 2004;140:346–55.
11 McHutchison JG, Gordon SC, Schiff ER, Shiffman ML, Lee WM, Rustgi VK, et al. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. N Engl J Med. 1998;339:1485–92.
12 Hasan F, Asker H, Al-Khaldi J, Siddique I, Al-Ajmi M, Owaid S, et al. Peginterferon alfa-2b plus ribavirin for the treatment of chronic hepatitis C genotype 4. Am J Gastroenterol. 2004;99:1733–7.
13 McHutchison JG, Lawitz EJ, Shiffman ML, Muir AJ, Galler GW, McCone J, et al. Peginterferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. N Engl J Med. 2009;361:580–93.
14 Mangia A, Santoro R, Minerva N, Ricci GL, Carretta V, Persico M, et al. Peginterferon alfa-2b and ribavirin for 12 vs. 24 weeks in HCV genotype 2 or 3. N Engl J Med. 2005;352:2609–17.
15 Muir AJ, Bornstein JD, Killenberg PG. Peginterferon alfa-2b and ribavirin for the treatment of chronic hepatitis C in blacks and non-Hispanic whites. N Engl J Med. 2004;350:2265–71.
16 Poynard T, Ratziu V, McHutchison J, Manns M, Goodman Z, Zeuzem S, et al. Effect of treatment with peginterferon or interferon alfa-2b and ribavirin on steatosis in patients infected with hepatitis C. Hepatology. 2003;38:75–85.
17 Gao B, Hong F, Radaeva S. Host factors and failure of interferon-alpha treatment in hepatitis C virus. Hepatology. 2004;39:880–90.
18 Lauer GM, Walker BD. Hepatitis C virus infection. N Engl J Med. 2001;345:41–52.
19 Strader DB, Wright T, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C. Hepatology. 2004;39: 1147–71.
20 Sulkowski M, Shiffman M, Afdhal N, Reddy R, McCone J, Lee W, et al. Hemoglobin decline is associated with SVR among HCV genotype 1-infected persons treated with peginterferon/ribavirin: analysis from the ideal study. J Hepatol. 2009;50:S51–S52.
21 Borden EC, Sen GC, Uze G, Silverman RH, Ransohoff RM, Foster GR, et al. Interferons at age 50: past, current and future impact on biomedicine. Nat Rev Drug Discov. 2007;6:975–90.
22 Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. Nature. 2001;413:732–8.
23 Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, Miyagishi M, et al. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. Nat Immunol. 2004;5:730–7.
24 Kato H, Takeuchi O, Sato S, Yoneyama M, Yamamoto M, Matsui K, et al. Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. Nature. 2006;441:101–5.
25 Darnell JE Jr, Kerr IM, Stark GR. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science. 1994;264:1415–21.
26 Heim MH. Intracellular signalling and antiviral effects of interferons. Dig Liver Dis. 2000;32:257–63.
27 Carroll SS, Chen E, Viscount T, Geib J, Sardana MK, Gehman J, et al. Cleavage of oligoribonucleotides by the 2',5'-oligoadenylate- dependent ribonuclease L. J Biol Chem. 1996;271:4988–92.
28 Marschall M, Zach A, Hechtfischer A, Foerst G, Meier-Ewert H, Haller O. Inhibition of influenza C viruses by human MxA protein. Virus Res. 2000;67:179–88.
29 Tilg H. New insights into the mechanisms of interferon alfa: an immunoregulatory and anti-inflammatory cytokine. Gastroenterology. 1997;112:1017–21.
30 Lohmann V, Korner F, Koch J, Herian U, Theilmann L, Bartenschlager R. Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. Science. 1999;285:110–3.
31 Lindenbach BD, Evans MJ, Syder AJ, Wolk B, Tellinghuisen TL, Liu CC, et al. Complete replication of hepatitis C virus in cell culture. Science. 2005;309:623–6.
32 Wakita T, Pietschmann T, Kato T, Date T, Miyamoto M, Zhao Z, et al. Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. Nat Med. 2005;11:791–6.
33 Jiang D, Guo H, Xu C, Chang J, Gu B, Wang L, et al. Identification of three interferon-inducible cellular enzymes that inhibit the replication of hepatitis C virus. J Virol. 2008;82:1665–78.
34 Helbig KJ, Lau DT, Semendric L, Harley HA, Beard MR. Analysis of ISG expression in chronic hepatitis C identifies viperin as a potential antiviral effector. Hepatology. 2005;42:702–10.
35 Gale M Jr, Blakely CM, Kwieciszewski B, Tan SL, Dossett M, Tang NM, et al. Control of PKR protein kinase by hepatitis C virus nonstructural 5A protein: molecular mechanisms of kinase regulation. Mol Cell Biol. 1998;18:5208–18.
36 Taylor DR, Shi ST, Romano PR, Barber GN, Lai MM. Inhibition of the interferon-inducible protein kinase PKR by HCV E2 protein. Science. 1999;285:107–10.
37 Heim MH, Moradpour D, Blum HE. Expression of hepatitis C virus proteins inhibits signal transduction through the Jak-STAT pathway. J Virol. 1999;73:8469–75.

38 Blindenbacher A, Duong FH, Hunziker L, Stutvoet ST, Wang X, Terracciano L, et al. Expression of hepatitis c virus proteins inhibits interferon alpha signaling in the liver of transgenic mice. Gastroenterology. 2003;124:1465–75.
39 Duong FH, Filipowicz M, Tripodi M, La Monica N, Heim MH. Hepatitis C virus inhibits interferon signaling through up-regulation of protein phosphatase 2A. Gastroenterology. 2004;126:263–77.
40 Bode JG, Ludwig S, Ehrhardt C, Albrecht U, Erhardt A, Schaper F, et al. IFN-alpha antagonistic activity of HCV core protein involves induction of suppressor of cytokine signaling-3. Faseb J. 2003;17:488–90.
41 Lin W, Choe WH, Hiasa Y, Kamegaya Y, Blackard JT, Schmidt EV, et al. Hepatitis C virus expression suppresses interferon signaling by degrading STAT1. Gastroenterology. 2005;128: 1034–41.
42 Conzelmann KK. Transcriptional activation of alpha/beta interferon genes: interference by nonsegmented negative-strand RNA viruses. J Virol. 2005;79:5241–8.
43 Li K, Foy E, Ferreon JC, Nakamura M, Ferreon AC, Ikeda M, et al. Immune evasion by hepatitis C virus NS3/4A protease-mediated cleavage of the Toll-like receptor 3 adaptor protein TRIF. Proc Natl Acad Sci. U S A 2005;102:2992–7.
44 Meylan E, Curran J, Hofmann K, Moradpour D, Binder M, Bartenschlager R, et al. Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. Nature. 2005;437:1167–72.
45 Di Bisceglie AM, Conjeevaram HS, Fried MW, Sallie R, Park Y, Yurdaydin C, et al. Ribavirin as therapy for chronic hepatitis C. A randomized, double-blind, placebo-controlled trial. Ann Intern Med. 1995;123:897–903.
46 Sarasin-Filipowicz M, Oakeley EJ, Duong FH, Christen V, Terracciano L, Filipowicz W, et al. Interferon signaling and treatment outcome in chronic hepatitis C. Proc Natl Acad Sci. U S A 2008;105:7034–9.
47 Shiffman ML, Suter F, Bacon BR, Nelson D, Harley H, Sola R, et al. Peginterferon alfa-2a and ribavirin for 16 or 24 weeks in HCV genotype 2 or 3. N Engl J Med. 2007;357:124–34.
48 Lanford RE, Guerra B, Bigger CB, Lee H, Chavez D, Brasky KM. Lack of response to exogenous interferon-alpha in the liver of chimpanzees chronically infected with hepatitis C virus. Hepatology. 2007;46:999–1008.
49 Bigger CB, Guerra B, Brasky KM, Hubbard G, Beard MR, Luxon BA, et al. Intrahepatic gene expression during chronic hepatitis C virus infection in chimpanzees. J Virol. 2004;78:13779–92.
50 MacQuillan GC, Mamotte C, Reed WD, Jeffrey GP, Allan JE. Upregulation of endogenous intrahepatic interferon stimulated genes during chronic hepatitis C virus infection. J Med Virol. 2003; 70:219–27.
51 Chen L, Borozan I, Feld J, Sun J, Tannis LL, Coltescu C, et al. Hepatic gene expression discriminates responders and non-responders in treatment of chronic hepatitis C viral infection. Gastroenterology. 2005;128:1437–44.
52 Feld JJ, Nanda S, Huang Y, Chen W, Cam M, Pusek SN, et al. Hepatic gene expression during treatment with peginterferon and ribavirin: Identifying molecular pathways for treatment response. Hepatology. 2007;46:1548–63.
53 Asselah T, Bieche I, Narguet S, Sabbagh A, Laurendeau I, Ripault MP, et al. Liver gene expression signature to predict response to pegylated interferon plus ribavirin combination therapy in patients with chronic hepatitis C. Gut. 2008;57:516–24.
54 Randall G, Chen L, Panis M, Fischer AK, Lindenbach BD, Sun J, et al. Silencing of USP18 potentiates the antiviral activity of interferon against hepatitis C virus infection. Gastroenterology. 2006;131:1584–91.
55 Sarasin-Filipowicz M, Wang X, Yan M, Duong FH, Poli V, Hilton DJ, et al. Alpha interferon induces long-lasting refractoriness of JAK-STAT signaling in the mouse liver through induction of USP18/UBP43. Mol Cell Biol. 2009; in press.
56 Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;116:281–97.
57 Pillai RS, Bhattacharyya SN, Artus CG, Zoller T, Cougot N, Basyuk E, et al. Inhibition of translational initiation by Let-7 MicroRNA in human cells. Science. 2005;309:1573–6.
58 Friedman RC, Farh KK, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res. 2009;19:92–105.
59 Pfeffer S, Sewer A, Lagos-Quintana M, Sheridan R, Sander C, Grasser FA, et al. Identification of microRNAs of the herpesvirus family. Nat Methods. 2005;2:269–76.
60 Jopling CL, Yi M, Lancaster AM, Lemon SM, Sarnow P. Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. Science. 2005;309:1577–81.
61 Jopling CL, Schutz S, Sarnow P. Position-dependent function for a tandem microRNA miR-122-binding site located in the hepatitis C virus RNA genome. Cell Host Microbe. 2008;4:77–85.
62 Pedersen IM, Cheng G, Wieland S, Volinia S, Croce CM, Chisari FV, et al. Interferon modulation of cellular microRNAs as an antiviral mechanism. Nature. 2007;449:919–22.
63 Sarasin-Filipowicz M, Krol J, Markiewicz I, Heim MH, Filipowicz W. Decreased levels of microRNA miR-122 in individuals with hepatitis C responding poorly to interferon therapy. Nat Med. 2009;15:31–3.
64 Hezode C, Forestier N, Dusheiko G, Ferenci P, Pol S, Goeser T, et al. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. N Engl J Med. 2009;360:1839–50.
65 Kwo P, Lawitz E, McCone J, Schiff E, Vierling J, Pound D, et al. HCV SPRINT-1 final results : SVR 24 from a phase 2 study of boceprevir plus pegintron (peginterferon alfa-2b)/ribavirin in treatment-naïve subjects with genotype-1 chronic hepatitis C. J Hepatol. 2009;50:S4.
66 Reesink HW, Zeuzem S, Weegink CJ, Forestier N, van Vliet A, van de Wetering de Rooij J, et al. Rapid decline of viral RNA in hepatitis C patients treated with VX-950: a phase Ib, placebo-controlled, randomized study. Gastroenterology. 2006;131:997–1002.
67 McHutchison JG, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, et al. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. N Engl J Med. 2009;360:1827–38.
68 Subramanian GM, Fiscella M, Lamouse-Smith A, Zeuzem S, McHutchison JG. Albinterferon alpha-2b: a genetic fusion protein for the treatment of chronic hepatitis C. Nat Biotechnol. 2007;25:1411–9.
69 Duong FH, Christen V, Filipowicz M, Heim MH. S-Adenosylmethionine and betaine correct hepatitis C virus induced inhibition of interferon signaling in vitro. Hepatology. 2006; 43:796–806.