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(57) Abstract: The application relates to treatments for improving antiviral therapies and to method for determining whether or not antiviral therapies will be effective. In particular, the present application provides a method for determining the likelihood that a subject having a viral infection of the liver will be responsive to antiviral therapy that includes stimulation of Interferon (IFN) activity, and kits for the performance of said determination.

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METHOD FOR PREDICTING THE RESPONSE OF A SUBJECT SUFFERING FROM A VIRAL INFECTION OF THE LIVER TO AN ANTIVIRAL THERAPY

The present invention relates to treatments for improving antiviral therapies and to method for determining whether or not antiviral therapies will be effective.

Viral infections represent a serious threat to health and are known to be a major cause of morbidity to animals and man. For instance, Hepatitis C virus (HCV) infection is a major cause of chronic liver disease worldwide. An important and striking feature of hepatitis C is its tendency towards chronicity. In over 70% of infected individuals, HCV establishes a persistent infection over decades that may lead to cirrhosis and hepatocellular carcinoma.

An interesting hypothesis in HCV biology proposes that the viral NS3-4A protease not only processes the viral proteins but also cleaves and inactivates components of the intracellular sensory pathways that detect viral infection and induce the transcriptional activation of type I interferons (IFN). One of the targets of NS3-4A is TRIF (TIR domain-containing adapter inducing IFN β), an essential link between dsDNA detection in endosomes by TLR3 (toll-like receptor 3) and the induction of IFN β . More recently, retinoic acid inducible gene-I (RIG-I) and MDA5 (helicard) were identified as intracellular sensors of dsRNA. Both sensors signal through Cardif (also known as IPS-1, MAVS, VISA) to induce IFN β production. Cardif can be cleaved and inactivated by HCV NS3-4A. Two RNA helicases, RIG-I and MDA5, identified as intracellular sensors of dsRNA act through Cardif to induce IFN β production.

Type I IFNs are not only crucial components of the innate immune system, but are also the most important components of current therapies against CHC. The current standard therapy consists of pegylated IFN α (pegIFN α) injected once weekly subcutaneously and daily intake of the oral antiviral agent ribavirin. This regimen achieves an overall sustained virological response (SVR) in about 55% of the patients, with significant differences between genotypes. An SVR is defined as the loss of detectable HCV RNA during treatment and its continued absence for at least 6 months after stopping therapy. Several studies of long-term follow-up on patients who achieve an SVR demonstrate that this response is durable in over 95% of patients. The probability of a SVR strongly depends on the early response to treatment. Patients who do not show an early virological response (EVR), defined as a decline of the viral load by at least 2 log₁₀ after 12 weeks of therapy, are highly unlikely to

develop an SVR, and treatment can be stopped in these patients. On the other hand, patients with an EVR have a good chance to be cured, with 65% of them achieving a SVR. The prognosis is even better for patients who have a rapid virological response (RVR), defined as serum HCV RNA undetectable after 4 weeks of treatment. Over 85% of them will achieve a SVR. Unfortunately, less than 20% of patients with genotype 1 and about 60% of patients with genotypes 2 or 3 show a RVR. The host factors that are important for an early response to therapy are currently not known.

Type I IFNs achieve their potent antiviral effects through the regulation of hundreds of genes (ISG, interferon stimulated genes). The transcriptional activation of ISGs induces proteins that are usually not synthesized in resting cells and that establish a non-virus-specific antiviral state within the cell. Interferons induce their synthesis by activating the Jak-STAT pathway, a paradigm of cell signaling used by many cytokines and growth factors. All type I IFNs bind to the same cell surface receptor (IFNAR) and activate the receptor-associated Janus kinase family members Jak1 and Tyk2. The kinases then phosphorylate and activate signal transducer and activator of transcription 1 (STAT1) and STAT2. The activated STATs translocate into the nucleus where they bind specific DNA elements in the promoters of ISGs. Many of the ISGs have antiviral activity but others are involved in lipid metabolism, apoptosis, protein degradation and inflammatory cell responses. As is the case with many viruses, HCV interferes with the IFN system, probably at multiple levels. IFN induced Jak-STAT signaling is inhibited in cells and transgenic mice that express HCV proteins, and in liver biopsies of patients with CHC. *In vitro*, HCV proteins NS5A and E2 bind and inactivate protein kinase R (PKR), an important non-specific antiviral protein. However, the molecular mechanisms that are important for the response to therapeutically applied IFN in patients with CHC are currently unknown.

The capacity of HCV to interfere with the IFN pathway at many different levels is a likely mechanism underlying HCV success to establish a chronic infection (2). However, quite paradoxically, in chimpanzees acutely or chronically infected with HCV hundreds of ISGs are induced in the liver (16, 17). Nevertheless, despite the activation of the endogenous IFN system, the virus is not cleared from chronically infected animals (18). The results obtained with chimpanzees are difficult to extrapolate to humans since there are some important differences in the pathobiology of HCV infection between these species. Whereas most chimpanzees acutely infected with HCV clear the virus spontaneously, infections in men

mostly become chronic. On the other hand, chronically infected chimpanzees can rarely be cured with IFN, whereas more than half of the patients with CHC are successfully treated (19).

Induction of ISGs was also found in pre-treatment liver biopsies of many patients with CHC, again demonstrating that HCV infection can lead to activation of the endogenous IFN system (20). Notably, patients with pre-elevated expression of ISGs tended to respond poorly to therapy when compared to patients having low initial expression (20). The cause of this differential response to therapy is not understood.

The present invention is based upon studies in which the inventors investigated IFN induced signaling and ISG induction in paired samples of liver biopsies and peripheral blood mononuclear cells (PBMCs) of patients with chronic hepatitis before and during therapy with pegIFN α . They further correlated biochemical and molecular data with the response to treatment. Their work is set out in more detail in the accompanying Example.

The inventors established that some subjects with a viral infection of the liver are in a state of "pre-activation", such that the IFN signalling pathway is in a state of stimulation with activated ISGs. The inventors have found that such individuals, when subsequently treated with IFN and an antiviral agent, had a poor, or no, response to the antiviral treatment. In contrast, another group of infected subjects appeared to have no prior stimulation of IFN receptors (and stimulation of ISGs) and this group responded well to the antiviral therapy (i.e. they had a rapid virological response (RVR)). Moreover it is possible to determine whether a subject would be a poor responder to treatment or have a RVR according to the expression level of a number of specific genes, some of which are ISG genes. In other words, the inventors identified a set of genes that are prognostic genetic markers, the expression levels of which predict whether a subject will respond to antiviral treatment.

This lead the inventors to realise that a method could be developed to help a clinician decide on a treatment regimen for subjects suffering from a viral infection of the liver. Gene expression from an infected individual can be compared with gene expression from a control (i.e. a subject without viral infection).

Infected subjects with altered gene expression (compared to the control) would be unlikely to benefit from the use of IFN in a treatment regimen (i.e. these individuals would not be expected to have an RVR) whereas infected subjects for whom gene expression was mostly unaltered, compared to control expression, are likely to benefit from IFN therapy and have an RVR. The inventors were surprised to make these correlations because a skilled person would expect activation of ISGs to be associated with better viral clearance and not with a subset of subjects who respond poorly to treatment.

While there have been previous studies of gene expression levels in "responder" and "non-responder" subjects with a viral infection of the liver, e.g. Chen *et al* (2005) Gastroenterology 128, 1437-1444, the research conducted as part of the present invention studied a very much broader set of genes in order to determine which would act as prognostic markers. Moreover, the inventors also analysed gene expression levels in samples taken before and after antiviral treatment, and used this information to identify prognostic genetic markers, while previous studies only attempted to correlate treatment outcome to gene expression levels present in samples taken before treatment. Thus the data set which lead to the identification of the prognostic genetic markers set out below are considered to be much more complete and robust than that in previous studies.

Accordingly in the first aspect of the invention there is provided a method for determining the likelihood that a subject having a viral infection of the liver will be responsive to antiviral therapy that includes stimulation of Interferon (IFN) activity, the method comprising:

- (a) analysing a sample from the subject for expression of at least one gene from each of the following groups of genes:
 - (i) KYNU; PAH; LOC129607; DDC; FOLH1; YBX1; BCHE; ACADL; ACSM3; NARF; SLPI; RPS5; RPL3; RPLP0; TRIM5 and HERC5;
 - (ii) HTATIP2; CDK4; IFI44L; and KLHDC3;
 - (iii) C7; IF; IFI27; IFIT1; OAS2; G1P2; OAS1; IRF7; RSAD2; IFI44; OAS3; SIGIRR; and IFIT2;

(iv) RAB4A; PPP1R1A; PPM1E; ENPP2; CAP2; ADCY1; CABYR; EVI1; PTGFRN; TRIM55; and IL28RA;

(v) MME; KCNN2; SLC16A10; AMOTL1; SPP2; LRCH4; HIST1H2BG; TSPYL5; HIST1H2AC; HIST1H2BD; PHTF1; ZNF684; GSTM5; FLJ20035; FIS; PARP12; C14orf21; PNPT1; FLJ39051; GALNTL1; OSBPL1A; LGALS3BP; TXNRD2; LOC201725, TOMM7; SRPX2; DCN; PSMAL; MICAL-L2; FLJ30046; SAMD9; ANKRD35; LOC284013; LOC402560; and LOC147646; and,

- (b) comparing expression of the genes in the sample to expression of the same genes in a control sample.

One embodiment of the invention is wherein altered expression of the genes in the sample compared to expression of the same genes in the control sample indicates that the subject is not likely to be responsive to said antiviral therapy.

An alternative embodiment of the invention is wherein unaltered expression of the genes in the sample compared to expression of the same genes in the control sample indicates that the subject is likely to be responsive to said antiviral therapy.

Further information regarding each of the genes assessed in the first aspect of the invention is provided in Table 2 in the accompanying Example. In particular, we provide the Affimetrix identification number for each of the genes, thus allowing each gene to be specifically identified.

It will be appreciated that the method of the first aspect of the invention will be of great benefit to clinicians. IFN is a protein growth factor and pharmaceutical preparations containing IFN are expensive to manufacture. It is therefore very important for a clinician to be confident that IFN is being used in an appropriate and cost-effective way. Furthermore, independent of the cost, it is often desirable to eliminate a viral infection of the liver as quickly as possible. It is therefore wasting time (which could be spent utilising alternative therapies) if a clinician administers IFN and subsequently discovers that it has no beneficial effects. The method of the first aspect of the invention is therefore of great assistance to a clinician because he can identify two populations of subjects. One population will show

altered expression of the genes listed above and in table 2 and will therefore not benefit from treatment with IFN. The other population, with expression of the genes listed above and in table 2 that do not significantly differ from control samples, will benefit from therapy with IFN.

In an alternative embodiment, it is considered that the expression of the genes of Table 3 (differentially expressed 4 hours after treatment) can be used in a similar way.

By "antiviral therapy" we mean any treatment regimen for reducing viral infection that involves the stimulation of IFN activity. Such a regimen may involve the use of compounds that stimulate Type I IFN activity and/or induce IFN stimulated genes (ISGs). The therapy may involve treatment with IFN *per se* or other IFN receptor agonists. For example the therapy may utilise pegylated IFN α (pegIFN α).

The therapy may involve the stimulation of IFN activity alone. However the inventors have found that the method according to the first aspect of the invention is particularly useful for predicting the effectiveness of an antiviral therapy that comprises the use of a combination therapy comprising a stimulator of IFN activity in conjunction with a known antiviral agent. Many antiviral agents are known to the art and the method of the invention can be used to evaluate the effectiveness of a number of different combination therapies. However the inventors have found that the method of the first aspect of the invention has particular value for predicting the effectiveness of therapy with a stimulator of IFN activity used in conjunction with the antiviral agent ribavirin.

It is most preferred that the method of the first aspect of the invention is used to predict the usefulness of pegIFN α and ribavirin as an antiviral therapy.

The method of the first aspect of the invention may be utilised to evaluate the effectiveness of treatments for a number of different viral infections of the liver, including Hepatitis B virus and Hepatitis C virus infections. It is most preferred that the method is utilised to evaluate the effectiveness of therapies for Hepatitis C Virus (HCV) infection. The inventors have found that the method of the invention is particularly useful for distinguishing between subjects that will be expected to have a rapid virological response (RVR) and those which will not (non-RVR).

Samples representative of gene expression in a subject that may be used in accordance with the present invention encompass any sample that may provide information as to genes being expressed by the subject.

Examples of suitable samples include biopsies, samples excised during surgical procedures, blood samples, urine samples, sputum samples, cerebrospinal fluid samples, and swabbed samples (such as saliva swab samples). It will be appreciated that the source of the sample will depend upon which type of viral infection the subject may have.

It is most preferred samples are from liver tissue. Liver samples have been found to be particularly instructive when the method is applied to assessing subjects with HCV infection. The inventors were surprised to find that RVR could be distinguished from non-RVR subjects by analysing gene expression from liver samples whereas peripheral blood leukocytes exhibited no significant changes in gene expression before or after exposure to IFN.

Suitable samples may include tissue sections such as histological or frozen sections. Methods by which such sections may be prepared in such a way as to be able to provide information representative of gene expression in the subject from which the section is derived will be well known to those skilled in the art, and should be selected with reference to the technique that it is intended to use when investigating gene expression.

Although the use of samples comprising a portion of tissue from the subject is contemplated, it may generally be preferred that the sample representative of gene expression comprise a suitable extract taken from such a tissue, said extract being capable of investigation to provide information regarding gene expression in the subject. Suitable protocols which may be used for the production of tissue extracts capable of providing information regarding gene expression in a subject will be well known to those skilled in the art. Preferred protocols may be selected with reference to the manner in which gene expression is to be investigated.

In the case of samples derived from liver suitable preparation steps are disclosed in 1.1.1 and 1.1.3 of the Example.

By "control sample" we mean a sample, equivalent to that from the subject, that has been derived from an individual that is not suffering from a viral infection of the liver. Although

equivalent tissue or organ samples, constituting control samples, or extracts from such samples, may be used directly as the source of information regarding gene expression in the control sample, it will be appreciated, and generally be preferred, that information regarding the expression of the selected gene (or genes) in an "ideal" control sample be provided in the form of reference data. Such reference data may be provided in the form of tables indicative of gene expression in the chosen control tissue. Alternatively, the reference data may be supplied in the form of computer software containing retrievable information indicative of gene expression in the chosen control tissue. The reference data may, for example, be provided in the form of an algorithm enabling comparison of expression of at least one selected gene(s) from each groups of genes in the subject with expression of the same genes in the control tissue sample.

In the event that expression of genes listed above and in Table 2 in a control sample is to be investigated via processing of a tissue or organ sample constituting the control sample, it is preferred that such processing is conducted using the same methods used to process the sample from the subject. Such parallel processing of subject samples and control samples allows a greater degree of confidence that comparisons of gene expression in these tissues will be normalised relative to one another (since any artefacts associated with the selected method by which tissue is processed and gene expression investigated will be applied to both the subject and control samples).

The method according to the first aspect of the invention may involve the analysis of gene expression of at least one gene, selected from each of the groups of genes. The finding that altered expression of the genes listed above and in Table 2 or 3 may be used in determining the effectiveness of an antiviral therapy is surprising, since although the expression of certain genes (such as those encoding STAT1) has been linked to HCV infection, most of the genes listed above and in Table 2 had never previously been identified as being associated IFN regulated gene expression or with the likelihood of a therapy being effective for treating viral infections. Furthermore, irrespective of the association of these genes with IFN activity, it was total unexpected that increased expression of ISGs would be associated with poor response to subsequent IFN treatment.

The inventors have identified a total of 83 different genes, the expression levels of which can be prognostic markers for the outcome of antiviral therapy. These genes have been

distributed into five different groups according to their function: group (i) are considered to be involved in cell metabolism; group (ii) are considered to be involved in cell cycle; group (iii) are considered to be involved in immune response; group (iv) are considered to be involved in signal transduction; group (v) are each unassigned to any particular group set out above. This distribution is shown in the method of the invention, in which the expression level of at least one gene from each of the groups of genes is assessed in order to determine the likelihood that the subject will be responsive to antiviral therapy. The inventors have further found that these subsets of the genes have particular value and can be effective for that purpose when the expression level of at least one member of each of those groups is analysed.

It is preferred that the method is based on the analysis of at least five, six, seven, eight, nine, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 71, 72, 73, 74, 75, 76, 77 or 78 genes listed above.

Expression of genes listed above and in Table 2 or 3 may be investigated by analysis of target molecules representative of gene expression in the sample. The presence or absence of target molecules in a sample will generally be detected using suitable probe molecules. Such detection will provide information as to gene expression, and thereby allow comparison between gene expression occurring in the subject and expression occurring in the control sample. Probes will generally be capable of binding specifically to target molecules directly or indirectly representative of gene expression. Binding of such probes may then be assessed and correlated with gene expression to allow an effective prognostic comparison between gene expression in the subject and in the control.

By "altered expression" we include where the gene expression is both elevated or reduced in the sample when compared to the control, as discussed above.

Conversely by "unaltered expression" we include where the gene expression is not elevated or reduced in the sample when compared to the control, as discussed above.

An assessment of whether a gene expression is altered or unaltered can be made using routine methods of statistical analysis.

The target molecule may be peptide or polypeptide. Preferably the amount of peptide or polypeptide is determined using a specific binding molecule, most preferably an antibody. In a preferred instance, the amount of certain target proteins present in a sample may be assessed with reference to the biological activity of the target protein in the sample. Assessment and comparison of expression in this manner is particularly suitable in the case of protein targets having enzyme activity. Suitable techniques for the measurement of the amount of a protein target present in a sample include, but are not limited to, aptamers and antibody-based techniques, such as radio-immunoassays (RIAs), enzyme-linked immunoassays (ELISAs) and Western blotting.

Nucleic acids represent preferred target molecules for assaying gene expression according to the third aspect of the invention.

It will be understood that "nucleic acids" or "nucleic acid molecules" for the purposes of the present invention refer to deoxyribonucleotide or ribonucleotide polymers in either single- or double-stranded form. Furthermore, unless the context requires otherwise, these terms should be taken to encompass known analogues of natural nucleotides that can function in a similar manner to naturally occurring nucleotides.

Furthermore it will be understood that target nucleic acids suitable for use in accordance with the invention need not comprise "full length" nucleic acids (e.g. full length gene transcripts), but need merely comprise a sufficient length to allow specific binding of probe molecules.

It is preferred that the nucleic acid target molecule is a mRNA gene transcript and artificial products of such transcripts. Preferred examples of artificial target molecules generated from gene transcripts include cDNA and cRNA, either of which may be generated using well known protocols or commercially available kits or reagents.

In a preferred embodiment of the method of the first aspect of the invention, samples may be treated to isolate RNA target molecules by a process of lysing cells taken from a suitable sample (which may be achieved using a commercially available lysis buffer such as that produced by Qiagen Ltd.) followed by centrifugation of the lysate using a commercially available nucleic acid separation column (such as the RNeasy midi spin column produced by Qiagen Ltd). Other methods for RNA extraction include variations on the phenol and

guanidine isothiocyanate method of Chomczynski, P. and Sacchi, N. (1987) *Analytical Biochemistry* 162, 156. "Single Step Method of RNA Isolation by Acid Guanidinium Thiocyanate-Phenol-Chloroform Extraction." RNA obtained in this manner may constitute a suitable target molecule itself, or may serve as a template for the production of target molecules representative of gene expression.

It may be preferred that RNA derived from a subject or control sample may be used as substrate for cDNA synthesis, for example using the Superscript System (Invitrogen Corp.). The resulting cDNA may then be converted to biotinylated cRNA using the BioArray RNA Transcript labelling Kit (Enzo Life Sciences Inc.) and this cRNA purified from the reaction mixture using an RNeasy mini kit (Qiagen Ltd).

mRNA, representative of gene expression, may be measured directly in a tissue derived from a subject or control sample, without the need for mRNA extraction or purification. For example, mRNA present in, and representative of gene expression in, a subject or control sample of interest may be investigated using appropriately fixed sections or biopsies of such a tissue. The use of samples of this kind may provide benefits in terms of the rapidity with which comparisons of expression can be made, as well as the relatively cheap and simple tissue processing that may be used to produce the sample. *In situ* hybridisation techniques represent preferred methods by which gene expression may be investigated and compared in tissue samples of this kind. Techniques for the processing of tissues of interest that maintain the availability of RNA representative of gene expression in the subject or control sample are well known to those of skill in the art.

However, techniques by which mRNAs representative of gene expression in a subject or control sample may be extracted and collected are also well known to those skilled in the art, and the inventors have found that such techniques may be advantageously employed in accordance with the present invention. Samples comprising extracted mRNA from a subject or control sample may be preferred for use in the method of the third aspect of the invention, since such extracts tend to be more readily investigated than is the case for samples comprising the original tissues. For example, suitable target molecules allowing for comparison of gene expression may comprise the total RNA isolated from a sample of tissue from the subject, or a sample of control tissue.

Furthermore, extracted RNA may be readily amplified to produce an enlarged mRNA sample capable of yielding increased information on gene expression in the subject or control sample. Suitable examples of techniques for the extraction and amplification of mRNA populations are well known, and are considered in more detail below.

By way of example, methods of isolation and purification of nucleic acids to produce nucleic acid targets suitable for use in accordance with the invention are described in detail in Chapter 3 of Laboratory Techniques in Biochemistry and Molecular Biology: Hybridization With Nucleic Acid Probes, Part I. Theory and Nucleic Acid Preparation, P. Tijssen, ed. Elsevier, N.Y. (1993).

In a preferred method, the total nucleic acid may be isolated from a given sample using, the techniques described in the Example.

In the event that it is desired to amplify the nucleic acid targets prior to investigation and comparison of gene expression it may be preferred to use a method that maintains or controls for the relative frequencies of the amplified nucleic acids in the subject or control tissue from which the sample is derived.

Suitable methods of "quantitative" amplification are well known to those of skill in the art. One well known example, quantitative PCR, involves simultaneously co-amplifying a control sequence whose quantities are known to be unchanged between control and subject samples. This provides an internal standard that may be used to calibrate the PCR reaction.

In addition to the methods outlined above, the skilled person will appreciate that any technology coupling the amplification of gene-transcript specific product to the generation of a signal may also be suitable for quantitation. A preferred example employs convenient improvements to the polymerase chain reaction (US 4683195 and 4683202) that have rendered it suitable for the exact quantitation of specific mRNA transcripts by incorporating an initial reverse transcription of mRNA to cDNA. Further key improvements enable the measurement of accumulating PCR products in real-time as the reaction progresses.

In many cases it may be preferred to assess the degree of gene expression in subject or control samples using probe molecules capable of indicating the presence of target

molecules (representative of one or more of the genes listed above and in Table 2) in the relevant sample.

Probes for use in the method of the invention may be selected with reference to the product (direct or indirect) of gene expression to be investigated. Examples of suitable probes include oligonucleotide probes, antibodies, aptamers, and binding proteins or small molecules having suitable specificity.

Oligonucleotide probes constitute preferred probes suitable for use in accordance with the method of the invention. The generation of suitable oligonucleotide probes is well known to those skilled in the art (Oligonucleotide synthesis: Methods and Applications, Piet Herdewijn (ed) Humana Press (2004).). Oligonucleotide and modified oligonucleotides are commercially available from numerous companies.

For the purposes of the present invention an oligonucleotide probe may be taken to comprise an oligonucleotide capable of hybridising specifically to a nucleic acid target molecule of complementary sequence through one or more types of chemical bond. Such binding may usually occur through complementary base pairing, and usually through hydrogen bond formation. Suitable oligonucleotide probes may include natural (ie., A, G, C, or T) or modified bases (7-deazaguanosine, inosine, etc.). In addition, a linkage other than a phosphodiester bond may be used to join the bases in the oligonucleotide probe(s), so long as this variation does not interfere with hybridisation of the oligonucleotide probe to its target. Thus, oligonucleotide probes suitable for use in the methods of the invention may be peptide nucleic acids in which the constituent bases are joined by peptide bonds rather than phosphodiester linkages.

The phrase "hybridising specifically to" as used herein refers to the binding, duplexing, or hybridising of an oligonucleotide probe preferentially to a particular target nucleotide sequence under stringent conditions when that sequence is present in a complex mixture (such as total cellular DNA or RNA). Preferably a probe may bind, duplex or hybridise only to the particular target molecule.

The term "stringent conditions" refers to conditions under which a probe will hybridise to its target subsequence, but minimally to other sequences. Preferably a probe may hybridise to

no sequences other than its target under stringent conditions. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridise specifically at higher temperatures.

In general, stringent conditions may be selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength, pH, and nucleic acid concentration) at which 50% of the oligonucleotide probes complementary to a target nucleic acid hybridise to the target nucleic acid at equilibrium. As the target nucleic acids will generally be present in excess, at T_m , 50% of the probes are occupied at equilibrium. By way of example, stringent conditions will be those in which the salt concentration is at least about 0.01 to 1.0 M Na^+ ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30° C for short probes (e.g., 10 to 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide.

Oligonucleotide probes may be used to detect complementary nucleic acid sequences (i.e., nucleic acid targets) in a suitable representative sample. Such complementary binding forms the basis of most techniques in which oligonucleotides may be used to detect, and thereby allow comparison of, expression of particular genes. Preferred technologies permit the parallel quantitation of the expression of multiple genes and include technologies where amplification and quantitation of species are coupled in real-time, such as the quantitative reverse transcription PCR technologies and technologies where quantitation of amplified species occurs subsequent to amplification, such as array technologies.

Array technologies involve the hybridisation of samples, representative of gene expression within the subject or control sample, with a plurality of oligonucleotide probes wherein each probe preferentially hybridises to a disclosed gene or genes. Array technologies provide for the unique identification of specific oligonucleotide sequences, for example by their physical position (e.g., a grid in a two-dimensional array as commercially provided by Affymetrix Inc.) or by association with another feature (e.g. labelled beads as commercially provided by Illumina Inc or Luminex Inc). Oligonucleotide arrays may be synthesised *in situ* (e.g by light directed synthesis as commercially provided by Affymetrix Inc) or pre-formed and spotted by contact or ink-jet technology (as commercially provided by Agilent or Applied Biosystems). It

will be apparent to those skilled in the art that whole or partial cDNA sequences may also serve as probes for array technology (as commercially provided by Clontech).

Oligonucleotide probes may be used in blotting techniques, such as Southern blotting or northern blotting, to detect and compare gene expression (for example by means of cDNA or mRNA target molecules representative of gene expression). Techniques and reagents suitable for use in Southern or northern blotting techniques will be well known to those of skill in the art. Briefly, samples comprising DNA (in the case of Southern blotting) or RNA (in the case of northern blotting) target molecules are separated according to their ability to penetrate a gel of a material such as acrylamide or agarose. Penetration of the gel may be driven by capillary action or by the activity of an electrical field. Once separation of the target molecules has been achieved these molecules are transferred to a thin membrane (typically nylon or nitrocellulose) before being immobilized on the membrane (for example by baking or by ultraviolet radiation). Gene expression may then be detected and compared by hybridisation of oligonucleotide probes to the target molecules bound to the membrane.

In certain circumstances the use of traditional hybridisation protocols for comparing gene expression may prove problematic. For example blotting techniques may have difficulty distinguishing between two or more gene products of approximately the same molecular weight since such similarly sized products are difficult to separate using gels. Accordingly, in such circumstances it may be preferred to compare gene expression using alternative techniques, such as those described below.

Gene expression in a sample representing gene expression in a subject may be assessed with reference to global transcript levels within suitable nucleic acid samples by means of high-density oligonucleotide array technology. Such technologies make use of arrays in which oligonucleotide probes are tethered, for example by covalent attachment, to a solid support. These arrays of oligonucleotide probes immobilized on solid supports represent preferred components to be used in the methods and kits of the invention for the comparison of gene expression. Large numbers of such probes may be attached in this manner to provide arrays suitable for the comparison of expression of large numbers of genes selected from those listed above and in Table 2. Accordingly it will be recognised that such oligonucleotide arrays may be particularly preferred in embodiments of the methods of the

invention where it is desired to compare expression of more than one gene selected from each of the groups of genes listed above and in Table 2.

Other suitable methodologies that may be used in the comparison of nucleic acid targets representative of gene expression include, but are not limited to, nucleic acid sequence based amplification (NASBA); or rolling circle DNA amplification (RCA).

It is usually desirable to label probes in order that they may be easily detected. Examples of detectable moieties that may be used in the labelling of probes or targets suitable for use in accordance with the invention include any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Suitable detectable moieties include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials and colourimetric materials. These detectable moieties are suitable for incorporation in all types of probes or targets that may be used in the methods of the invention unless indicated to the contrary.

Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride, phycoerythrin, texas red, rhodamine, green fluorescent protein, and the like; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S , ^3H , ^{14}C , or ^{32}P ; examples of suitable colorimetric materials include colloidal gold or coloured glass or plastic (e.g., polystyrene, polypropylene, latex, etc.) beads.

Means of detecting such labels are well known to the skilled person. For example, radiolabels may be detected using photographic film or scintillation counters; fluorescent markers may be detected using a photodetector to detect emitted light. Enzymatic labels are typically detected by providing the enzyme with a substrate and detecting the reaction product produced by the action of the enzyme on the substrate, and colorimetric labels are detected by simply visualizing the coloured label.

In a preferred embodiment of the invention fluorescently labelled probes or targets may be scanned and fluorescence detected using a laser confocal scanner.

In the case of labelled nucleic acid probes or targets suitable labelling may take place before, during, or after hybridisation. In a preferred embodiment, nucleic acid probes or targets for use in the methods of the invention are labelled before hybridisation. Fluorescence labels are particularly preferred and, where used, quantification of the hybridisation of the nucleic acid probes to their nucleic acid targets is by quantification of fluorescence from the hybridised fluorescently labelled nucleic acid. More preferably quantitation may be from a fluorescently labelled reagent that binds a hapten incorporated into the nucleic acid.

In a preferred embodiment of the invention analysis of hybridisation may be achieved using suitable analysis software, such as the Microarray Analysis Suite (Affymetrix Inc.).

Effective quantification may be achieved using a fluorescence microscope which can be equipped with an automated stage to permit automatic scanning of the array, and which can be equipped with a data acquisition system for the automated measurement, recording and subsequent processing of the fluorescence intensity information. Suitable arrangements for such automation are conventional and well known to those skilled in the art.

In a preferred embodiment, the hybridised nucleic acids are detected by detecting one or more detectable moieties attached to the nucleic acids. The detectable moieties may be incorporated by any of a number of means well known to those of skill in the art. However, in a preferred embodiment, such moieties are simultaneously incorporated during an amplification step in the preparation of the sample nucleic acids (probes or targets). Thus, for example, polymerase chain reaction (PCR) using primers or nucleotides labelled with a detectable moiety will provide an amplification product labelled with said moiety. In a preferred embodiment, transcription amplification using a fluorescently labelled nucleotide (e.g. fluorescein-labelled UTP and/or CTP) incorporates the label into the transcribed nucleic acids.

Alternatively, a suitable detectable moiety may be added directly to the original nucleic acid

sample (e.g., mRNA, polyA mRNA, cDNA, etc. from the tissue of interest) or to an amplification product after amplification of the original nucleic acid is completed. Means of attaching labels such as fluorescent labels to nucleic acids are well known to those skilled in the art and include, for example nick translation or end-labelling (e.g. with a labeled RNA) by kinasing of the nucleic acid and subsequent attachment (ligation) of a nucleic acid linker joining the sample nucleic acid to a label (such as a suitable fluorophore).

Although the method of the first aspect of the invention is most suitable for use in association with human subjects it will be appreciated that it may also be useful in determining a course of treatment of viral infection in non-human animals (e.g. horses, dogs, cattle).

An alternative method of the invention comprises a method for determining the likelihood that a subject having a viral infection of the liver will be responsive to antiviral therapy that includes stimulation of Interferon (IFN) activity, the method comprising:

- (a) analysing a sample from the subject for expression of at least one gene selected from the genes listed in Table 3 below.
- (b) comparing expression of the genes in the sample to expression of the same genes in a control sample;

One embodiment of the method is wherein altered expression of the genes in the sample compared to expression of the same genes in the control sample indicates that the subject is not likely to be responsive to said antiviral therapy.

An alternative embodiment of the method is wherein unaltered expression of the genes in the sample compared to expression of the same genes in the control sample indicates that the subject is likely to be responsive to said antiviral therapy.

Techniques used for performing this aspect of the invention are provided above in relation to the first aspect of the invention. While the specific genes are different, the skilled person would appreciate and be able to identify target molecules to be assessed according to this method, as well as identify specific binding agents that can be used.

The inventors expanded their work investigating the differences between infected subjects that respond well to IFN treatment with those that do not, to examine IFN Induced Jak-STAT signalling.

IFN binds to interferon receptors and activates the Jak-STAT pathway. A central event in this activation is the phosphorylation of STAT1. The inventors found that STAT1 phosphorylation was induced in most subjects when they were treated with pegIFN α 2b. However there seemed to be no correlation between STAT1 phosphorylation and the responsiveness of a subject to IFN treatment in an antiviral therapy. However the inventors were surprised to find that there were differences in responders and non-responders with regards the location of STAT1 when examined in samples. STAT1 is known to translocate into the nucleus and bind as a dimer to specific response elements in the promoters of ISGs. All rapid responding subjects had an IFN induced shift in STAT1 location following treatment with pegIFN α 2b. In contrast, the non-responsive subjects (i.e. those with pre-activated IFN signalling) had no detectable STAT1 shifts; rather a large proportion of hepatocytes already had appreciable nuclear staining.

Therefore according to a second aspect of the invention, there is provided a method for determining the likelihood that a subject having a viral infection of the liver will be responsive to antiviral therapy that includes stimulation of Interferon (IFN) activity, the method comprising, examining a sample from the subject to identify the subcellular location of STAT1.

As set out in the accompanying examples, the inventors have determined that the location of STAT1 in liver cells is a prognostic marker for the responsiveness of a subject to antiviral therapy that includes stimulation of Interferon (IFN) activity. In that data it is shown that a large proportion of hepatocytes in liver samples taken from non-RVR subjects (i.e. non responders to antiviral therapy) already had an appreciable nuclear staining for STAT1 prior to antiviral therapy, whereas hepatocytes in liver samples from RVR subjects only have minimal nuclear staining. This totally unexpected finding is neither disclosed nor suggested in the art.

Thus, if a majority of hepatocytes in liver samples have nuclear staining for STAT1, then that subject is likely to be non-responsive to antiviral therapy that includes stimulation of

Interferon (IFN) activity. Conversely, if a minimal number of the hepatocytes in liver samples have nuclear staining for STAT1 is likely to be responsive to antiviral therapy that includes stimulation of Interferon (IFN) activity.

Preferably the sample is a liver sample. Also preferably the method examines the subcellular location of STAT1 in hepatocyte cells.

Methods for determining the location of STAT1 protein in liver samples are routine in the art. An example of such a method is standard immunohistochemistry using commercially available anti-STAT antibodies or other specific binding entities. The accompanying example provides a detailed method for determining the location of STAT1 protein in a liver sample. Preferably the STAT1 protein examined in the method of the invention is phospho-STAT1.

By "subject" we include those subjects defined above in relation to the first aspect of the invention. Preferably the subject is human.

As set out above, the present invention is based upon studies in which the inventors investigated IFN induced signaling and ISG induction in paired samples of liver biopsies and peripheral blood mononuclear cells (PBMCs) of patients with chronic hepatitis before and during therapy with pegIFN α ; this is described in more detail in the accompanying Example.

The inventors established that the endogenous IFN system is constantly activated in many infected patients. Moreover, the inventors were surprised to correlate patients with a pre-activated IFN system with a poor response to IFN therapy. This finding is counter-intuitive, because one would expect that an active innate immune system would help to eliminate the virus during IFN α therapy.

The inventors analysed ISG expression in liver biopsies and further concluded that there are patients where HCV surprisingly induces (does not block) the endogenous IFN system, and there are patients where HCV does not induce (may be by cleaving TRIF and/or Cardif) the endogenous IFN system, but that this difference has no impact on the ability of HCV to maintain a chronic infection.

In patients without a pre-activation of the IFN system, the inventors found that pegIFN α 2b induced within 4 hours a robust (sub-) maximal up-regulation of many ISGs in the liver. Surprisingly, such high ISG expression levels were already present in the pretreatment biopsies of patients that later did not show a rapid virological response at week 4.

It was also found that the pre-activation of the endogenous IFN system was found more frequently in liver biopsies of patients infected with HCV genotype 1 (and 4) than with genotype 2 or 3. This is intriguing because it is well known that genotype 2 and 3 infections can be cured in over 80% of the patients, compared to less than 50% of the patients with genotype 1. The inventors finding that the frequency and degree of pre-activation of the endogenous IFN system depends on the HCV genotype provides an explanation for the different treatment susceptibility of HCV genotypes.

The inventors realised that these data establish that HCV interferes with IFN signalling and thereby impairs the response to therapy. Moreover, an inhibition of IFN α signalling by HCV explains why the strong pre-activation of the endogenous IFN system does not lead to a spontaneous elimination of HCV. The inventors do not wish to be bound by any hypothesis but believe this means that IFN α would not induce an antiviral state in the hepatocytes that are infected with HCV. The up-regulation of ISGs observed in the liver of many patients would then occur only in the non-infected hepatocytes. The strong induction of ISGs found in liver biopsies is compatible with such a model, because there are more non-infected than infected hepatocytes. IFN β production would occur in the hepatocytes infected with a virus that is not successful in cleaving Cardif and/or TRIF. Because of the HCV induced inhibition of the Jak-STAT pathway, the secreted IFN β would not induce an antiviral state in these infected hepatocytes, but only in non-infected neighbor cells.

The inventor realised that their new understanding of the interaction between HCV and the immune system was highly relevant to the design and selection of treatment regimens for viral infections such as HCV infection. It is therefore an aim of certain embodiments of the invention to provide novel means of treating viral infections.

According to a third aspect of the present invention, there is provided a use of an agent that reduces the activation of the IFN system for the prevention or treatment of a viral infection of the liver.

According to a fourth aspect of the present invention, there is provided an agent that reduces the activation of the IFN system in the manufacture of a medicament for the prevention or treatment of a viral infection of the liver.

The inventors, as explained above and in the Example, have realised that some subjects with a viral infection have activation of the IFN system (and associated upregulation of ISGs) and this is associated with a poor response to subsequent antiviral therapy with IFN. This lead them to realise that agents according to the third or fourth aspect of the invention, which will prevent such preactivation, are useful for reducing the activity of the IFN pathway and will effectively "prime" a subject such that they will respond better to subsequent antiviral therapies which utilise IFN. The inventors were surprised to make these correlations because a skilled person would expect increased IFN activity in a subject to be associated with better viral clearance and not with a subset of subjects who respond poorly to treatment.

It is therefore preferred that the agents are used according to the third or fourth aspects of the invention are used to treat subjects with viral infections that also have increased (relative to uninfected control subjects) activation of IFN system.

By "reduces" we mean that agent is effective for reducing the stimulation of ISGs such that the expression levels of ISGs are not significantly different to expression levels in control tissues.

The agents may be used in the treatment of a number of different viral infections of the liver, including Hepatitis B virus and Hepatitis C virus infections. It is most preferred that the agents are used to prevent or reduce Hepatitis C Virus (HCV) infection.

Examples of agents which may be used according to the invention include where the agent may bind to the IFN α polypeptide and prevent IFN functional activity, e.g. antibodies and fragments and derivatives thereof (e.g. domain antibodies or Fabs). Alternatively the agent may act as a competitive inhibitor to IFN system by acting as an antagonist at IFN α receptors (e.g. IFNAR1, IFNAR2a, b, or c). Alternatively the agent may inhibit enzymes or other molecules in the IFN pathway. Alternatively the agent may bind to mRNA encoding IFN α polypeptide in such a manner as to lead to a reduction in that mRNA and hence a

reduction in IFN α polypeptide. Alternatively the agent may bind to a nucleic sequence encoding IFN α in such a manner that it leads to a reduction in the amount of transcribed mRNA encoding IFN α polypeptide. For instance the agent may bind to coding or non-coding regions of the IFN α gene or to DNA 5' or 3' of the IFN and thereby reduce expression of the protein.

It is preferred that the agent of the third or fourth aspect of the invention binds to IFN α polypeptide, an IFN α receptor or to a nucleic acid encoding IFN α polypeptide.

There are a number of different human Interferon α polypeptide sequences. An alignment of these sequences is shown in Figure 8. From this alignment the following consensus sequence has been determined. This information can be used by the skilled person to develop a binding agent to IFN α polypeptide.

When the agents binds to IFN α polypeptide, it is preferred that the agent binds to an epitope defined by the protein that has been correctly folded into its native form. It will be appreciated, that there can be some sequence variability between species and also between genotypes. Accordingly other preferred epitopes will comprise equivalent regions from variants of the gene. Equivalent regions from further IFN polypeptides can be identified using sequence similarity and identity tools, and database searching methods, outlined above in the first aspect of the invention.

It is most preferred that the agent binds to a conserved region of the IFN α polypeptide or a fragment thereof. As can be seen from the alignment of IFN α polypeptide sequences in Figure 8, there are a number of regions of amino acid sequence which are conserved between the different polypeptides. An example of such a conserved region would be positions 161 to 174 of the "consensus" sequence shown in that figure.

Agents which bind to such a region have a particularly dramatic effect on IFN α activity and are therefore particularly effective for preventing pre-activation of the IFN system and thereby improving elimination of HCV from subjects receiving antiviral therapy.

When the agents binds to an IFN receptor, it is preferred that the agent binds to and inhibits the binding of IFN α to the IFN receptor.

There are a number of different Interferon receptors. The amino acid sequences of these are shown in Figure 9. This information can be used by the skilled person to develop a binding agent to IFN receptor polypeptide.

It is preferred that the agent binds to an epitope on the receptor defined by the IFN receptor protein that has been correctly folded into its native form. It will be appreciated, that there can be some sequence variability between species and also between genotypes. Accordingly other preferred epitopes will comprise equivalent regions from variants of the receptor gene. Equivalent regions from further IFN polypeptides can be identified using sequence similarity and identity tools, and database searching methods, outlined above in the first aspect of the invention.

An embodiment of the third or fourth aspects of the invention is wherein the agent is an antibody or fragment thereof.

The use of antibodies as agents to modulate polypeptide activity is well known. Indeed, therapeutic agents based on antibodies are increasingly being used in medicine. As set out above, the inventors realised that an antibody may be used to neutralise IFN system by binding thereto or may act as an inhibitor of an IFN receptor. It is therefore apparent that such agents have great utility as medicaments for the improving the treatment of HCV infections. Moreover, such antibodies can be used in the prognostic methods set out above in further aspects of the invention.

Antibodies, for use in treating human subjects, may be raised against:

- (a) IFN α polypeptide *per se* or a number of peptides derived from the IFN α polypeptide, or peptides comprising amino acid sequences corresponding to those found in the IFN α polypeptide; or
- (b) the IFN receptor or a number of peptides derived from the IFN receptor, or peptides comprising amino acid sequences corresponding to those found in the IFN receptor.

It is preferred that the antibodies are raised against antigenic structures from human IFN α polypeptide, the human IFN receptor and peptide derivatives and fragments thereof.

Antibodies may be produced as polyclonal sera by injecting antigen into animals. Preferred polyclonal antibodies may be raised by inoculating an animal (e.g. a rabbit) with antigen (e.g. all or a fragment of the IFN α polypeptide) using techniques known to the art.

Alternatively the antibody may be monoclonal. Conventional hybridoma techniques may be used to raise such antibodies. The antigen used to generate monoclonal antibodies for use in the present invention may be the same as would be used to generate polyclonal sera.

In their simplest form, antibodies or immunoglobulin proteins are Y-shaped molecules usually exemplified by the γ -immunoglobulin (IgG) class of antibodies. The molecule consists of four polypeptide chains two identical heavy (H) chains and two identical (L) chains of approximately 50kD and 25kD each respectively. Each light chain is bound to a heavy chain (H-L) by disulphide and non-covalent bonds. Two identical H-L chain combinations are linked to each other by similar non-covalent and disulphide bonds between the two H chains to form the basic four chain immunoglobulin structure (H-L)₂.

Light chain immunoglobulins are made up of one V-domain (V_L) and one constant domain (C_L) whereas heavy chains consist of one V-domain and, depending on H chain isotype, three or four C-domains (C_{H1}, C_{H2}, C_{H3} and C_{H4}).

At the N-terminal region of each light or heavy chain is a variable (V) domain that varies greatly in sequence, and is responsible for specific binding to antigen. Antibody specificity for antigen is actually determined by amino acid sequences within the V-regions known as hypervariable loops or Complementarity Determining Regions (CDRs). Each H and L chain V regions possess 3 such CDRs, and it is the combination of all 6 that forms the antibody's antigen binding site. The remaining V-region amino acids which exhibit less variation and which support the hypervariable loops are called framework regions (FRs).

The regions beyond the variable domains (C-domains) are relatively constant in sequence. It will be appreciated that the characterising feature of antibodies according to the invention is the V_H and V_L domains. It will be further appreciated that the precise nature of the C_H and C_L domains is not, on the whole, critical to the invention. In fact preferred antibodies for use in the invention may have very different C_H and C_L domains. Furthermore, as discussed more

fully below, preferred antibody functional derivatives may comprise the Variable domains without a C-domain (e.g. scFV antibodies).

Preferred antibodies considered to be agents according to the third or fourth aspect of the invention may have the V_L (first domain) and V_H (second domain) domains. A derivative thereof may have 75% sequence identity, more preferably 90% sequence identity and most preferably has at least 95% sequence identity. It will be appreciated that most sequence variation may occur in the framework regions (FRs) whereas the sequence of the CDRs of the antibodies, and functional derivatives thereof, should be most conserved.

A number of preferred embodiments of the agent of the third or fourth aspects of the invention relate to molecules with both Variable and Constant domains. However it will be appreciated that antibody fragments (e.g. scFV antibodies or FAbs) are also encompassed by the invention that comprise essentially the Variable region of an antibody without any Constant region.

An scFV antibody fragment considered to be an agent of the third or fourth aspect of the invention may comprise the whole of the V_H and V_L domains of an antibody raised against IFN polypeptide. The V_H and V_L domains may be separated by a suitable linker peptide.

Antibodies, and particularly mAbs, generated in one species are known to have several serious drawbacks when used to treat a different species. For instance when murine antibodies are used in humans they tend to have a short circulating half-life in serum and may be recognised as foreign proteins by the immune system of a patient being treated. This may lead to the development of an unwanted human anti-mouse antibody (HAMA) response. This is particularly troublesome when frequent administration of an antibody is required as it can enhance its clearance, block its therapeutic effect, and induce hypersensitivity reactions. These factors limit the use of mouse monoclonal antibodies in human therapy and have prompted the development of antibody engineering technology to generate humanised antibodies.

Therefore, where the antibody capable of reducing IFN activity is to be used as a therapeutic agent for treating HCV infections in a human subject, then it is preferred that antibodies and fragments thereof of non-human source are humanised.

Humanisation may be achieved by splicing V region sequences (e.g. from a monoclonal antibody generated in a non-human hybridoma) with C region (and ideally FRs from V region) sequences from human antibodies. The resulting 'engineered' antibodies are less immunogenic in humans than the non-human antibodies from which they were derived and so are better suited for clinical use.

Humanised antibodies may be chimaeric monoclonal antibodies, in which, using recombinant DNA technology, rodent immunoglobulin constant regions are replaced by the constant regions of human antibodies. The chimaeric H chain and L chain genes may then be cloned into expression vectors containing suitable regulatory elements and induced into mammalian cells in order to produce fully glycosylated antibodies. By choosing an appropriate human H chain C region gene for this process, the biological activity of the antibody may be pre-determined. Such chimaeric molecules may be used to treat or prevent cancer according to the present invention.

Further humanisation of antibodies may involve CDR-grafting or reshaping of antibodies. Such antibodies are produced by transplanting the heavy and light chain CDRs of a non-human antibody (which form the antibody's antigen binding site) into the corresponding framework regions of a human antibody.

Humanised antibody fragments represent preferred agents for use according to the invention. Human FAbs recognising an epitope on IFN α polypeptide or an IFN receptor may be identified through screening a phage library of variable chain human antibodies. Techniques known to the art (e.g. as developed by Morphosys or Cambridge Antibody Technology) may be employed to generate Fabs that may be used as agents according to the invention. In brief a human combinatorial Fab antibody library may be generated by transferring the heavy and light chain variable regions from a single-chain Fv library into a Fab display vector. This library may yield 2.1×10^{10} different antibody fragments. The peptide may then be used as "bait" to identify antibody fragments from then library that have the desired binding properties.

Domain antibodies (dAbs) represent another preferred agent that may be used according to this embodiment of the invention. dAbs are the smallest functional binding unit of antibodies

and correspond to the variable regions of either the heavy or light chains of human antibodies. Such dAbs may have a molecule weight of around 13kDa (corresponding to about 1/10 (or less) the size of a full antibody).

According to another embodiment of the third and fourth aspects of the invention, peptides may be used to reduce IFN α polypeptide activity. Such peptides represent other preferred agents for use according to the invention. These peptides may be isolated, for example, from libraries of peptides by identifying which members of the library are able to reduce the activity or expression of IFN α polypeptide. Suitable libraries may be generated using phage display techniques.

Aptamers represent another preferred agent of the third or fourth aspect of the invention. Aptamers are nucleic acid molecules that assume a specific, sequence-dependent shape and bind to specific target ligands based on a lock-and-key fit between the aptamer and ligand. Typically, aptamers may comprise either single- or double-stranded DNA molecules (ssDNA or dsDNA) or single-stranded RNA molecules (ssRNA). Aptamers may be used to bind both nucleic acid and non-nucleic acid targets. Accordingly aptamers may be generated that recognise and so reduce the activity or expression of IFN α . Suitable aptamers may be selected from random sequence pools, from which specific aptamers may be identified which bind to the selected target molecules with high affinity. Methods for the production and selection of aptamers having desired specificity are well known to those skilled in the art, and include the SELEX (systematic evolution of ligands by exponential enrichment) process. Briefly, large libraries of oligonucleotides are produced, allowing the isolation of large amounts of functional nucleic acids by an iterative process of *in vitro* selection and subsequent amplification through polymerase chain reaction.

Antisense molecules represent another preferred agent for use according to the third or fourth aspects of the invention. Antisense molecules are typically single-stranded nucleic acids, which can specifically bind to a complementary nucleic acid sequence produced by a gene and inactivate it, effectively turning that gene "off". The molecule is termed "antisense" as it is complementary to the gene's mRNA, which is called the "sense" sequence, as appreciated by the skilled person. Antisense molecules are typically are 15 to 35 bases in length of DNA, RNA or a chemical analogue. Antisense nucleic acids have been used experimentally to bind to mRNA and prevent the expression of specific genes. This has lead

to the development of "antisense therapies" as drugs for the treatment of cancer, diabetes and inflammatory diseases. Antisense drugs have recently been approved by the US FDA for human therapeutic use. Accordingly, by designing an antisense molecule to polynucleotide sequence encoding IFN polypeptide it would be possible to reduce the expression of IFN α polypeptide in a cell and thereby reduce in IFN α activity and reduce the preactivation seen in HCV infection. A polynucleotide sequence encoding an IFN α polypeptide is provided in Figure 8.

Small interfering RNA (siRNA), sometimes known as short interfering RNA or silencing RNA, represent further preferred agents for use according to the third or fourth aspects of the invention. As set out above, the inventors realised that preactivation of the IFN system is associated with a resistance to antiviral therapy. It is therefore apparent that siRNA molecules that can reduce IFN α expression have great utility in the preparation of medicaments for the treatment of HCV infection. siRNA are a class of 20-25 nucleotide-long RNA molecules are involved in the RNA interference pathway (RNAi), by which the siRNA can lead to a reduction in expression of a specific gene, or specifically interfere with the translation of such mRNA thereby inhibiting expression of protein encoded by the mRNA. siRNAs have a well defined structure: a short (usually 21-nt) double-strand of RNA (dsRNA) with 2-nt 3' overhangs on either end. Each strand has a 5' phosphate group and a 3' hydroxyl (-OH) group. *In vivo* this structure is the result of processing by Dicer, an enzyme that converts either long dsRNAs or hairpin RNAs into siRNAs. siRNAs can also be exogenously (artificially) introduced into cells by various transfection methods to bring about the specific knockdown of a gene of interest. Essentially any gene of which the sequence is known can thus be targeted based on sequence complementarity with an appropriately tailored siRNA. Given the ability to knockdown essentially any gene of interest, RNAi via siRNAs has generated a great deal of interest in both basic and applied biology. There is an increasing number of large-scale RNAi screens that are designed to identify the important genes in various biological pathways. As disease processes also depend on the activity of multiple genes, it is expected that in some situations turning off the activity of a gene with a siRNA could produce a therapeutic benefit. Hence their discovery has led to a surge in interest in harnessing RNAi for biomedical research and drug development. Recent phase I results of therapeutic RNAi trials demonstrate that siRNAs are well tolerated and have suitable pharmacokinetic properties. siRNAs and related RNAi induction methods therefore stand to become an important new class of drugs in the foreseeable future. siRNA molecules

designed to nucleic acid encoding IFN α polypeptide can be used to reduce the expression of IFN α and therefore result in a reduction in the preactivation of the IFN system. Hence an embodiment of this aspect of the invention is wherein the agent is a siRNA molecule having complementary sequence to IFN α polynucleotide.

A polynucleotide sequence encoding an IFN α polypeptide is provided in Figure 8.

Using such information it is straightforward and well within the capability of the skilled person to design siRNA molecules having complementary sequence to IFN α polynucleotide. For example, a simple internet search yields many websites that can be used to design siRNA molecules.

By "siRNA molecule" we include a double stranded 20 to 25 nucleotide-long RNA molecule, as well as each of the two single RNA strands that make up a siRNA molecule.

It is most preferred that the siRNA is used in the form of hairpin RNA (shRNA). Such shRNA may comprise two complementary siRNA molecules that are linked by a spacer sequence (e.g. of about 9 nucleotides). The complementary siRNA molecules may fold such that they bind together.

A ribozyme capable of cleaving RNA or DNA encoding IFN α polypeptide represent another preferred agent of the third or fourth aspect of the invention.

It is preferred that the agent of the third or fourth aspect of the invention is able to reduce the activation of the IFN system in a subject to be treated but not to reduce the activity of subsequent antiviral therapy supplied to the subject.

For example, where the agent of the third or fourth aspect of the invention is an antibody or fragment thereof, then it is preferred that the agent can bind to and reduce the activity of endogenous IFN α polypeptide but not exogenously supplied IFN α polypeptide. It is possible to derive such antibodies using methods routine in the art, and the information provided previously in this aspect of the invention.

It will be appreciated that the amount of an agent needed according to the invention is determined by biological activity and bioavailability which in turn depends on the mode of administration and the physicochemical properties of the agent. The frequency of administration will also be influenced by the abovementioned factors and particularly the half-life of the agent within the target tissue or subject being treated.

Known procedures, such as those conventionally employed by the pharmaceutical industry (e.g. *in vivo* experimentation, clinical trials etc), may be used to establish specific formulations of the agents and precise therapeutic regimes (such as daily doses and the frequency of administration).

Generally, a daily dose of between 0.01 µg/kg of body weight and 0.1g/kg of body weight of an agent may be used in a treatment regimen for treating HCV infection; more preferably the daily dose is between 0.01mg/kg of body weight and 100mg/kg of body weight.

By way of example a suitable dose of an antibody according to the invention is 10 µg/kg of body weight – 100mg/kg of body weight, more preferably about 0.1mg/kg of body weight – 10mg/kg of body weight and most preferably about 6mg/kg of body weight.

Daily doses may be given as a single administration (e.g. a single daily injection or a single dose from an inhaler). Alternatively the agent (e.g. an antibody or aptamer) may require administration twice or more times during a day.

Medicaments according to the invention should comprise a therapeutically effective amount of the agent and a pharmaceutically acceptable vehicle.

A “therapeutically effective amount” is any amount of an agent according to the invention which, when administered to a subject inhibits or prevents cancer growth or metastasis.

A “subject” may be a vertebrate, mammal, domestic animal or human being. It is preferred that the subject to be treated is human. When this is the case the agents may be designed such that they are most suited for human therapy (e.g. humanisation of antibodies as discussed above). However it will also be appreciated that the agents may also be used to treat other animals of veterinary interest (e.g. horses, dogs or cats).

A "pharmaceutically acceptable vehicle" as referred to herein is any physiological vehicle known to those skilled in the art as useful in formulating pharmaceutical compositions.

In one embodiment, the medicament may comprise about 0.01 μ g and 0.5 g of the agent. More preferably, the amount of the agent in the composition is between 0.01 mg and 200 mg, and more preferably, between approximately 0.1mg and 100 mg, and even more preferably, between about 1mg and 10mg. Most preferably, the composition comprises between approximately 2mg and 5mg of the agent.

Preferably, the medicament comprises approximately 0.1% (w/w) to 90% (w/w) of the agent, and more preferably, 1% (w/w) to 10% (w/w). The rest of the composition may comprise the vehicle.

Nucleic acid agents can be delivered to a subject by incorporation within liposomes, Alternatively the "naked" DNA molecules may be inserted into a subject's cells by a suitable means e.g. direct endocytotic uptake. Nucleic acid molecules may be transferred to the cells of a subject to be treated by transfection, infection, microinjection, cell fusion, protoplast fusion or ballistic bombardment. For example, transfer may be by ballistic transfection with coated gold particles, liposomes containing the DNA molecules, viral vectors (e.g. adenovirus) and means of providing direct DNA uptake (e.g. endocytosis) by application of the DNA molecules directly to the target tissue topically or by injection.

The antibodies, or functional derivatives thereof, may be used in a number of ways. For instance, systemic administration may be required in which case the antibodies or derivatives thereof may be contained within a composition which may, for example, be ingested orally in the form of a tablet, capsule or liquid. It is preferred that the antibodies, or derivatives thereof, are administered by injection into the blood stream. Injections may be intravenous (bolus or infusion) or subcutaneous (bolus or infusion). Alternatively the antibodies may be injected directly to the liver.

Nucleic acid or polypeptide therapeutic entities may be combined in pharmaceutical compositions having a number of different forms depending, in particular on the manner in which the composition is to be used. Thus, for example, the composition may be in the form

of a powder, tablet, capsule, liquid, ointment, cream, gel, hydrogel, aerosol, spray, micelle, transdermal patch, liposome or any other suitable form that may be administered to a person or animal. It will be appreciated that the vehicle of the composition of the invention should be one which is well tolerated by the subject to whom it is given, and preferably enables delivery of the therapeutic to the target cell, tissue, or organ.

In a preferred embodiment, the pharmaceutical vehicle is a liquid and the pharmaceutical composition is in the form of a solution. In another embodiment, the pharmaceutical vehicle is a gel and the composition is in the form of a cream or the like.

Compositions comprising such therapeutic entities may be used in a number of ways. For instance, systemic administration may be required in which case the entities may be contained within a composition that may, for example, be ingested orally in the form of a tablet, capsule or liquid. Alternatively, the composition may be administered by injection into the blood stream. Injections may be intravenous (bolus or infusion) or subcutaneous (bolus or infusion). The entities may be administered by inhalation (e.g. intranasally).

Therapeutic entities may also be incorporated within a slow or delayed release device. Such devices may, for example, be inserted on or under the skin, and the compound may be released over weeks or even months. Such devices may be particularly advantageous when long term treatment with an entity is required and which would normally require frequent administration (e.g. at least daily injection).

The agents of the first aspect of the invention are particularly useful for pretreating patients about to undergo treatment with antiviral therapy with IFN (e.g. pegIFN) and an antiviral agent such as ribavirin. It is therefore preferred that the agent is administered to a virally infected individual before therapy with IFN and ribavirin is initiated.

The length of time between the pre-treatment with the agents defined in relation to the third and fourth aspect of the invention and the antiviral therapy can depend on the agents used. For example, where the agent is able to reduce the activation of the IFN system in a subject to be treated but not to reduce the activity of subsequent antiviral therapy supplied to the subject, then the length of time can be very short. For example, the subject could be treated concurrently, or even with a combined treatment regime.

If the agent is not distinguishing, then the length of time can depend on the nature of the agent. For example, it is known that exogenously supplied antibody takes around 4 to 6 weeks in order to be cleared from the human body. Therefore, where the agent is an antibody to the IFN α polypeptide or receptor, or other such member of the IFN system, then preferably the subsequent antiviral therapy is supplied to the patient 4 to 6 weeks later, preferably at least 6 weeks.

The various elements required for a technician to perform the method of the first aspect of the invention may be incorporated in to a kit.

Thus, according to a fifth aspect of the invention there is provided a kit for determining the likelihood that a subject having a viral infection of the liver will be responsive to antiviral therapy that includes stimulation of Interferon (IFN) activity, comprising:

- (i) means for analysing in a sample from a subject the expression of at least one gene from each of the groups of genes listed above and shown in Table 2; and, optionally,
- (ii) means for comparing expression of the genes in the sample to expression of the same genes in a control sample.

By "means for analysing in a sample from a subject the expression of at least one gene from each of the groups of genes listed above and shown in Table 2" we include the specific binding molecules given in the first aspect of the invention that can target molecules representative of gene expression in the sample. Preferably the specific binding molecule is an oligonucleotide probe, antibody, aptamers, or binding proteins or small molecules mentioned above.

By "means for comparing expression of the genes in the sample to expression of the same genes in a control sample" we include the control samples mentioned above in the first aspect of the invention. We also include the control reference data mentioned therein.

The kit of the fifth aspect of the invention may also comprise:

(iii) relevant buffers and reagents for analysing the expression of said genes.

The buffers and reagents provided with the kit may be in liquid form and preferably provided as pre-measured aliquots. Alternatively, the buffers and reagents may be in concentrated (or even powder form) for dilution.

The various elements required for a technician to perform the method of the second aspect of the invention may be incorporated in to a kit.

Thus, according to a sixth aspect of the invention there is provided a kit for determining the likelihood that a subject having a viral infection of the liver will be responsive to antiviral therapy that includes stimulation of Interferon (IFN) activity, comprising means for examining a sample from the subject to identify the subcellular location of STAT1.

By "means for examining a sample from the subject to identify the subcellular location of STAT1" we include the specific binding molecules given in the second aspect of the invention that can identify the subcellular location of STAT1. Preferably said specific binding molecule is an anti-STAT antibody; preferably an anti-phospho-STAT1 antibody.

The kit of the sixth aspect of the invention may also comprise:

(iii) relevant buffers and reagents for identifying the subcellular location of STAT1.

The buffers and reagents provided with the kit may be in liquid form and preferably provided as pre-measured aliquots. Alternatively, the buffers and reagents may be in concentrated (or even powder form) for dilution.

All of the features described herein (including any accompanying claims, abstract and drawings), and/or all of the steps of any method or process so disclosed, may be combined with any of the above aspects in any combination, except combinations where at least some of such features and/or steps are mutually exclusive.

The invention will now be further described with reference to the following Example and figures in which:

Figure 1. PegIFN- α 2b induced regulation of gene expression in liver and PBMCs.

- (A) Rapid responders up- or down-regulate significantly more genes in response to pegIFN- α 2b than non-RVR patients. Shown are numbers of genes significantly changed in >60% of patients ($p < 0.05$) >2-, >1.5- or > 1.3-fold in liver and >5- and >2fold in PBMCs.
- (B) Venn diagram of genes significantly ($p < 0.05$) up- or down-regulated >1.5-fold in response to pegIFN- α in at least 60% of patients in the RVR versus the non-RVR group.
- (C) Venn diagram of genes significantly ($p < 0.05$) up- or down-regulated >2-fold in response to pegIFN- α in at least 60% of patients in liver versus PBMCs of RVR patients.

Figure 2. PegIFN- α 2b induced gene regulation in HCV-infected patients shows major differences between livers of RVR and non-RVR patients and between liver and PBMCs.

- (A) Five ISGs (Mx1, viperin, Mda5/helicard, OAS1, USP18) were chosen from the list of genes significantly regulated >1.5-fold between B-1 and B-2 in RVR patients. In liver of non-RVR patients, expression of these genes is already high before treatment (lanes 9-13), and does not further increase after pegIFN α (lanes 20-24). In RVR patients, pre-treatment expression (lanes 3-8) is similar to controls (lanes 1-2), and pegIFN α induces a strong upregulation (lanes 3-8 versus 14-19). No pre-activation is found in PBMCs (lanes 25-46). The y-axes display raw expression values.
- (B) An example of a gene (CCL8) upregulated in liver in response to pegIFN- α 2b in both RVR and non-RVR patients.

Figure 3. RT-qPCR analysis of selected ISGs and of the catalytic subunit of PP2A.

- (A) RT-qPCR analysis of the USP18 mRNA corroborates the array data. Depicted is the fold induction of USP18 mRNA between B-1 and B-2 in individual patients.
- (B) PP2Ac expression is significantly lower in EVR than PNR patients.
- (C) The expression level of selected ISGs in pre-treatment biopsies is lower in EVR than in PNR patients.
- (D) Expression levels of USP18 and IFI27 in pre-treatment biopsies are significantly higher in patients infected with genotype 1 compared to genotypes 2 or 3. In panels B, C and D, the y axis shows expression relative to that of GAPDH. Statistical significance was tested with the

unpaired t-test for USP18 and with the Mann-Whitney test for all other genes. *N* = number of patients in each group.

Figure 4. Analysis of Jak-STAT signaling in liver biopsies.

(A) STAT1 phosphorylation in extracts of liver biopsies collected before (B-1) and after (B-2) pegIFN- α 2b treatment. Extracts were analyzed by Western blot using antibodies specific for PY(701)-STAT1. Most patients showed increase in phosphorylated STAT1 in the liver in response to pegIFN- α 2b. The signals were quantified using the Odyssey Imaging Software to calculate the integrated intensity (kilo counts $\times$ mm²). The values represent the fold increase of phosphorylation in B-2 samples. Blots were stripped and reprobed for total STAT1 used as a loading control for each pair of samples.

(B) Immunohistochemical staining of phospho-STAT1 in liver biopsies reveals a weak nuclear staining in pre-treatment biopsies of non-RVR patients.

Shown are representative examples of B-1 and B-2 of RVR and non-RVR patients. No nuclear staining is evident in pre-treatment biopsies of RVR patients (Pat. 4). The light blue color of nuclei originates from the counterstaining with haematoxylin. 4 h after pegIFN α , most hepatocytes of RVR patients show a strong nuclear staining. In non-RVR patients, a weak nuclear staining is already present in pre-treatment biopsies, and pegIFN α induces little changes in hepatocytes. The visible increased nuclear staining is confined to Kupffer cells.

Figure 5.

The predominant pattern of gene expression in all patient biopsy samples is shown as a heat map. The map was generated using a list of 176 genes that are altered > 2 fold in at least 60% of RVRs with a *p* value of < 0.05 . The colour-coding of the raw expression values is shown on the left. Many genes have a low expression level in the control patients and the pre-treatment biopsies of the RVR patients (B-1), and comparable high expression levels in B-1 of non-RVR patients and in all B-2 samples.

Figure 6.

Supervised classifier prediction in liver biopsy samples and PBMCs with response to treatment at week 4 as grouping criterium.

- (A and B) Supervised classifier prediction of PBMC-1 and PBMC-2 samples did not generate a useful list of predictive genes with any of the 4 statistical tests used (Support Vector Machine, Sparse Linear Discriminant Analysis, Fisher Linear Discriminant Analysis, K Nearest Neighbors). The misclassification rates were 52% for PBMC-1 and 47% for PBMC-2.
- (C) Supervised classifier prediction using the B-2 biopsies of the two response groups revealed a list of 173 genes (180 transcripts) as best predictors of treatment outcome with a misclassification rate of 14%.
- (D) Supervised classifier prediction using the B-1 biopsies of the two response groups resulted in a list of 83 genes (91 transcripts) as best predictors of treatment outcome with a misclassification rate of 9%.

Figure 7

(A) Semiquantitative assessment of immunohistochemical staining of phospho-STAT1 in liver biopsies. Nuclear staining of hepatocytes was quantified by repeated counting (5 times) in 200 hepatocytes in B-1 (blue) and B-2 (red) samples of the indicated patients (patient numbers correspond to the numbers in table 1). In four out of five non-RVR patients, a considerable proportion of hepatocytes had a weak but clear nuclear staining already in the pre-treatment biopsies. All the RVR patients had no phospho-STAT1 signals in the nuclei before treatment, but showed a strong induction after pegIFN α .

(B) The induction of STAT-DNA binding in response to pegIFN α 2b is impaired in most of the non-RVR patients. Nuclear extracts from B-1 and B-2 samples were analyzed with EMSAs using the radiolabeled SIE-m67 oligonucleotide probe. The asterisk (*) depicts the signal of the activated STAT1 dimers that have bound the oligonucleotide sequence. The numbers above the gel shift panels represent the patient numbers. The upper panel shows the 6 patients with a rapid response at week 4 (numbers 1-6). The lower panel shows the 5 patients without a virological response at week 4 (numbers 7-11).

Figure 8: Amino acid and nucleotide sequences of human Interferon α .

Figure 9: Amino acid and nucleotide sequence of human Interferon Receptor 1.

Figure 10: Amino acid and nucleotide sequence of human Interferon Receptor 2.

Figure 11: Amino acid and nucleotide sequence of human Interferon Receptor 2b.

Figure 12: Amino acid and nucleotide sequence of human Interferon Receptor 2c.

Example 1

1.1 Methods

1.1.1 Subject samples and treatment

Paired human liver biopsy samples from 11 chronically infected HCV subjects were obtained. From January 2006 to April 2007, all subjects with chronic hepatitis C referred to the outsubject liver clinic of the University Hospital Basel were asked for their permission to use part of their diagnostic liver biopsy for research purposes. Liver biopsies were obtained by ultrasound-guided technique using a coaxial needle.

After removal of two 20- to 25-mm long biopsy specimens for routine histopathological workup for grading and staging of the liver disease according to the Metavir scoring system, the remaining 5- to 20-mm long biopsy cylinders were labeled as B1 (for biopsy 1) and stored as pretreatment samples of future study participants. Pegylated-IFN α 2b (Essex Chemie AG, Switzerland) was prescribed to all subjects participating in this study. Second biopsy (B2) was performed 4 hours following the first pegIFN α 2b injection. The first dose of ribavirin was given after this second biopsy to avoid further confounding factors. The protocol was approved by the Ethics Committee of the University Hospitals in Basel. Written informed consent was obtained from all subjects.

In addition, blood for peripheral blood mononuclear cell (PBMC) isolation was collected before treatment and 4 hours after the first pegIFN α 2b injection.

The HCV subjects underwent a standard combination treatment with pegIFN α 2b (1.5 μ g/kg body weight) and ribavirin (weight based dosing: <65 kg: 800 mg/d; 65-85 kg: 1 g/d; >85 kg: 1,2 g/d). HCV-RNA was quantified before treatment initiation, at week 4 and week 12 of the treatment (Table 1). Treatment duration is 24 weeks for subjects with genotypes 2/3 and 48 weeks for genotype 1. From the 11 subjects included in the study, 2 subjects (Nr. 10 and 11)

had a primary non-response and treatment was stopped at week 12. From the remaining 9 subjects, 2 (Nr. 1, 2) have accomplished the therapy with an end of treatment response.

As non-HCV controls, two subjects that underwent ultrasound-guided liver biopsies of focal lesions (metastasis of carcinomas) gave informed consent for a biopsy from the normal liver tissue outside the focal lesion. Again, a part of the biopsy was used for routine histopathological diagnosis, and the remaining tissue for the extraction of RNA, as described later. Both control samples showed confirmed absence of liver disease in the routine histopathological workup.

1.1.2 Measurement of IFN alpha serum concentrations

Pretreatment hIFN α serum levels and the serum concentration of pegIFN α 2b 4 h after the first injection were measured using the human interferon alpha ELISA kit from PBL Biomedical Laboratories according to manufacturer's instructions. This ELISA kit has previously been shown to recognize both unpegylated and pegylated human IFN α ³⁴.

1.1.3 Preparation of extracts from human liver biopsies

Liver biopsy samples were used for the preparation of whole cell, cytoplasmic and nuclear extracts. For whole cell extracts, samples were dounce homogenized in 100 μ l of lysis buffer containing 100 mmol/l NaCl, 50 mmol/l Tris pH 7.5, 1 mmol/l EDTA, 0.1% Triton X-100, 10 mmol/l NaF, 1 mmol/l phenylmethyl sulfonyl fluoride, and 1 mmol/l vanadate. Lysates were centrifuged at 14,000 rpm at 4°C for 5 minutes. Protein concentration was determined by Lowry (BioRad Protein Assay).

For nuclear and cytoplasmic extracts, livers were lysed in a low-salt buffer containing 200 mmol/l Hepes pH 7.6, 10 mmol/l KCl, 1 mmol/l EDTA, 1 mmol/l EGTA, 0.2% NP-40, 10% glycerol, and 0.1 mmol/l vanadate. After centrifugation at 15,000 rpm for 5 minutes, the pellet was resuspended in high-salt buffer (low-salt buffer supplemented with 420 mmol/L NaCl). After centrifugation, aliquots of nuclear extracts were made for electrophoretic mobility shift assays (EMSAs).

1.1.4 Western blots and Electrophoretic Mobility Shift Assays

10 μ g of total protein from human liver lysates was loaded for sodium dodecyl sulphate–polyacrylamide gel electrophoresis and transferred onto a nitrocellulose membrane

(Schleicher & Schuell, Bottmingen, Switzerland). The membranes were blocked in 3% BSA/milk (1:1)-0.1% Triton X-100 for 1 hour, washed with Tris-buffered saline Tween-20 (TBST), and incubated with the primary antibody overnight at 4°C.

Proteins were detected with primary antibodies specific to phosphorylated STAT1 (PY(701)-STAT1; Cell Signaling, Bioconcept, Allschwil, Switzerland) and STAT1 (carboxy-terminus; Transduction Laboratories, BD Biosciences, Pharmingen). After 3 washes with TBST, the membranes were incubated with infrared fluorescent secondary goat anti-mouse (IRDye 680) or anti-rabbit (IRDye 800) antibodies (both from LI-COR Biosciences) for 1 hour at room temperature. Blots were analyzed by the Odyssey Infrared Imaging System from LI-COR. The infrared image was obtained in a single scan and the signal was quantified using the integrated intensity.

For loading controls, the membranes were stripped and incubated with anti- β -Actin antibody (Sigma).

EMSA were performed using 2 μ g of nuclear extracts and 32 P-radiolabeled DNA-oligonucleotide serum inducible element (SIE)-m67 corresponding to STAT response element sequences²⁵.

1.1.5 Immunohistochemistry

Standard indirect immunoperoxidase procedures were used for immunohistochemistry (ABC-Elite, Vectra Laboratories). 4-mm-thick sections were cut from paraffin blocks, rehydrated, pretreated (20' in ER2 solution) incubated with a monoclonal rabbit antibody against phospho-STAT1 (dilution 1:200, #9167 Cell Signaling) and counterstained with haematoxylin. The whole staining procedure (dehydration, pre-treatment, incubation, counterstaining and mounting) was performed with an automated stainer (Bond®, Vision BioSystems Europe, Newcastle-upon-Tyne, UK). For quantification of nuclear phospho-STAT1 staining, 5 times 200 hepatocytes were counted for each B-1 and B-2 sample of each patient. In supplementary Fig. 3, the mean values with the standard deviations are shown.

1.1.6 RNA isolation and Microarray analysis

Total RNA was extracted from liver and PBMC samples using the RNeasy Mini Kit (Qiagen) according to manufacturer's instructions. RNA was aliquoted and stored at - 80°C. Gene expression was assessed in liver and PBMCs by microarray analysis using Affymetrix Human Genome U133 Plus 2.0 arrays representing over 56,000 transcripts and variants with 11 perfect-match/mis-match probe pairs per transcript. The microarray hybridizations were performed at the functional genomics facility of the Friedrich Miescher Institute for Biomedical Research in Basel. Total RNA (1-2 µg) from each sample was reverse transcribed and biotinylated using the Affymetrix 1-cycle amplification kit as per manufacturer's instructions. Biotinylated cRNA (20 µg) was fragmented by heating with magnesium (as per Affymetrix's instructions) and 15 µg of fragmented cRNA was hybridized to Human U133 Plus 2.0 GeneChips according to the manufacturer's instructions. Quality control and background normalization was performed using Refiner 4.1 from Genedata AG (Basel, Switzerland). Expression value estimates were obtained using the GC-RMA implementation in Refiner 4.1. LOWESS-normalization and median scaling of the genes called present (detection P-value < 0.04) to a value of 500 was performed in Genedata's Analyst 4.1 package. The LOWESS-normalized data are referred to as 'raw' expression values in this paper. We also performed a point-wise division on the genes by dividing each gene by its median in order to centre its expression level at 1.0. This scaled data shows only the magnitude and direction of change but not of absolute expression level. Scaled data were used for clustering analyses. Unless noted, all other analyses were performed using the raw data.

Data analysis was performed using Expressionist® Analyst 4.1 from Genedata AG. Genes were required to pass a t-test with a $P < 0.05$ and have a median fold change of 1.3, 1.5, 2 and 5 or greater between the paired patient samples in at least 60% of patients within each group. For the supervised classifier prediction of liver biopsy samples and PBMCs using the response at week 4 as a grouping criterion 4 statistical tests were used (Support Vector Machine, Sparse Linear Discriminant Analysis, Fisher Linear Discriminant Analysis, K Nearest Neighbors). The misclassification rates could be determined for every test used and the one with the lowest rate was selected.

1.1.7 RNA isolation, reverse transcription and SYBR-PCR

The array data were validated by quantitative real-time RT-PCR analysis of several IFN regulated genes including STAT1, IP10, USP18, IFI27 SOCS1 and SOCS3.

Total RNA was extracted from liver using the RNeasy Mini Kit (Qiagen) according to manufacturer's instructions. The RNA was reverse transcribed by Moloney murine leukemia virus reverse transcriptase (Promega Biosciences, Inc., Wallisellen, Switzerland) in the presence of random hexamers (Promega) and deoxynucleoside triphosphate. The reaction mixture was incubated for 5 min at 70°C and then for 1 h at 37°C. The reaction was stopped by heating at 95°C for 5 min. SYBR-PCR was performed based on SYBR green fluorescence (SYBR green PCR master mix; Applied Biosystems, Foster City, CA). Primers for GAPDH (glyceraldehyde-3-phosphate dehydrogenase), STAT1, inducible protein 10 (IP10), SOCS1, SOCS3, USP18, IFI27 and PP2Ac were designed across exon-intron junctions. The primer sequences are shown in Table 4. The difference in the cycle threshold (ΔC_T) value was derived by subtracting the C_T value for GAPDH, which served as an internal control, from the C_T value for STAT1 or other transcripts of interest. All reactions were run in duplicate by using an ABI 7000 sequence detection system (Applied Biosystems). mRNA expression levels of the transcripts were calculated relative to GAPDH from the ΔC_T values using the formula $2^{-\Delta C_T}$. The change of expression in paired liver biopsy samples was calculated as a fold change according to the formula $2^{(\Delta C_T \text{ B-1} - \Delta C_T \text{ B-2})}$.

Box plot diagrams, unpaired t-tests and Mann Whitney tests were performed using GraphPad Prism version 4.00 for Macintosh, GraphPad Software, San Diego California USA, www.graphpad.com.

1.2 Results

Patients and Response to Treatment

11 patients included in this study, 4 women and 7 men, were treated with a weight-adjusted combination of subcutaneously injected pegIFN α 2b once weekly and oral ribavirin twice daily. All of them had two liver biopsies, the pre-treatment biopsy (B-1) and the second biopsy (B-2) obtained 4 h after the first injection of pegIFN α 2b. We have chosen to analyze gene expression 4 h after pegIFN α 2b injection since kinetics of the induction of ISGs by pegIFN α in liver of chimpanzees was maximal at this time and was followed by a rapid down-regulation of many genes (22). We realize that we probably missed the up-regulation of

some late induced ISGs, but because of the rapid down-regulation, we would have missed more ISGs when using later time-points.

Five of the patients were infected with HCV genotype 1, and 3 patients each with genotype 2 and 3 (Table 1). 6 patients had negative serum HCV RNA after 4 weeks of treatment and were classified as RVRs, whereas 5 patients were still positive for HCV RNA at week 4 (non-RVRs). All 6 patients with an RVR were still HCV RNA negative at week 12 (EVRs). 2 of the 5 non-RVR patients showed $>2 \log_{10}$ drop of the viral load after 12 weeks of therapy, and were classified as EVR. 3 patients were non-responders at week 12 (primary non-responders, PNR) (Table 1).

Serum IFN α concentrations were below the limit of detection in all patients before treatment, and, in accordance with previously published pharmacokinetic data (24), between 34 and 360 pg/ml in samples obtained at 4 h after the pegIFN α 2b injection (data not shown). There was no significant correlation between the virological response at week 4 and the serum IFN α concentration at 4 h post-injection. Furthermore, despite the differences in the serum IFN α levels, all patients showed similar ISG induction in PMBCs (see below).

IFN-induced regulation of target genes

Gene expression was analyzed with Affymetrix U133plus2.0 arrays in B-1 and B-2 samples, and also in PMBCs isolated from blood obtained before (PMBC-1) and 4 h after the first pegIFN α 2b injection (PMBC-2). For each patient, the genes that were up- or down-regulated more than 1.3-, 1.5-, 2- or 5-fold in post-treatment samples were identified and saved in gene lists. These lists were used to identify all genes that were significantly ($p < 0.05$) up- or down-regulated in at least 60% of the patients in the RVR and non-RVR groups. In this analysis, RVR patients were found to up- or down-regulate a larger number of genes than patients without a RVR (Fig. 1A). For example, in the RVR group, 500 genes were significantly changed >1.5 -fold, but only 149 in the non-RVR samples. Overall, 3-5 times more genes were induced in the liver within the first 4 h after pegIFN α 2a injections in RVR versus non-RVR patients. There was also a difference in the PMBC samples, but only between 1.4- and 1.8-fold (Fig. 1A). There was an overlap in the significantly regulated genes found in RVR samples and non-RVR samples. For example, 90 of the 149 genes that changed more >1.5 -fold in non-RVR samples were also present among 500 genes changed in the RVR group (Fig. 1B).

Not surprisingly, many of the regulated genes represent known ISGs. However, contrary to our expectations, expression levels of these ISGs were not higher in post pegIFN α 2b treatment biopsies from RVR patients as compared to non-RVRs. Rather, non-RVR patient samples had a higher level of ISG expression already in B-1, and the fold change in the B-2 samples was therefore only minor. This is illustrated in Fig. 2A at the example of five ISGs: Mx1, viperin, Mda5/helicard, oligoadenylate synthetase 1 (OAS1), and USP18. The genes show a very low expression in biopsies from individuals without hepatitis C and in B-1's of RVR patients. The 5 non-RVR patients had high expression of these genes before treatment, and pegIFN α 2a administration not or only minimally increased their expression. There were very few exceptions to this rule (an example is shown in Fig. 2B). These genes had low expression in the pre-treatment biopsies, and pegIFN α 2b induced them in all patients. Nevertheless, the predominant pattern of gene expression resembled this shown in Fig. 2A. A heat map of the expression of 176 genes significantly changed > 2 fold between B-1 and B-2 in the RVR group has been done for all biopsy samples.

There was a considerable overlap of pegIFN α 2b-regulated genes in liver and PBMCs (Fig. 1C). 133 of the 176 ISGs significantly regulated >2-fold in RVRs in the liver were also significantly changed in the PBMCs of these patients. Interestingly, in all patients pegIFN α 2b regulated more genes in PBMCs than in liver. However, there was no significant difference in the upregulation of ISGs in PBMCs between RVRs and non-RVRs. No pre-activation of ISGs was found in PBMCs of non-RVRs, and pegIFN α 2b treatment had the same effect on ISG regulation in RVR and non-RVR patients (for representative ISG examples, see Fig. 2). This indicates that chronic HCV infection has strong local effects on the IFN system in liver, but little effect in PBMCs.

A subset of genes that predicts response to treatment

Supervised classifier analysis of array data allows the identification of a subset of genes that best predicts the outcome, in our case rapid response versus non-response at week 4. All liver biopsy and PBMC data sets were subjected to supervised classifier prediction using the response at 4 weeks of treatment as grouping criteria. For PBMC samples the analysis did not identify a subset of genes that could predict the treatment outcome. In contrast, a subset of 173 genes was identified in the liver B-2 samples that allowed to predict the response to treatment with an error rate of 14%. Even better prediction was possible with a subset of 83

genes in the pre-treatment biopsies B-1 where the error rate was 9%. In this set there were 22 genes up-regulated and 5 genes down-regulated by pegIFN α 2b (Table 2). Therefore, 27 of the 83 (33%) best predictor genes represent IFN-regulated genes.

Contrary to the predominance of IFN-regulated genes in the best predictor set from pre-treatment biopsies, only few IFN-regulated genes were found in the set of best predictor genes derived from an analysis of the B-2 biopsies. Of the 173 different genes in this set, only 4 known IFN-regulated genes were up-regulated and 1 was down-regulated by pegIFN α 2b (Table 3). These results support the findings shown in Fig. 2 that expression levels of the IFN-regulated genes in B-2 do not differ between RVR and non-RVR samples and therefore are not suited for the discrimination of responders from non-responders. Among the non-IFN-regulated genes present in the B1 and B2 liver biopsies lists discussed above are genes having functions in signal transduction, cell cycle regulation, apoptosis, and amino acid and lipid metabolism.

RT-qPCR analysis of ISG expression in liver biopsies

Array analysis of the paired liver biopsies emphasized the importance of ISG expression in B-1 biopsies for the outcome of therapy. To confirm these data, we measured by real time quantitative PCR (RT-qPCR) the expression of selected ISGs (USP18, Stat1, IP10, IFI27) in 11 patients with B1 and B2 biopsies, and in pre-treatment biopsies of 51 additional patients with CHC. In the 11 patients with the paired biopsies, the RT-qPCR values matched well the array expression, validating the quality of the array data (Fig. 3A, and data not shown). The expression of all four ISGs in pre-therapy biopsies was significantly different between the EVR and PNR groups (Fig. 3C), further supporting the conclusion that there is an inverse correlation between the pre-treatment expression of ISGs in liver and the response to IFN α therapy.

Pre-treatment ISG expression levels correlate with HCV genotype

We also analyzed the expression of selected ISGs with regard to the HCV genotype (Fig. 3D). Interestingly, the investigated ISGs showed significantly higher expression in patients infected with the "difficult-to-treat" genotypes 1 and 4 than with genotypes 2 and 3, which can be successfully treated in over 80% of patients. Increased expression of ISGs in liver of patients infected with HCV genotypes 1 and 4 provides a plausible explanation for the poor response of these patients to IFN therapy.

Non-responders have higher expression of PP2Ac

We have previously shown that the catalytic subunit of PP2A (PP2Ac) is over-expressed in liver of patients with CHC compared to controls, and that over-expression of PP2Ac inhibits IFN α signaling (14, 25). We therefore analyzed the PP2Ac mRNA levels in a group of patients with known treatment responses at week 12. Patients of the EVR group expressed significantly less PP2Ac mRNA than PNR patients (Fig. 3B).

IFN-induced Jak-STAT signaling

The injected pegIFN α 2b binds to IFN receptors and activates the Jak-STAT pathway. A central event in this activation is the phosphorylation of STAT1 on tyrosine 701 (26). We analyzed extracts from all B-1 and B-2 biopsies by Western blot using a phospho-specific STAT1 antibody (Fig. 4A). In most patients, STAT1 phosphorylation was induced in response to pegIFN α 2b injection. The strongest induction was observed in two patients (1 and 4) later RVRs, while the weakest induction was found in patients 8 and 10 who did not clear the virus within the first 4 weeks. However, the remaining patients had similar 2- to 3.8-fold induction regardless of their virological response at week 4. Hence, STAT1 phosphorylation is not significantly impaired in non-RVR patients.

Phosphorylated STAT1 translocates into the nucleus and binds as a dimer to specific response elements of ISG promoters (26). Assessment of nuclear translocation by immunohistochemistry, using anti-phospho-STAT1 antibodies, should potentially allow to discriminate between STAT1 activation in hepatocytes and other cells present in the biopsy material. Analysis of paired biopsies of RVR patients revealed a minimal nuclear staining in B-1 samples and a strong staining in most hepatocyte nuclei in B-2 samples, following injection of pegIFN α (Fig. 4B). In contrast, all but one (number 7) non-RVR patients showed a remarkably different staining pattern. In the pre-treatment biopsies, a large proportion of hepatocytes already had an appreciable nuclear staining, which did not increase in B-2 samples. The visible increase in nuclear staining in B-2 samples of non-RVR patients originated from nuclear translocation of STAT1 in Kupffer cells (liver macrophages), and not hepatocytes (Fig. 4B). Activation of STAT1 in Kupffer cells, and possibly contaminating blood cells, may have contributed to the increased STAT1 phosphorylation observed in Western blotting (Fig. 4A).

The next step in the signaling pathway is the binding of nuclear phospho-STAT1 to promoter elements of ISGs. We therefore assessed the STAT1 DNA-binding in extracts of B-1 and B-2 biopsies by performing electrophoretic mobility shift assays (EMSAs). All rapid responders showed a marked increase in the STAT1 DNA binding in the B-2 samples. In contrast, most non-RVR patients showed a minimal or no increase of the gel shift signal upon pegIFN α application.

These data indicate that results of immunohistochemistry and EMSA assays correlate better with the therapy outcome than results of Western analysis for phospho-STAT1. Taken together, the data demonstrate substantial differences in the IFN-induced Jak-STAT signaling between RVR and non-RVR patients.

1.3. Discussion

To learn more about possible mechanisms underlying differential response of HCV-infected patients to IFN therapy, we investigated the IFN-induced signaling and ISG induction in paired liver biopsies collected from patients with CHC before and during therapy pegIFN α . Comparison of IFN signaling in two liver samples obtained from the same patient, and comparison with the ISG induction in matching PBMC samples originating from the same patient, allowed us to obtain unequivocal evidence that patients who respond poorly to the therapy show pre-activation of their IFN system, and that the pre-activation is confined to the liver and is not evident in PBMCs. Importantly, in patients with a low initial ISG expression, representing future responders to therapy, activation of the IFN system in response to pegIFN α did not exceed that seen in non-responders, either before or after therapy. This could suggest that patients with the initial pre-activation of the IFN system, future non-responders, have some defects at steps downstream of ISG expression, making them refractory to both endogenous IFN and IFN therapy.

IFN α treatment induced the STAT1 phosphorylation in all but one patient. There was a tendency for stronger STAT1 activation in RVR compared to non-RVR samples. However, the immunohistochemical analysis revealed a more pronounced difference. In non-RVR samples, pegIFN α strongly induced the nuclear STAT1 translocation in Kupffer cells, contrary to RVR samples, where nuclear STAT1 accumulation was induced predominantly in hepatocytes. Interestingly, non-RVR patients (with one exception) had nuclear phospho-STAT1 already present in pre-treatment biopsies. This is consistent with the observation that

ISG transcripts are up-regulated in pre-treatment biopsies of later non-responders. How this preactivation of the Jak-STAT pathway is connected to the refractoriness of the IFN system in non-RVR patients requires further investigations.

Over the last few years, important insights into the interference of HCV with the innate immune system have been gained. Foremost, a series of elegant papers demonstrated the ability of HCV to inhibit both TLR3-TRIF-IRF3 and the RIG-I/MDA5-Cardif signaling pathways of IFN β induction (27-33). This capacity of HCV could help to explain why the virus often establishes a chronic infection. However, our data and previously published results (20) demonstrate that the endogenous IFN system is constantly activated in many patients. Moreover, patients with a pre-activated IFN system seem to respond poorly to IFN therapy. This finding is counter-intuitive (one would expect that an active innate immune system would help to eliminate the virus during IFN α therapy), but it is largely supported by other published data from chimpanzees and human patients (16, 17, 20). From the analyses of ISG expression in liver biopsies it is apparent that in some patients HCV induces (or at least does not block) the endogenous IFN system, while in others it successfully represses it, possibly by cleaving TRIF and/or Cardif. Paradoxically, this difference has no apparent impact on the ability of HCV to maintain a chronic infection.

In patients without pre-activated IFN system, pegIFN α 2b induced a robust up-regulation of many ISGs in the liver within 4 h. Similar high ISG expression was already present in the pre-treatment biopsies of patients that later did not show a rapid virological response at week 4. It is somewhat perplexing why the latter patients do not resolve the chronic HCV infection spontaneously despite the strong activation of the IFN system. One possibility is that ISG proteins that are up-regulated in both cases possess different post-transcriptional modifications. In an alternative scenario, non-response to both endogenous and exogenous IFN α may be caused by the lack of induction of a few critical ISGs that are specifically required for the elimination of HCV. We cannot exclude this possibility, but an array analysis performed on paired liver samples did not reveal ISGs that were specifically up-regulated in rapid responders. Furthermore, this model cannot explain why pre-activation of the endogenous IFN system is so closely linked to later non-response to treatment.

Alternatively, the kinetics of induction of the interferon response could be decisive. In the patients without pre-activated IFN system, the injection of exogenous IFN α during treatment

should induce an antiviral state very rapidly in most liver cells, and HCV would not have "enough" time to escape from the IFN-induced defense. On the other hand, the build-up of the antiviral state could be slow in the other group of patients which would give HCV enough time to adapt to and evade the intracellular antiviral defense system, making it also resistant to the subsequent IFN therapy.

How could the induction of the endogenous IFN system compromise the success of IFN α therapy? Clearly, the activation of negative feedback loops that inhibit IFN signaling could play a role. Prominent candidates amongst the negative regulators are: suppressors of cytokine signaling 1 (SOCS1) and SOCS3 (34), two IFN induced proteins that bind to the IFN receptor and inhibit the activity of Jak1 and Tyk2; and the more recently described regulator Ubp43, an IFN-stimulated protein that binds to IFN α receptor 2 (IFNAR2) and blocks the access of Jak1 to it (35). However, we could not find a significant difference in the expression levels of these negative regulators in the pegIFN α 2b stimulated liver biopsies of RVR compared to non-RVR patients (data not shown). Moreover, a general up-regulation of negative regulators such as SOCSs and Ubp43 is not compatible with the observed strong constitutive expression of a large number of ISGs in the subset of patients that poorly respond to IFN therapy. If IFN α signaling were indeed inhibited by the induction of SOCSs and Ubp43 in the majority of liver cells, then one should not observe such a pronounced pre-activation of ISGs in pre-treatment livers.

Notably, the pre-activation of tested ISGs occurred more frequently in liver biopsies of patients infected with HCV genotype 1 and 4 than with genotypes 2 or 3. It is well known that genotype 2 and 3 infections can be cured in over 80% of patients, compared to less than 50% of infections with genotype 1 (4). Our finding that the frequency and degree of pre-activation of the endogenous IFN system depends on the HCV genotype could provide an explanation for this differential susceptibility. Perhaps HCV genotypes 2 and 3 are more successful in preventing the activation of innate immunity in the liver by a more effective cleavage of Cardif and/or TRIF. The success of the virus in preventing the induction of the endogenous IFN system would however come at the cost of being more susceptible to IFN α therapies. Of note, a single chimpanzee infected with the genotype 3 HCV has been shown to have lower ISG expression levels than animals infected with genotype 1 (17).

We have shown previously that HCV inhibits the IFN α -induced signaling via the Jak-STAT pathway by up-regulating a protein phosphatase PP2A (12, 14, 25, 36). PP2A is a heterotrimeric complex of a scaffolding A, a regulatory B, and a catalytic C subunits. The PP2Ac subunit expression is significantly higher in livers of patients infected with genotype 1 than genotype 3 (25). As shown in this work, the expression of PP2Ac mRNA is higher in biopsies of later non-responders than responders. These data support a model where HCV interference with the IFN signaling impairs the response to therapy. Moreover, inhibition of the IFN α signaling by HCV could also explain why the strong pre-activation of the endogenous IFN system does not lead to a spontaneous elimination of HCV. If one assumes that not all hepatocytes are infected by HCV, but rather a minority, then the induction of ISGs observed in pre-treatment biopsies of non-RVR patients could occur predominantly in non-infected hepatocytes. In the infected cells, IFN would be ineffective because of the inhibition of the Jak-STAT signaling pathway. The IFN responsible for the pre-activation of the system would be secreted by hepatocytes that are infected with a virus that is not successful in cleaving Cardif and/or TRIF. Because of the HCV-induced inhibition of the Jak-STAT pathway, the secreted IFN β would not induce an antiviral state in the infected hepatocytes, but rather in non-infected neighbor cells. To gain further insights into the pathobiology of CHC, future studies should focus on analysis at the single-cell level. Unfortunately, the detection of HCV infected hepatocytes in liver biopsies is still unsatisfactory, making such studies difficult.

Although the precise mechanism of the HCV escape from the immune defense system still remains to be elucidated, the impairment of the hepatitis C therapy by pre-activation of the endogenous IFN system is now well established. It would be interesting to investigate if this pre-activation is a reversible process. The injection of neutralizing anti-IFN α/β antibodies or other factors blocking the IFN response before treatment could return the endogenous IFN system to a "naïve" state, and potentially enhance the response to IFN α based therapies.

Table 1

Patient Nr.	4 week response	Sex (m/f)	Age (years)	HCV genotype	Baseline VL (log IU/ml)	4 week VL (log IU/ml)	12 week VL (log IU/ml)	12 week response	Metavir (grade/stage)	Weight (kg)
1	RVR	m	52	3a	7.14	neg.	neg.	EVR	A2/F2	75
2	RVR	m	37	3a	4.90	neg.	neg.	EVR	A1/F2	73
3	RVR	m	38	1a	6.91	neg.	neg.	EVR	A2/F1	85
4	RVR	m	33	2b	6.27	neg.	neg.	EVR	A1/F2	57
5	RVR	m	48	2b	6.67	neg.	neg.	EVR	A3/F4	110
6	RVR	f	53	2a/c	4.95	neg.	neg.	EVR	A3/F3	74
7	Non- RVR	f	54	3a	4.52	4.08	1.3	EVR	A3/F4	69
8	Non- RVR	m	64	1b	6.24	4.83	3.46	EVR	A3/F4	74
9	Non- RVR	m	56	1b	6.89	6.76	6.01	PNR	A2/F3	60
10	Non- RVR	f	50	1a	7.11	6.58	6.35	PNR	A1/F2	77
11	Non- RVR	f	47	1a	6.16	5.99	5.52	PNR	A2/F2	81

Table 2

Analysis of gene expression in pre-treatment biopsies (B-1). List of 83 genes best predicting treatment outcome at week 4 (IFN regulated genes shaded in grey, up-regulated genes in bold, down-regulated genes italic; genes that differ between RVR and non-RVR but are not regulated by IFN are not shaded).

Gene Symbol	Description	Affy-ID	Function
RPS5	ribosomal protein S5, ribosomal protein S5	200024_at	protein biosynthesis
LGALS3BP	lectin, galactoside-binding, soluble, 3 binding protein	200923_at	response to stress
RPL3	ribosomal protein L3	201217_x_at	protein biosynthesis
LOC201725, TOMM7	translocase of outer mitochondrial membrane 7 homolog (yeast), hypothetical protein LOC201725	201812_s_at	Unknown
CDK4	cyclin-dependent kinase 4	202246_s_at	cell cycle
IFI27	interferon, alpha-inducible protein 27	202411_at	innate immune response
C7	Complement component 7	202992_at	complement activation
SLPI	secretory leukocyte peptidase inhibitor	203021_at	Peptidase
IFIT1	interferon-induced protein with tetratricopeptide repeats 1	203153_at	innate immune response
MME	membrane metallo-endopeptidase (neutral endopeptidase, enkephalinase, CALLA, CD10)	203434_s_at	cell communication
RAB4A	RAB4A, member RAS oncogene family	203582_s_at	signal transduction
IFI44L	interferon-induced protein 44-like	204439_at	cell cycle
OAS2	2'-5'-oligoadenylate synthetase 2,	204972_at	innate immune

Gene Symbol	Description	Affy-ID	Function
	69/71kDa		response
<i>BCHE</i>	butyrylcholinesterase	205433_at	cholinesterase
PPP1R1A	protein phosphatase 1, regulatory (inhibitor) subunit 1A	205478_at	signal transduction
G1P2	interferon, alpha-inducible protein (clone IFI-15K)	205483_s_at	innate immune response
SRPX2	sushi-repeat-containing protein, X-linked 2	205499_at	Unknown
OAS1	2',5'-oligoadenylate synthetase 1, 40/46kDa	205552_s_at	innate immune response
GSTM5	glutathione S-transferase M5	205752_s_at	Glutathione S-transferase
<i>FOLH1</i>	folate hydrolase (prostate-specific membrane antigen) 1	205860_x_at	cellular macromolecule metabolism
PPM1E	protein phosphatase 1E (PP2C domain containing)	205938_at	signal transduction
ACSM3	acyl-CoA synthetase medium-chain family member 3	205942_s_at	fatty acid synthesis
ACADL	acyl-Coenzyme A dehydrogenase, long chain	206068_s_at	fatty acid metabolism
IRF7	interferon regulatory factor 7	208436_s_at	innate immune response
YBX1	Y box binding protein 1	208628_s_at	cellular metabolism
DCN	Decorin	209335_at	Unknown
HTATIP2	HIV-1 Tat interactive protein 2, 30kDa	209448_at	Apoptosis
OSBPL1A	oxysterol binding protein-like 1A	209485_s_at	alcohol metabolism
PHTF1	putative homeodomain transcription factor 1	210191_s_at	gene transcription

Gene Symbol	Description	Affy-ID	Function
HIST1H2BG	histone 1, H2bg	210387_at	DNA packaging
KYNU	kynureninase (L-kynurenine hydrolase)	210663_s_at	amino acid metabolism
TRIM5	tripartite motif-containing 5	210705_s_at	protein ubiquitination
ENPP2	ectonucleotide pyrophosphatase/phosphodiesterase 2 (autotaxin)	210839_s_at	signal transduction
TXNRD2	thioredoxin reductase 2	211177_s_at	response to stress
<i>PSMAL</i>	prostate-specific membrane antigen-like	211303_x_at	Unknown
RPLP0	ribosomal protein, large, P0, ribosomal protein, large, P0	211720_x_at	protein biosynthesis
CAP2	CAP, adenylate cyclase-associated protein, 2 (yeast)	212554_at	signal transduction
TSPYL5	TSPY-like 5	213122_at	DNA packaging
ADCY1	adenylate cyclase 1 (brain)	213245_at	signal transduction
RSAD2	radical S-adenosyl methionine domain containing 2 (viperin)	213797_at	innate immune response
KLHDC3	kelch domain containing 3	214383_x_at	cell cycle
IFI44	interferon-induced protein 44	214453_s_at	innate immune response
SPP2	secreted phosphoprotein 2, 24kDa	214478_at	development
HIST1H2AC	histone 1, H2ac	215071_s_at	DNA packaging
<i>PAH</i>	phenylalanine hydroxylase	217583_at	amino acid metabolism
OAS3	2'-5'-oligoadenylate synthetase 3, 100kDa	218400_at	innate immune response
PARP12	poly (ADP-ribose) polymerase family, member 12	218543_s_at	poly (adp-ribose) polymerase family
SIGIRR	single immunoglobulin and toll-interleukin 1 receptor (TIR) domain	218921_at	innate immune response

Gene Symbol	Description	Affy-ID	Function
FLJ20035	Hypothetical protein FLJ20035	218986_s_at	Helicase
MICAL-L2	MICAL-like 2	219332_at	Unknown
NARF	nuclear prelamin A recognition factor	219862_s_at	iron metabolism
HERC5	hect domain and RLD 5	219863_at	protein ubiquitination
SLC16A10	solute carrier family 16 (monocarboxylic acid transporters), member 10	219915_s_at	Transport
CABYR	calcium binding tyrosine-(Y)-phosphorylation regulated (fibrousheathin 2)	219928_s_at	signal transduction
KCNN2	potassium intermediate/small conductance calcium-activated channel, subfamily N, member 2	220116_at	cell communication
EVI1	ecotropic viral integration site 1	221884_at	signal transduction
LRCH4	leucine-rich repeats and calponin homology (CH) domain containing 4	90610_at	development
PTGFRN	prostaglandin F2 receptor negative regulator	224937_at	signal transduction
PNPT1	polyribonucleotide nucleotidyltransferase 1	225291_at	RNA catabolism
AMOTL1	angiomotin like 1	225450_at	tight junction
FLJ30046	Hypothetical protein FLJ30046	225619_at	Unknown
LOC129607	Hypothetical protein LOC129607	226702_at	amino acid metabolism
IFIT2	interferon-induced protein with tetratricopeptide repeats 2	226757_at	innate immune response
LOC402560	Hypothetical LOC401384	227554_at	
FLJ39051	Hypothetical gene supported by AK096370	227925_at	RNA catabolism
SAMD9	sterile alpha motif domain containing 9	228531_at	Unknown

Gene Symbol	Description	Affy-ID	Function
GALNTL1	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase-like 1	230417_at	UDP-glycosyltransferase
ANKRD35	ankyrin repeat domain 35	231118_at	Unknown
HIST1H2BD	Histone 1, H2bd	235456_at	DNA packaging
	Transcribed locus	235945_at	
TRIM55	tripartite motif-containing 55	236175_at	signal transduction
	Full-length cDNA clone CS0DF012YD09 of Fetal brain of Homo sapiens (human)	236331_at	
DDC	Dopa decarboxylase (aromatic L-amino acid decarboxylase)	236774_at	amino acid metabolism
FIS	FIS	239380_at	hypothetical protein
LOC284013	secretory protein LOC284013	241894_at	Unknown
	Transcribed locus	243278_at	
IL28RA	interleukin 28 receptor, alpha (interferon, lambda receptor)	244261_at	signal transduction
ZNF684	zinc finger protein 684	244398_x_at	gene transcription
C14orf21	Chromosome 14 open reading frame 21	1555390_at	RNA binding
IF	I factor (complement)	1555564_a_at	complement activation
	gb:H41167	1559776_at	sphingolipid metabolism
LOC147646	Hypothetical protein LOC147646	1560830_a_at	
	Homo sapiens, clone IMAGE:3934814, mRNA	1563298_at	

Table 3

Analysis of gene expression in biopsies obtained 4 hours after pegIFN α (B-2). List of 173 genes best predicting treatment outcome at week 4 (IFN regulated genes shaded in grey, up-regulated genes in bold, down-regulated genes italic; genes that differ between RVR and non-RVR but are not regulated by IFN are not shaded).

Gene Symbol	Description	Affy-ID
ACSL3	acyl-CoA synthetase long-chain family member 3	201662_s_at
ACSM3	acyl-CoA synthetase medium-chain family member 3	205942_s_at
ADCY1	adenylate cyclase 1 (brain)	213245_at
ADRB2	adrenergic, beta-2-, receptor, surface	206170_at
AMOTL1	angiomotin like 1	225450_at
ANXA10	annexin A10	210143_at
ARHGEF16	Rho guanine exchange factor (GEF) 16	208009_s_at
ATP2A2	ATPase, Ca ⁺⁺ transporting, cardiac muscle, slow twitch 2	209186_at
BCHE	butyrylcholinesterase	205433_at
BRUNOL5	bruno-like 5, RNA binding protein (Drosophila)	230497_at
C10orf125	chromosome 10 open reading frame 125	230259_at
C1orf96	chromosome 1 open reading frame 96	225904_at
C21orf106	chromosome 21 open reading frame 106	1561286_a_at
C22orf3	chromosome 22 open reading frame 3	217622_at
C3orf4	chromosome 3 open reading frame 4	239146_at
C6orf71	chromosome 6 open reading frame 71	231070_at

Gene Symbol	Description	Affy-ID
C8orf47	chromosome 8 open reading frame 47	1552389_at
C9orf95	chromosome 9 open reading frame 95	219147_s_at
CAMK2D	calcium/calmodulin-dependent protein kinase (CaM kinase) II delta	225019_at
CAPN3	calpain 3, (p94)	211890_x_at
CCL14, CCL15	chemokine (C-C motif) ligand 14, chemokine (C-C motif) ligand 15	210390_s_at
CCT5	chaperonin containing TCP1, subunit 5 (epsilon)	208696_at
CDKN2A	cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)	207039_at
CES7	carboxylesterase 7	1553465_a_at
CFHL5	complement factor H-related 5, complement factor H-related 5	208088_s_at
CHMP4A, MGC5987	chromatin modifying protein 4A, hypothetical protein MGC5987	228764_s_at
CNDP1	carnosine dipeptidase 1 (metallopeptidase M20 family)	223699_at
CTNNA1	catenin (cadherin-associated protein), alpha 1, 102kDa	210844_x_at
CUEDC1	CUE domain containing 1	219468_s_at
CXCL2	chemokine (C-X-C motif) ligand 2	209774_x_at
CYP11A1	cytochrome P450, family 11, subfamily A, polypeptide 1, cytochrome P450, family 11, subfamily A, polypeptide 1	204309_at
DAAM1	Dishevelled associated activator of morphogenesis 1	1555989_at
DBT	Dihydrolipoamide branched chain transacylase E2	231919_at
DHRS10	dehydrogenase/reductase (SDR family) member 10	228713_s_at

Gene Symbol	Description	Affy-ID
DPAGT1	dolichyl-phosphate (UDP-N-acetylglucosamine) N-acetylglucosaminophosphotransferase 1 (GlcNAc-1-P transferase)	209509_s_at
EEF1G	eukaryotic translation elongation factor 1 gamma	200689_x_at
EFHD1	EF-hand domain family, member D1	209343_at
ENPP2	ectonucleotide pyrophosphatase/phosphodiesterase 2 (autotaxin)	210839_s_at
ESR1	estrogen receptor 1	205225_at
EXOC4	Exocyst complex component 4	1557772_at
FIS	FIS	239380_at
FLJ21511	hypothetical protein FLJ21511	220723_s_at
FLJ34790	hypothetical protein FLJ34790	230012_at
FOLH1	folate hydrolase (prostate-specific membrane antigen) 1	205860_x_at
FZD3	frizzled homolog 3 (Drosophila)	219683_at
GLRX2	glutaredoxin 2	219933_at
GPC3	glypican 3	209220_at
GPR143	G protein-coupled receptor 143	206696_at
HCG4	HLA complex group 4	206685_at
HERC4	hect domain and RLD 4	225988_at
HIST1H2AC	histone 1, H2ac	215071_s_at
HKDC1	hexokinase domain containing 1	227614_at
HLX1	H2.0-like homeo box 1 (Drosophila)	214438_at
HLXB9	homeo box HB9	214614_at
HSPA5	heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa)	211936_at
HTATIP2	HIV-1 Tat interactive protein 2, 30kDa	209448_at
HYI	hydroxypyruvate isomerase homolog (E. coli), hydroxypyruvate isomerase	221435_x_at

Gene Symbol	Description	Affy-ID
	homolog (E. coli)	
IDH3A	isocitrate dehydrogenase 3 (NAD+) alpha	202070_s_at
IDS	iduronate 2-sulfatase (Hunter syndrome)	206342_x_at
IFI27	interferon, alpha-inducible protein 27	202411_at
ITGA6	integrin, alpha 6	201656_at
KCNN2	potassium intermediate/small conductance calcium-activated channel, subfamily N, member 2	220116_at
KIAA0090	KIAA0090	212395_s_at
KIAA0446	KIAA0446 gene product	212683_at
KIAA0888	KIAA0888 protein	235048_at
KIAA1522	KIAA1522	224746_at
KIAA1609	KIAA1609 protein	65438_at
KIAA2002	KIAA2002 protein	225913_at
KPNA2	karyopherin alpha 2 (RAG cohort 1, importin alpha 1), karyopherin alpha 2 (RAG cohort 1, importin alpha 1)	211762_s_at
LGALS3BP	lectin, galactoside-binding, soluble, 3 binding protein	200923_at
LOC201725, TOMM7	translocase of outer mitochondrial membrane 7 homolog (yeast), hypothetical protein LOC201725	201812_s_at
LOC202775, LOC402617	hypothetical LOC202775, hypothetical LOC402617	241353_s_at
LOC221710	hypothetical protein LOC221710	1564651_at
LOC286434, LOC389906, LOC389908	hypothetical protein LOC286434, similar to Serine/threonine-protein kinase PRKX (Protein kinase PKX1), hypothetical LOC389908	222031_at
LOC402560	Hypothetical LOC401384	227554_at

Gene Symbol	Description	Affy-ID
LOC90268	hypothetical protein BC007706	229268_at
LONRF1	LON peptidase N-terminal domain and ring finger 1	226038_at
LRRC20	leucine rich repeat containing 20	218550_s_at
MAFB	v-maf musculoaponeurotic fibrosarcoma oncogene homolog B (avian)	218559_s_at
MDK	midkine (neurite growth-promoting factor 2)	209035_at
MEF2D	MADS box transcription enhancer factor 2, polypeptide D (myocyte enhancer factor 2D)	203003_at
MEGF10	MEGF10 protein	232523_at
MFN1	mitofusin 1	217043_s_at
MGC39545	hypothetical protein LOC403312	1555002_at
MICAL-L2	MICAL-like 2	219332_at
MME	membrane metallo-endopeptidase (neutral endopeptidase, enkephalinase, CALLA, CD10)	203434_s_at
MORC4	MORC family CW-type zinc finger 4	219038_at
MRAP	melanocortin 2 receptor accessory protein	1555741_at
MYH14	myosin, heavy polypeptide 14	234290_x_at
NEU4	sialidase 4	222957_at
NFASC	Neurofascin	213438_at
OAT	ornithine aminotransferase (gyrate atrophy)	201599_at
OSTalpha	organic solute transporter alpha	229230_at
PAPPA2	Pappalysin 2	213332_at
PARP6	poly (ADP-ribose) polymerase family, member 6	219639_x_at
PBX1	Pre-B-cell leukemia transcription	1562235_s_at

Gene Symbol	Description	Affy-ID
	factor 1	
PCBD2	6-pyruvoyl-tetrahydropterin synthase/dimerization cofactor of hepatocyte nuclear factor 1 alpha (TCF1) 2	223712_at
PCOLCE	procollagen C-endopeptidase enhancer	202465_at
PCYOX1	prenylcysteine oxidase 1	225274_at
PDE4DIP	phosphodiesterase 4D interacting protein (myomegalin)	210305_at
PPAPDC2	phosphatidic acid phosphatase type 2 domain containing 2	227385_at
PPARD	peroxisome proliferative activated receptor, delta	242218_at
PPGB	protective protein for beta-galactosidase (galactosialidosis)	200661_at
PPID	Peptidylprolyl isomerase D (cyclophilin D)	228469_at
PPP1R1A	protein phosphatase 1, regulatory (inhibitor) subunit 1A	205478_at
PPP2R1B	protein phosphatase 2 (formerly 2A), regulatory subunit A (PR 65), beta isoform	222351_at
PSMAL	prostate-specific membrane antigen-like	211303_x_at
PTGFRN	prostaglandin F2 receptor negative regulator	224950_at
RAB11FIP5	RAB11 family interacting protein 5 (class I)	210879_s_at
RABEPK	Rab9 effector protein with kelch motifs	1558021_at
RAD52	RAD52 homolog (S. cerevisiae), RAD52 homolog (S. cerevisiae)	211994_at

Gene Symbol	Description	Affy-ID
RAI14	retinoic acid induced 14	202052_s_at
RPL14, RPL14L	ribosomal protein L14, ribosomal protein L14, ribosomal protein L14-like, ribosomal protein L14-like	200074_s_at
RPL3	ribosomal protein L3, ribosomal protein L3	211666_x_at
RPLP0	ribosomal protein, large, P0	201033_x_at
RPLP0	ribosomal protein, large, P0	211972_x_at
RPS5	ribosomal protein S5, ribosomal protein S5	200024_at
RRBP1	ribosome binding protein 1 homolog 180kDa (dog)	201206_s_at
SAC3D1	SAC3 domain containing 1	205449_at
SDCBP2	syndecan binding protein (syntenin) 2	233565_s_at
SEMA4F	sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4F	210124_x_at
SERPINA7	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 7	206386_at
SET7	SET domain-containing protein 7	224928_at
SH3MD1	SH3 multiple domains 1	224817_at
SHD	Src homology 2 domain containing transforming protein D	227845_s_at
SIGIRR	single immunoglobulin and toll-interleukin 1 receptor (TIR) domain	52940_at
SLC16A10	solute carrier family 16 (monocarboxylic acid transporters), member 10	219915_s_at
SLC16A4	solute carrier family 16 (monocarboxylic acid transporters),	205234_at

Gene Symbol	Description	Affy-ID
	member 4	
SLC19A2	solute carrier family 19 (thiamine transporter), member 2	209681_at
SLC23A2	solute carrier family 23 (nucleobase transporters), member 2	209236_at
SLC25A16	Solute carrier family 25 (mitochondrial carrier; Graves disease autoantigen), member 16	235747_at
SLC25A37	solute carrier family 25, member 37	218136_s_at
SLC6A8	solute carrier family 6 (neurotransmitter transporter, creatine), member 8	210854_x_at
SLPI	secretory leukocyte peptidase inhibitor	203021_at
SMBP	SM-11044 binding protein	217758_s_at
SNF1LK2	SNF1-like kinase 2	213221_s_at
SPP2	secreted phosphoprotein 2, 24kDa	214478_at
SSTR1	somatostatin receptor 1	235591_at
ST13	suppression of tumorigenicity 13 (colon carcinoma) (Hsp70 interacting protein)	208667_s_at
ST7L	suppression of tumorigenicity 7 like	1552739_s_at
STMN1	Stathmin 1/oncoprotein 18	217253_at
STS	steroid sulfatase (microsomal), arylsulfatase C, isozyme S	203768_s_at
TM4SF4	transmembrane 4 L six family member 4	209937_at
TMED10	transmembrane emp24-like trafficking protein 10 (yeast)	200929_at
TRIM55	tripartite motif-containing 55	236175_at
UBE1L	ubiquitin-activating enzyme E1-like	203281_s_at
VISA	Virus-induced signaling adapter	229741_at
VKORC1L1	vitamin K epoxide reductase complex,	224881_at

Gene Symbol	Description	Affy-ID
	subunit 1-like 1	
WVOX	WW domain containing oxidoreductase	210695_s_at
WVOX	WW domain containing oxidoreductase	219077_s_at
YLPM1	YLP motif containing 1	214659_x_at
ZD52F10	Dermokine	226926_at
ZFP3	zinc finger protein 3 homolog (mouse)	235728_at
ZNF511	zinc finger protein 511	225307_at
ZNF710	Zinc finger protein 710	213658_at
	CDNA FLJ41000 fis, clone UTERU2016761, highly similar to Homo sapiens ES/130 mRNA	201204_s_at
	gb:AI341383 /DB_XREF=gi:4078310 /DB_XREF=qx91a06.x1 /CLONE=IMAGE:2009842 /FEA=EST /CNT=52 /TID=Hs.112751.2 /TIER=Stack /STK=42 /UG=Hs.112751 /LL=23383 /UG_GENE=KIAA0892 /UG_TITLE=KIAA0892 protein	227092_at
	CDNA FLJ38567 fis, clone HCHON2005166	228240_at
	CDNA: FLJ21462 fis, clone COL04744	228732_at
	gb:AI357655 /DB_XREF=gi:4109276 /DB_XREF=qy15d03.x1 /CLONE=IMAGE:2012069 /FEA=EST /CNT=15 /TID=Hs.292931.0 /TIER=Stack /STK=13 /UG=Hs.292931 /UG_TITLE=ESTs	228971_at
	gb:AU144136 /DB_XREF=gi:11005657	233799_at

Gene Symbol	Description	Affy-ID
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	CDNA FLJ41376 fis, clone BRCAN2008494	235133_at
	gb:AW167727 /DB_XREF=gi:6399252 /DB_XREF=xn48c05.x1 /CLONE=IMAGE:2696936 /FEA=EST /CNT=15 /TID=Hs.11873.0 /TIER=ConsEnd /STK=3 /UG=Hs.11873 /UG_TITLE=ESTs	235201_at
	Transcribed locus, strongly similar to XP_513428.1 PREDICTED: similar to hypothetical protein 4732467L16 [Pan troglodytes]	235651_at
	Transcribed locus	235945_at
	Transcribed locus	236220_at
	gb:BF437161 /DB_XREF=gi:11449494 /DB_XREF=7p67c05.x1 /CLONE=IMAGE:3650697 /FEA=EST /CNT=6 /TID=Hs.208261.0 /TIER=ConsEnd /STK=4 /UG=Hs.208261 /UG_TITLE=ESTs	239082_at
	gb:N57929 /DB_XREF=gi:1201819 /DB_XREF=yv61e06.s1 /CLONE=IMAGE:247234 /FEA=EST /CNT=4 /TID=Hs.48100.0	242978_x_at

Gene Symbol	Description	Affy-ID
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	Transcribed locus	243278_at
	Transcribed locus	243302_at
	gb:H41167 /DB_XREF=gi:917219 /DB_XREF=yp64g06.s1 /CLONE=IMAGE:192250 /TID=Hs2.191285.1 /CNT=4 /FEA=mRNA /TIER=ConsEnd /STK=0 /UG=Hs.191285 /UG_TITLE=Homo sapiens cDNA FLJ36989 fis, clone BRACE2006753.	1559776_at

Table 4

Primer sequences used for real-time RT-PCR analysis.

Gene	Primer 1 sequence	Primer 2 sequence
GAPDH	5' GTCCTCCTGTTTCGACAGTCA 3'	5' ACCTTCCCCATGGTG TCTGA 3'
STAT1	5' TCCCCA GGCCCTTGTTG 3'	5' CAAGCTGCTGAAGTTGGTACCA 3'
IP10	5' CGATTCTGATTTGCTGCCTTAT 3'	5' GCAGGTACAGCGTACGGTTCT 3'
SOCS1	5' CCCCTTCTGTAGGATGGTAGCA 3'	5' TGCTGT GGAGACTGCATTGTC 3'
SOCS3	5' TGCGCCTCAAGACCT TCAG 3'	5' CTTGCGCACTGCGTTCAC 3'
USP18	5' CTC AGTCCCGACGTGGAAC 3'	5' ATCTCTCAAGCGCCATGCA 3'
IFI27	5' CCTCGGGCAGCCTTG 3'	5' AATCCGAGAGTCCAGTTGCT 3'

(c) 1.4 References

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CLAIMS

1. A method for determining the likelihood that a subject having a viral infection of the liver will be responsive to antiviral therapy that includes stimulation of Interferon (IFN) activity, the method comprising:
 - (a) analysing a sample from the subject for expression of at least one gene from each of the following groups of genes:
 - (i) KYNU; PAH; LOC129607; DDC; FOLH1; YBX1; BCHE; ACADL; ACSM3; NARF; SLPI; RPS5; RPL3; RPLP0; TRIM5 and HERC5;
 - (ii) HTATIP2; CDK4; IFI44L; and KLHDC3;
 - (iii) C7; IF; IFI27; IFIT1; OAS2; G1P2; OAS1; IRF7; RSAD2; IFI44; OAS3; SIGIRR; and IFIT2;
 - (iv) RAB4A; PPP1R1A; PPM1E; ENPP2; CAP2; ADCY1; CABYR; EVI1; PTGFRN; TRIM55; and IL28RA;
 - (v) MME; KCNN2; SLC16A10; AMOTL1; SPP2; LRCH4; HIST1H2BG; TSPYL5; HIST1H2AC; HIST1H2BD; PHTF1; ZNF684; GSTM5; FLJ20035; FIS; PARP12; C14orf21; PNPT1; FLJ39051; GALNTL1; OSBPL1A; LGALS3BP; TXNRD2; LOC201725; TOMM7; SRPX2; DCN; PSMAL; MICAL-L2; FLJ30046; SAMD9; ANKRD35; LOC284013; LOC402560; and LOC147646; and,
 - (b) comparing expression of the genes in the sample to expression of the same genes in a control sample.
2. The method of Claim 1 wherein altered expression of the genes in the sample compared to expression of the same genes in the control sample indicates that the subject is not likely to be responsive to said antiviral therapy.

3. The method of Claim 1 wherein unaltered expression of the genes in the sample compared to expression of the same genes in the control sample indicates that the subject is likely to be responsive to said antiviral therapy.
4. The method of any of the previous claims wherein the antiviral therapy includes pegylated IFN α .
5. The method of Claim 4 wherein the antiviral therapy includes pegIFN α and ribavirin.
6. The method of any of the previous claims wherein the viral infection is Hepatitis B virus or Hepatitis C virus infection.
7. The method of claim 4 wherein the virus is Hepatitis C virus.
8. The method of any of the previous claims wherein the sample comprises liver tissue.
9. The method of any of the previous claims wherein the expression of five, six, seven, eight, nine, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 71, 72, 73, 74, 75, 76, 77 or 78 genes of claim 1 is analysed.
10. The method of any of the previous claims wherein gene expression is determined by measuring the amount of mRNA gene transcript in the sample, or the amount of cDNA derived from said mRNA.
11. The method of any of claims 1 to 9 wherein gene expression is determined by measuring the amount of peptide or polypeptide encoded by the gene in the sample.
12. The method of claim 11 wherein the amount of peptide or polypeptide is determined using a specific binding molecule.
13. The method of any of the previous claims wherein the subject is human.
14. A method for determining the likelihood that a subject having a viral infection of the liver will be responsive to antiviral therapy that includes stimulation of Interferon (IFN)

activity, the method comprising, examining a sample from the subject to identify the subcellular location of STAT1.

15. The method of Claim 14 wherein where the majority of cells have nuclear localised STAT1 then that subject is likely to be non-responsive to antiviral therapy.
16. Use of an agent that reduces the activation of the IFN system for the prevention or treatment of a viral infection of the liver.
17. An agent that reduces the activation of the IFN system in the manufacture of a medicament for the prevention or treatment of a viral infection of the liver.
18. A kit for performing the method of any of Claims 1 to 13 comprising:
 - (i) means for analysing in a sample from a subject the expression of at least one gene from each of the groups of genes listed in Claim 1; and, optionally,
 - (ii) means for comparing expression of the genes in the sample to expression of the same genes in a control sample.
19. The kit of Claim 18 comprising one or more specific binding molecules that can target molecules representative of said gene expression in the sample.
20. The kit of Claim 19 wherein said specific binding molecule is an oligonucleotide probe, antibody, or aptamer.

Figure 1

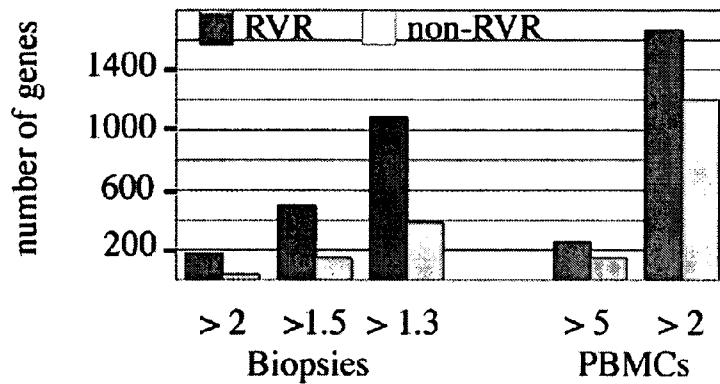
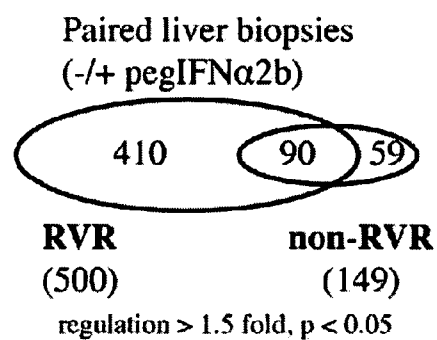
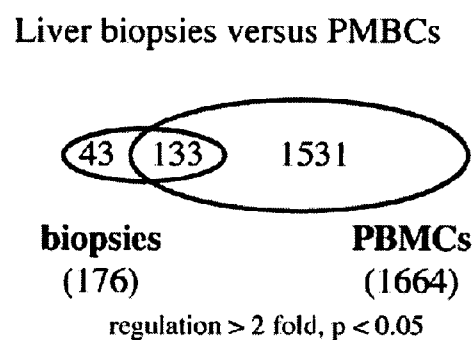
A**B****C**

Figure 2

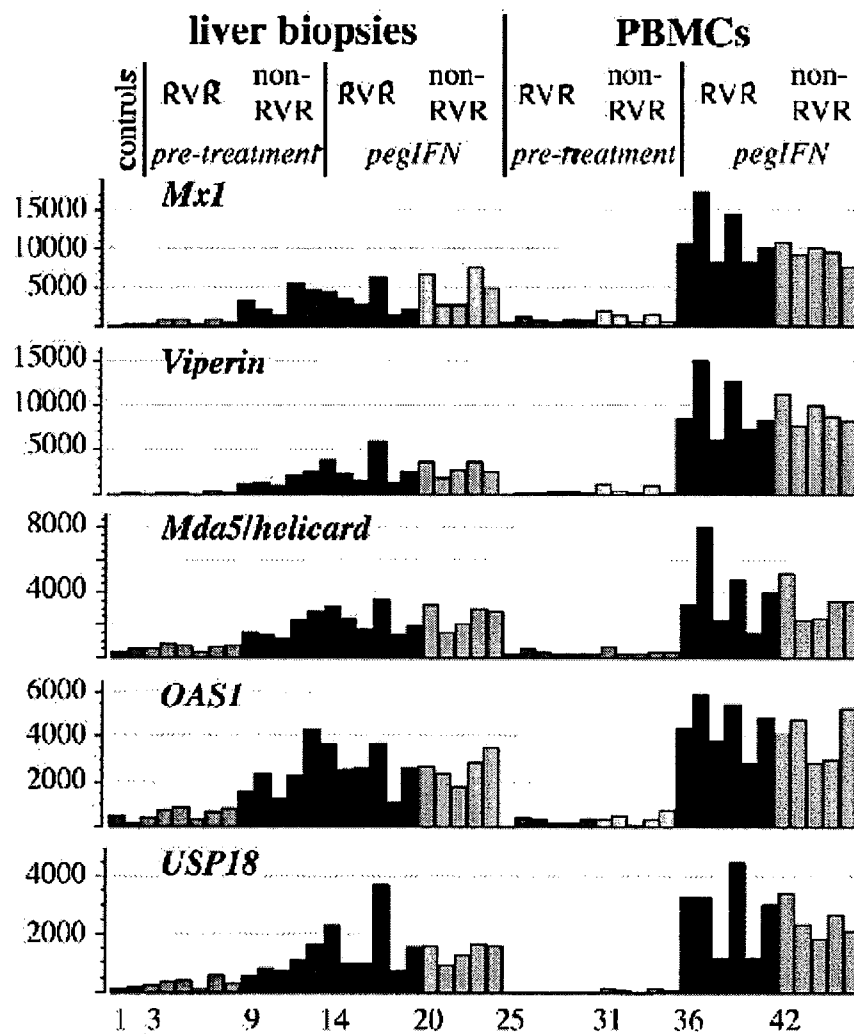
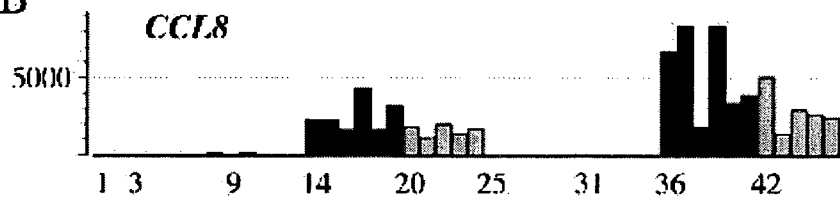
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Figure 3

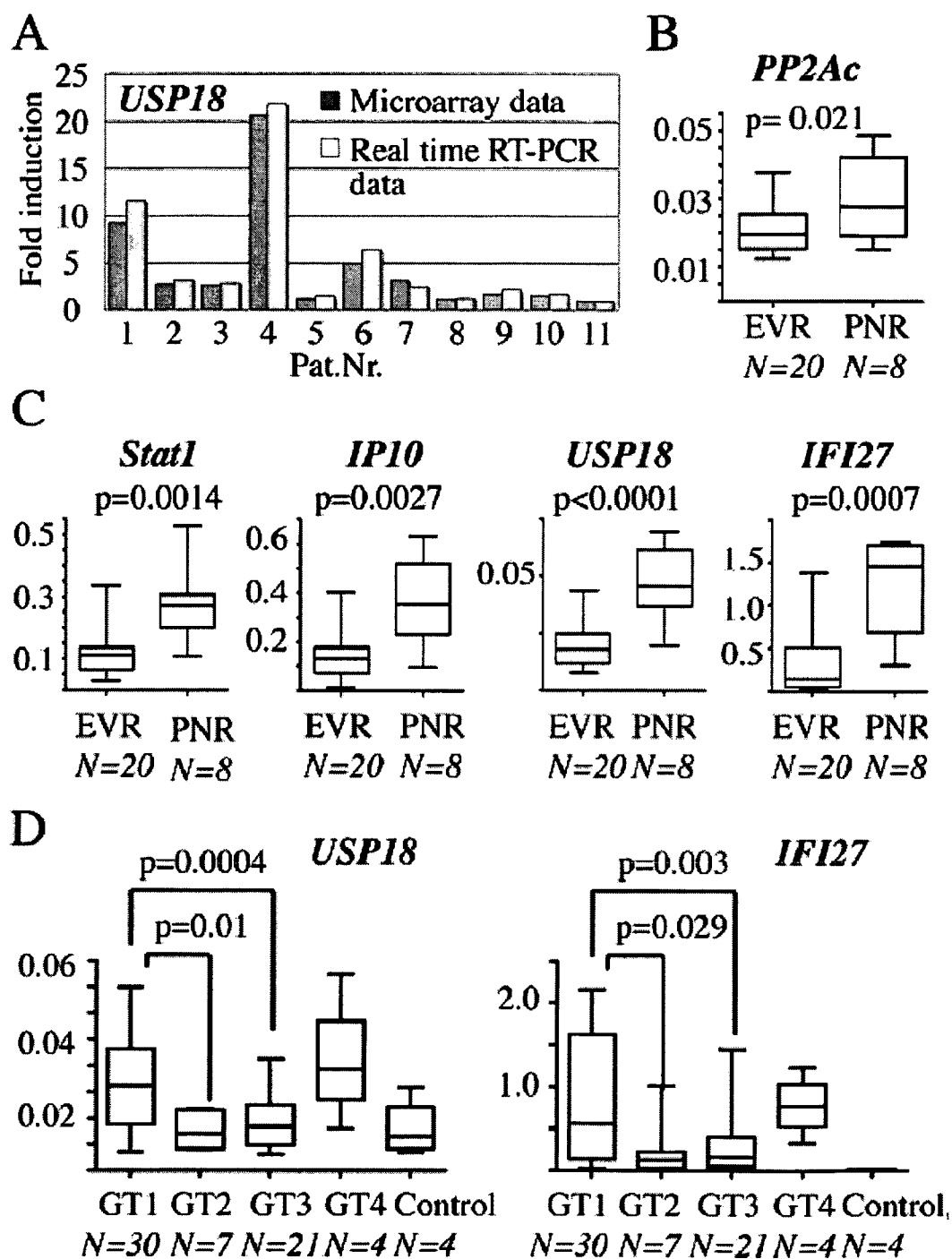
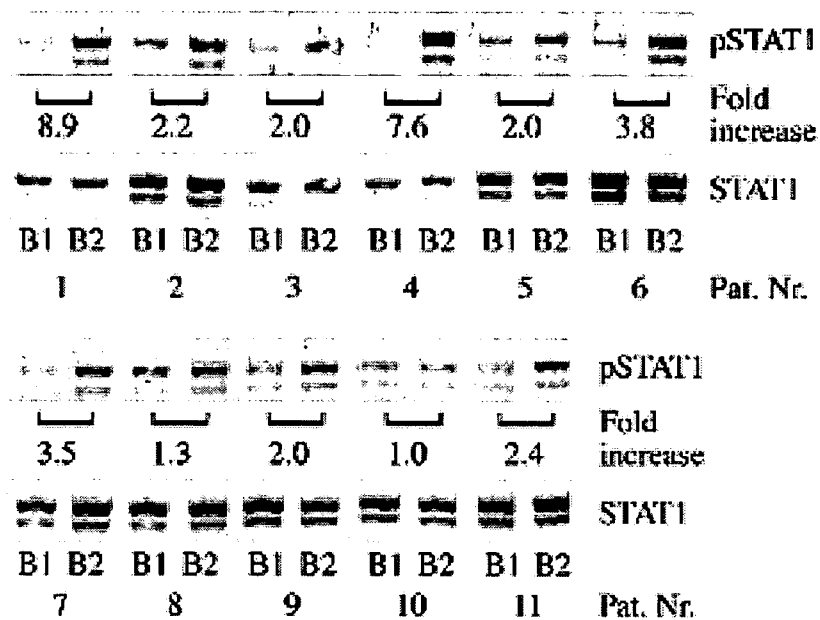


Figure 4

A



B

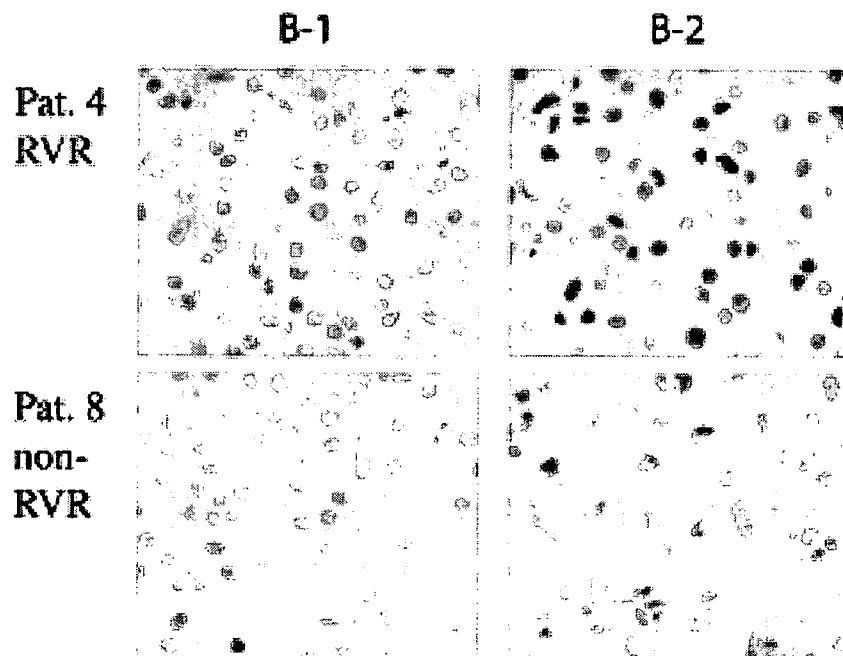


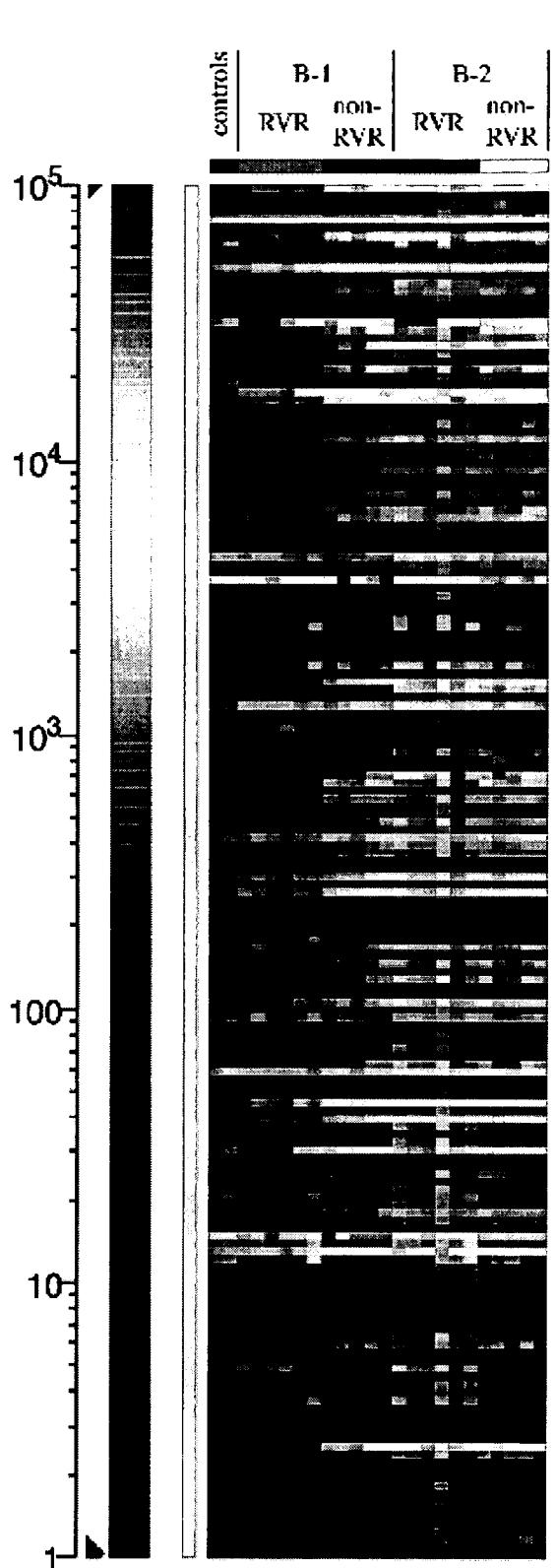
Figure 5

Figure 6

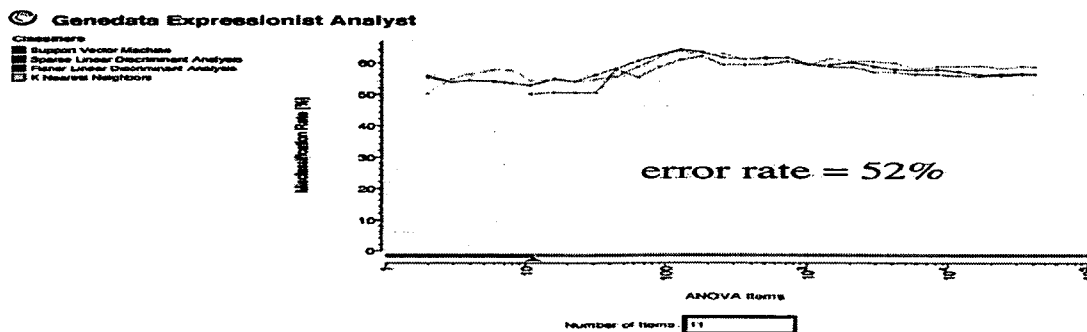
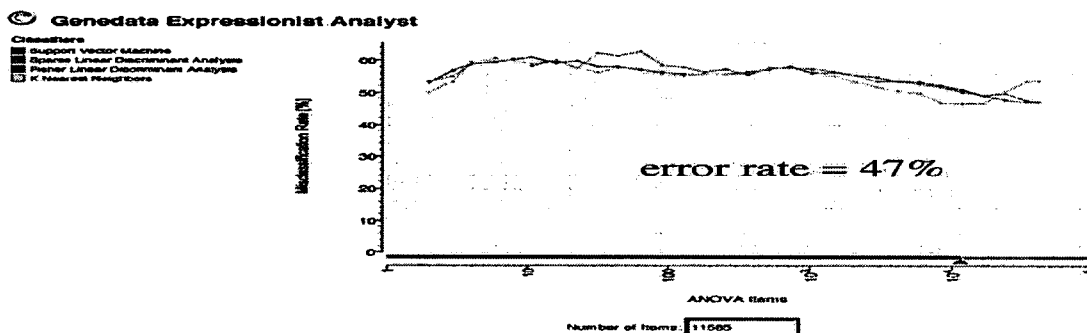
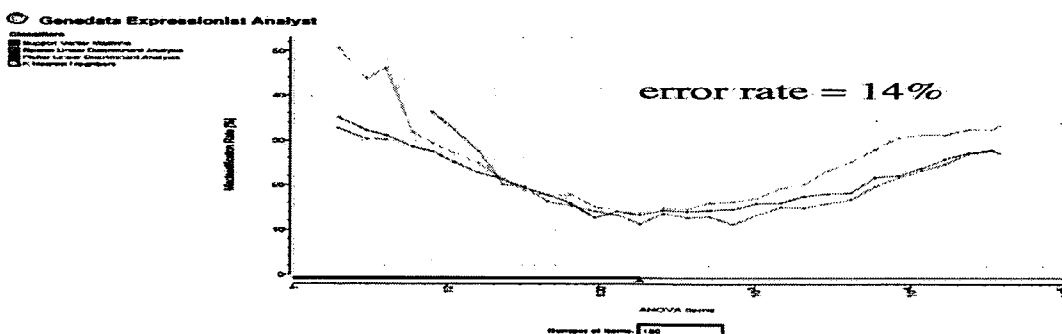
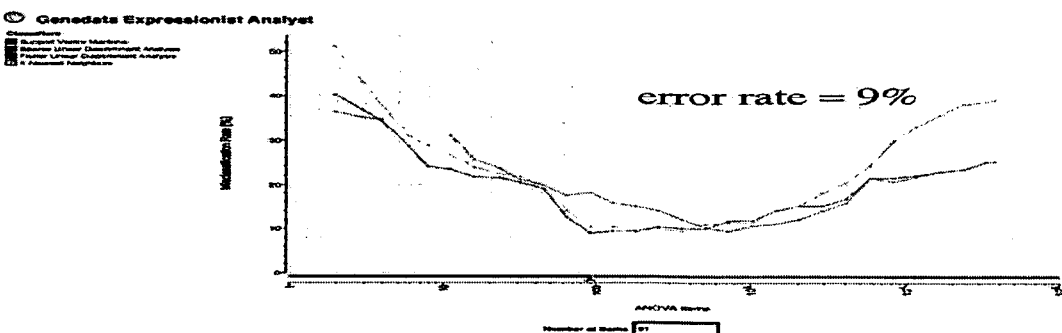
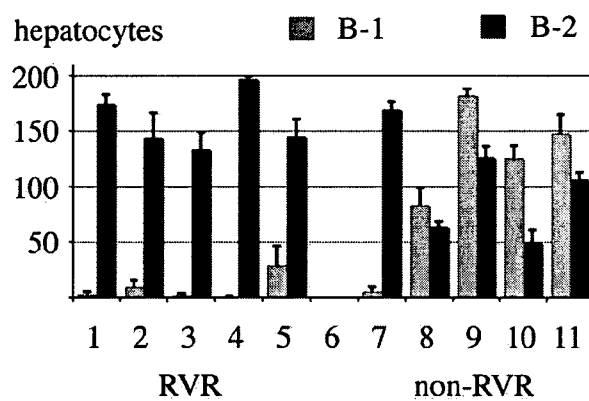
A**B****C****D**

Figure 7

A



B

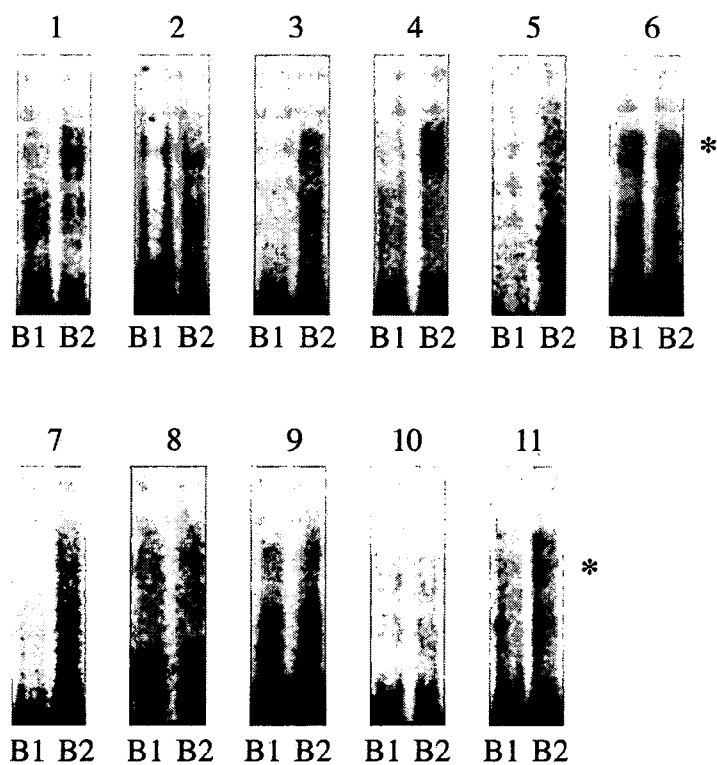


Figure 8

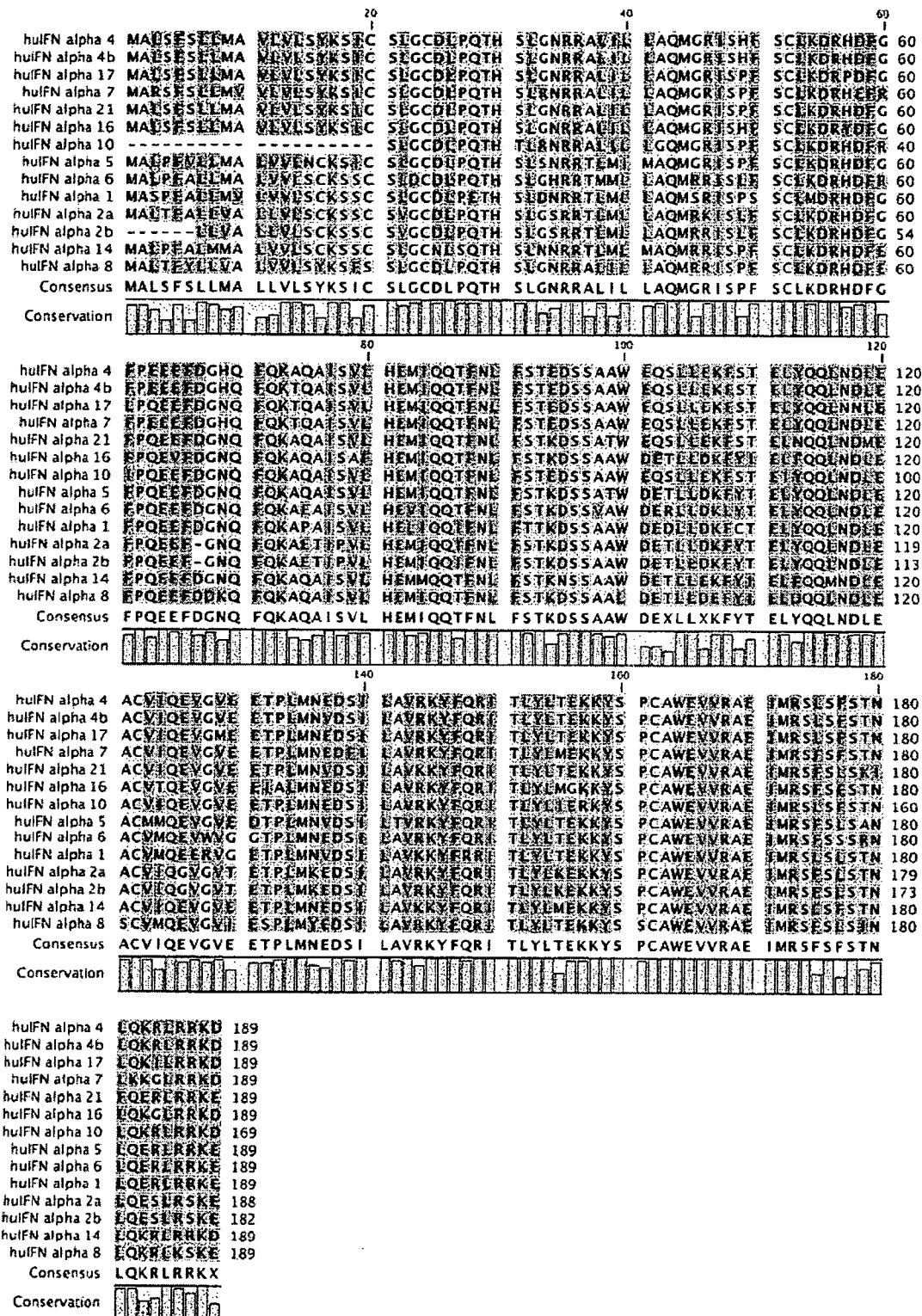


Figure 9:

(a) Human Interferon receptor 1 (GenBank accession NM_000629)

MMVLLGATTTLVLVAVAPWVLSAAAGGKNLKSPPQKVEVDIIDDN
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 L
 RIRAEKENTSSWYEVDSFTPFRKAQIGPPEVHLEAEDKAIVIHISPGTKDSVMWALD
 G
 LSFTYSLVIWKNSSGVEERIEINIYSRHKIYKLSPETTYCLKVKAALLTSWKIGVYSP
 V
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 K
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 P
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 T
 VPNLKPLTVYCVKARAHTMDEKLNKSSVFS DAVCEKTKPGNTSKIWLIVGICIALFA
 L
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 I
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1	AGCGGCGCGG	TGCGTAGAGG	GGCGGTGAGA	GCTAAGAGGG	GCAGCGCGTG	TGCAGAGGGG
61	CGGTGTGACT	TAGGACGGGG	CGATGGCGGC	TGAGAGGAGC	TGCGCGTGCG	CGAACATGTA
121	ACTGGTGGGA	TCTGCGGCGG	CTCCAGATG	ATGGTCGTCC	TCCTGGGCGC	GACGACCCTA
181	GTGCTCGTCG	CCGTGGCGCC	ATGGGTGTTG	TCCGCGAGCCG	CAGGTGGAAA	AAATCTAAAA
241	TCTCCTCAAA	AAGTAGAGGT	CGACATCATA	GATGACAAC	TTATCCTGAG	GTGGAACAGG
301	AGCGATGAGT	CTGTCGGGAA	TGTGACTTTT	TCATTCGATT	ATCAAAAAAC	TGGGATGGAT
361	AATTGGATAA	AATTGTCTGG	GTGTCAGAAT	ATTACTAGTA	CCAAATGCAA	CTTTTCTTCA
421	CTCAAGCTGA	ATGTTTATGA	AGAAATTAAA	TGCGGTATAA	GAGCAGAAAA	AGAAAAACACT
481	TCTTCATGGT	ATGAGGTTGA	CTCATTTACA	CCATTTTCGCA	AAGCTCAGAT	TGGTCTCTCCA
541	GAAGTACATT	TAGAAGCTGA	AGATAAGGCA	ATAGTGATAC	ACATCTCTCC	TGGAACAAAA
601	GATAGTGTTA	TGTGGGCTTT	GGATGGTTTA	AGCTTTACAT	ATAGCTTAGT	TATCTGGAAA
661	AACTCTTCAG	GTGTAGAAGA	AAGGATTGAA	AATATTTATT	CCAGACATAA	AATTTATAAA
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781	ATTGGTGTCT	ATAGTCCAGT	ACATTGTATA	AAGACCACAG	TTGAAAATGA	ACTACCTCCA
841	CCAGAAAATA	TAGAAGTCAG	TGTCCAAAA	CAGAACTATG	TTCTTAAATG	GGATTATACA
901	TATGCAAACA	TGACCTTTCA	AGTTCAAGTG	CTCCACGCC	TTTTAAAAAG	GAATCCTGGA
961	AACCATTGT	ATAAATGGAA	ACAAATACCT	GACTGTGAAA	ATGTCAAAAC	TACCCAGTGT
1021	GTCTTTTCCTC	AAAACGTTTT	CCAAAAAGGA	ATTTACCTTC	TCCGCGTACA	AGCATCTGAT
1081	GGAAATAACA	CATCTTTTTG	GTCTGAAGAG	ATAAAGTTTG	ATACTGAAAT	ACAAGCTTTC
1141	CTACTTCCTC	CAGTCTTTAA	CATTAGATCC	CTTAGTGATT	CATTCCATAT	CTATATCGGT
1201	GCTCCAAAAC	AGTCTGGAAA	CACGCCTGTG	ATCCAGGATT	ATCCACTGAT	TTATGAAATT
1261	ATTTTGTGGG	AAAACACTTC	AAATGCTGAG	AGAAAAATTA	TCGAGAAAAA	AACTGATGTT
1321	ACAGTTCCTA	ATTTGAAACC	ACTGACTGTA	TATTGTGTGA	AAGCCAGAGC	ACACACCATG
1381	GATGAAAAGC	TGAATAAAAG	CAGTGTTTTT	AGTGACGCTG	TATGTGAGAA	AACAAAACCA
1441	GGAAATACCT	CTAAAATTTG	GCTTATAGTT	GGAATTTGTA	TTGCATTATT	TGCTCTCCCG
1501	TTTGTCAATT	ATGCTGCGAA	AGTCTTCTTG	AGATGCATCA	ATTATGTCTT	CTTTCCATCA
1561	CTTAAACCTT	CTTCCAGTAT	AGATGAGTAT	TTCTCTGAAC	AGCCATTGAA	GAATCTTCTG
1621	CTTTCAACTT	CTGAGGAACA	AATCGAAAAA	TGTTTCATAA	TTGAAAATAT	AAGCACAATT
1681	GCTACAGTAG	AAGAAACTAA	TCAAACCTGAT	GAAGATCATA	AAAAATACAG	TCCCCAAACT
1741	AGCCAAGATT	CAGGAAATTA	TTCTAATGAA	GATGAAAGCG	AAAGTAAAC	AAGTGAAGAA
1801	CTACAGCAGG	ACTTTGTATG	ACCAGAAATG	AACGTGTGTC	AGTATAAGGT	TTTTTCAGCAG
1861	GAGTTACACT	GGGAGCCTGA	GGTCCTCACC	TTCTCTCAG	TAACACAGA	GAGGACGTTT
1921	CCCTGTTTAT	GGAAAGAAAA	AACATCTTCA	GATCATAGGT	CCTAAAAATA	CGGGCAAGCT
1981	CTTAACATAT	TAAAAATGAA	ATTACAGGCC	CGGGCACGGT	GGCTCACACC	TGTAATCCCA
2041	GCACTTTGGG	AGGCTGAGGC	AGGCAGATCA	TGAGGTCAAG	AGATCGAGAC	CAGCCTGGCC
2101	AACGTGGTGA	AACCCCATCT	CTACTAAAAA	TACAAAAATT	AGCCGGGTGT	GGTGGCGCGC

Figure 9 (cont.)

2161	GCCTGTTGTC	TTAGCTACTC	AGGAGGCTGA	GGCAGGAGAA	TCGCTTGAAA	ACAGGAGGTG
2221	GAGGTTGCAG	TGAGCCGAGA	TCACGCCACT	GCACTCCAGC	CTGGTGACAG	CGTGAGACTC
2281	TTTAAAAAAA	GAAATTAAAA	GAGTTGAGAC	AAACGTTTCC	TACATTCTTT	TCCATGTGTA
2341	AAATCATGAA	AAAGCCTGTC	ACCGGACTTG	CATTGGATGA	GATGAGTCAG	ACCAAAACAG
2401	TGGCCACCCG	TCTTCCTCCT	GTGAGCCTAA	GTGCAGCCGT	GCTAGCTGCG	CACCGTGGCT
2461	AAGGATGACG	TCTGTGTTCC	TGTCATCAC	TGATGCTGCT	GGCTACTGCA	TGTGCCACAC
2521	CTGTCTGTTT	GCCATTCCTA	ACATTCCTGT	TCATTCCTCC	TCGGGAGATA	TTTCAAACAT
2581	TTGGTCTTTT	CTTTTAACAC	TGAGGGTAGG	CCCTTAGGAA	ATTTATTTAG	GAAAGTCTGA
2641	ACACGTTATC	ACTTGGTTTT	CTGGAAGTA	GCTTACCCTA	GAAAACAGCT	GCAAATGCCA
2701	GAAAGATGAT	CCCTAAAAAT	GTTGAGGGAC	TTCTGTTTCAT	TCATCCCGAG	AACATTGGCT
2761	TCCACATCAC	AGTATCTACC	CTTACATGGT	TTAGGATTAA	AGCCAGGCAA	TC'TTTTACTA
2821	TGCATTAGAA	CCTCTGATTG	AAAACCTTAT	AGAACAGTAG	CTTCTGCTGG	AATTTGCAAT
2881	CACTGAAGTC	ATAGAAAATA	GGTAACTATC	TAATTAGAGA	AATAATTGTT	GTATTTTAAAG
2941	ATCTGAGAGT	GTGTACAAAGT	TTTAGTATAG	ATGCCATGCC	AGAAGATAGT	GTATGCAAGA
3001	AGTCTTGGGA	CCAGAAAATG	GCAATGATAG	GAGACTGACA	TAGAAGAAGA	ATGCTTCCCT
3061	AGGAAAAAGG	TCGCTGGCTT	TGGTGCAAGA	GGAAGAAGAA	TGTTCCACTG	GAAGCCTGAG
3121	CACCTAATCA	GCTCTCAGTG	ATCAACCCAT	TC'TGT'TATG	GGTGGTCTCT	GTCACTTTGA
3181	ATGCCAGGCT	GGCTTCTCGT	CTAGCAGTAT	TCAGATACCC	CTTCTGCTCA	GCCTGCTTGG
3241	CGTTAAATTA	CAAATCATTG	AACTGAGGGG	GAAAAATGTA	ACTAGGAAGA	AAAACCCAAT
3301	TTAAGAAATT	ACATAATGCT	TTCCAAAGGC	ACCTACAAC	TAGTTTTAAA	TTACTTGCTA
3361	CTGGGGATTA	CCCATGGATA	TCCTTAATAG	GCAGGAAGTC	TGGGAATTCT	GGTGGCCTCT
3421	AGGCGAGTGT	TCTCACAGCA	CCGTTCCGAC	AGGGACCAGT	GAAAAGAAAAG	AGACAAAAGT
3481	AGAACCTGCT	TGCGAGCGGC	CATTCTTAAG	GCCAGTCTGG	TTTAAAGTAGT	CATTTCTGCT
3541	GAAAAAACAG	ATGATCCTGG	TGGAAGAAAA	GGTTGAAGGC	AGCTGCCCTC	GGGAGGGCTG
3601	TGATGCTCGG	CACATCCTGC	CTGGCACATA	CACGTGTCTG	CAGGCCACAC	CGTGCATGTC
3661	CCCAGACCTG	CCGCCTGGCT	TCTGGAGTGC	TTCAAGCAGA	GCATGGTGGG	TCATPGAGGA
3721	GACCCAGGAA	TCTCATCTGA	GAACCCACTC	TC'TGCCGGAG	AACCCCATGG	TGACACATTT
3781	TCATCTTTCT	GACCAGAGGC	TGTTTTTTTT	TTTTTTTGGAG	ACAGTCTCAT	TCTGTTGCCC
3841	AGGCTGGAGT	GCACTGGCTT	GATCTCGGCT	CACTGCAACC	TCGCCTCCCG	GGTTCAGGCA
3901	ATTCTCTGCC	CCAGCCTCCA	GAGTAGCTGG	GATAACAGGT	GCCCACCACC	ACACCCCACT
3961	AA'TTTTGTGA	TTTGTATTTT	TAGTAGAGAT	GGGGTTTCAC	CATGTTGGTC	AGGCTGGTCT
4021	TGGACTCCTG	ACCTCATGCT	CCACCCGCTT	CGGCCTCCCA	AAGT'TCTGGG	ATTACAGGTG
4081	TGAGCCACCG	TGCACGGCCG	GCCTGACCTT	TGGAAAAGCC	TTGTCACTTT	GGACGTTTGC
4141	GTCTTTGAAG	AGGCGATGGG	AGCATATCAT	GACTGCCTGC	CACCATTGCT	TTTCAAGATA
4201	CCACAACCTCA	ATCATGCTGT	CCAGGACTTC	TGGCCCTGTG	TTCAACCACTG	GGAAAACGTA
4261	CTTCAGACTG	GATAGCCTAA	AAAGGAGCAA	TGCCCTTGTA	GGATGTGGAG	AAGGGAAAAAT
4321	ACGGACATTA	ACATTAAAAG	ACACCAAGTA	AAT'TGTTAGG	TCTCTAGGAA	GTTGGAGCAC
4381	AAGCCTTACG	CCTTTAAGAC	CATCTGTGGT	TTTCAAGTGA	CAAGCGCTGA	GCACCGACAG
4441	CAGAAAACAA	CAACAAAAAA	ACACCTCGTT	TTTACCTTGT	CTTCTAGACA	TGAAAAGGCA
4501	GTTGCATTCC	ACTCTGCATT	ATGTTCTACA	TGTTGCTTTA	TCAGTATATG	CTTAGCTGTA
4561	AGTGACAAGT	ATTTT'TTCTG	AACAGAAGTT	TACTTAGAAA	TACCATGCAC	TTGGGGGTAC
4621	CAATTAACCG	CCTGAAAATT	AGCATATTGA	TAGTTCTTAG	AGAGACCAGA	TATAATCTAA
4681	GAATTTATAT	GAAAGATTG	TATCATTAGA	GCCAGAAATA	ATTTTATATT	AATATATAAT
4741	ACAGATTAAC	ATTATATATA	ATAATGTACCT	GTGTCACTTC	TGACATGAGC	CTGTAAACAT
4801	ATATTCATAT	ATGTACCTGC	ACATGTACCC	ACCTGATGTA	GGTCTTATTC	CTTTAGTATG
4861	GACTTAAAGT	ACTTATTCAT	ATACCTTGTA	ACTAAAAATT	AGAACAGCTC	CCTAGAATTG
4921	TGAAC'TTTTA	AGAGTCTGAC	TAGAAA'TTTG	CAACTTATAA	AAAAGTTACT	TTTAAAAATA
4981	TAAGTTAGGG	CTAGGCACAG	TGGCTCATGC	CTATAATCTC	AGCACTTTTG	GGAGGCCAAG
5041	ACAGGAGGAT	CACTTCAGGC	CAGGAGTTCA	AGATCAACCA	ACCTGGGTAA	CATGGCCAGA
5101	CCCCATCTCT	ATTATATATA	ATATATATAA	AACTTAGAGT	TTTATCTTTC	CCCTAAAAAG
5161	GGCCGTGATA	TTTGCAGCAG	CCTCAAATTG	CTCTTAAGGG	GTTTAGGTGT	GCAGAAGCTT
5221	TCCTTTCCCT	ACCCAGTAAC	CATGTGACTA	CTAACGTGGT	ATATTGATTT	ATTTTGT'TTG
5281	CTGTCTGTCT	CCCTGCCCC	ACTGCTGGAA	CAGAGGCTCC	AAGAAAACAG	GGACCTTATT
5341	ATTCACTACT	GCATCCCCAG	TAATGAAAGT	ACTTAGAAAA	TAATTATTGA	ATGAATGAAA
5401	TCTAAACTGT	GAACCTGAGG	GTGTTTGTGG	CAGTGT'TTGT	TTTACTGAAT	TGTAGAAGGA
5461	CATAACCGTG	TTTTCAGTGT	TTCTATGGAA	CAAACCTGTA	CATTTTATTT	CAC'TTGTGTT
5521	TTGTCTTAAA	CCCTACTGCT	GGAAACAATT	TTATGTAATA	AGCAATGGGC	CCAAAAGTCT
5581	AGGAGTTTTT	TTGTACTTAG	TGAATTTGTA	TGCAACAGAG	ATGCTGCAGC	TGATGCCTTT
5641	AAAAGGTATT	CATCATGGAA	GAGCTGAGGC	CTGTGCTTGG	TGTTCCAGAG	CCGAGGGTTG
5701	AGCATCCTGA	AGGAGCCACT	GCAGCCGTCA	CTGTCCCCAG	AGCCTGTGGA	GATAGAGCCT
5761	GTTTGCTGCT	TTTTCTTCCC	GCTCTTAAGA	CATGGCTGGA	GCTCAGTCTT	CATTGAATGA
5821	AGTTTGCTGT	GGTATTGCAT	AGCCTTGCTT	TCTTGAACCTA	AACTGTTTGC	CCTTCACAAG
5881	TAGT'TCTTCT	TTCAGGATTA	GTTTCGT'TCCA	AGGAGGCTCT	TCAGTCTCAC	AGATAAGTAG
5941	ATCTCTCCTG	CTGTCTGGAC	ACATTTCACT	CGGAAATTGA	ATACAATTTG	TATTCAGGCT
6001	GGGAACCTGA	ACACACACTT	GTGTTT'TTAA	GCTTCCCTTT	TTTACAGTGG	ACAAGGACAC
6061	AAATAATAAA	TAAATCATCC	CTAATGCCCA	AGAAAAAA		

Figure 10:

(b) Human Interferon receptor 2a (GenBank accession NM_207585)

MLLSQNAFIFRSLNLVLMVYISLVFGISYDSPDYTDESCTFKIS
 LRNFRSILSWELKNHSIVPTHYTLTYTMSKPEDLKVVKNCAANTTRSFCDLTDEWRS
 T
 HEAYVTVLEGFSGNTTLFSCSHNFWLAIDMSFEPPEFEIVGFTNHINVMVKFPSIVE
 E
 ELQFDLSLVIEEQSEGIVKKHKPEIKGNMSGNFTYIIDKLIPNTNYCVSVYLEHSDE
 Q
 AVIKSPLKCTLLPPGQESESAESAIGGGIITVFLIALVLTSTIVTLKWIGYICLRNS
 L
 PKVLNFHNFLAWPFPNLPPEAMDMVEVIYINRKKKVDYNYDDESDSDTEAAPRTS
 G
 GGYTMHGLTVRPLGQASATSTESQLIDPESEEEPDLPEDVDELPTMPKDSPQQLELL
 S
 GPCERRKSPLQDPFPEEDYSSTEGSGGRITFNVDLNSVFLRVLDDESDDDLEAPLML
 S
 SHLEEMVDPEDPDNVQSNHLLASGEGTQPTFPSPSSEGLWSEDAPSDQSDTSESDVD
 L
 GDGYIMR

1	GCCCGCGCTT	CCGTATCGCT	CCTCGTAGGC	CGGGGCTCGG	CGCGCGCACC	CGCACTAAAG
61	ACGCTTCTTC	CCGGCGGGTA	GGAATCCCGC	CGGCGAGCCG	AACAGTTCCC	CGAGCGCAGC
121	CCGCGGACCA	CCACCCGGCC	GCACGGGCGC	CTTTTGTTCC	CCGCCCCCGC	CTTCTGTCCG
181	AGAGGCCGCC	CGCGAGGCGC	ATCCTGACCG	CGAGCGTCGG	GTCCCAGAGC	CGGGCGCGGC
241	TGGGGCCCGA	GGCTAGCATC	TCTCGGGAGC	CGCAAGGCGA	GAGCTGCAAA	GATGTAAAAG
301	TCAAGAGAAG	ACTCTAAAAA	TAGCAAAGAT	GCTTTTGAGC	CAGAATGCCT	TCATCTTCAG
361	ATCACTTAAT	TTGGTTCTCA	TGGTGTATAT	CAGCCTCGTG	TTTGGTATTT	CATATGATTC
421	GCCTGATTAC	ACAGATGAAT	CTTGCACTTT	CAAGATATCA	TTGCGAAATT	TCCGGTCCAT
481	CTTATCATGG	GAATTAATAA	ACCACTCCAT	TGTACCAACT	CACTATACAT	TGCTGTATAC
541	AATCATGAGT	AAACCAGAAG	ATTTGAAGGT	GGTTAAGAAC	TGTGCAATAA	CCACAAGATC
601	ATTTTGTGAC	CTCACAGATG	AGTGGAGAAG	CACACACGAG	GCCTATGTCA	CCGTCCTAGA
661	AGGATTCAGC	GGGAACACAA	CGTTGTTCAG	TTGCTCACAC	AATTTCTGGC	TGGCCATAGA
721	CATGTCTTTT	GAACCACCAG	AGTTTGAGAT	TGTTGGTTTT	ACCAACCACA	TTAATGTGAT
781	GGTGAAATTT	CCATCTATTG	TTGAGGAAGA	ATTACAGTTT	GATTTATCTC	TCGTCAATGA
841	AGAACAGTCA	GAGGGAATTG	TTAAGAAGCA	TAAACCCGAA	ATAAAAGGAA	ACATGAGTGG
901	AAATTTACCC	TATATCATTG	ACAAGTTAAT	TCCAAACACG	AACTACTGTG	TATCTGTTTA
961	TTTAGAGCAC	AGTGATGAGC	AAGCAGTAAT	AAAGTCTCCC	TTAAATATGA	CCCTCCTTCC
1021	ACCTGGCCAG	GAATCAGAA	CAGCAGAAAT	TGCCAAATAA	GGAGGAATAA	TTACTGTGTT
1081	TTTGATAGCA	TTGGTCTTGA	CAAGCACCAT	AGTGACACTG	AAATGGATTG	GTTATATATG
1141	CTTAAGAAAT	AGCCTCCCCA	AAGTCTTGAA	TTTTTCATAA	TTTTTAGCCT	AGCCATTTCC
1201	TAACCTGCCA	CCGTTGGAAG	CCATGGATAT	GGTGGAGGTC	ATTTACATCA	ACAGAAAGAA
1261	GAAAGTGTGG	GATTATAATT	ATGATGATGA	AAGTGATAGC	GATACTGAGG	CAGCGCCCAG
1321	GACAAGTGGC	GGTGGCTATA	CCATGCATGG	ACTGACTGTC	AGGCCTCTGG	GTCAGGCCTC
1381	TGCCACCTCT	ACAGAATCCC	AGTTGATAGA	CCCGGAGTCC	GAGGAGGAGC	CTGACCTGCC
1441	TGAGGTTGAT	GTGGAGCTCC	CCACGATGCC	AAAGGACAGC	CCTCAGCAGT	TGGAACCTCT
1501	GAGTGGGCCC	TGTGAGAGGA	GAAAGAGTCC	ACTCCAGGAC	CCTTTTCCCG	AAGAGGACTA
1561	CAGCTCCACG	GAGGGGTCTG	GGGGCAGAA	TACCTTCAAT	GTGGACTTAA	ACTCTGTGTT
1621	TTTGAGAGTT	CTTGATGACG	AGGACAGTGA	CGACTTAGAA	GCCCCCTGTA	TGCTATCGTC
1681	TCATCTGGAA	GAGATGGTTG	ACCCAGAGGA	TCCTGATAAT	GTGCAATCAA	ACCATTTGCT
1741	GGCCAGCGGG	GAAGGGACAC	AGCCAACCTT	TCCCAGCCCC	TCTTCAGAGG	GCCTGTGGTC
1801	CGAAGATGCT	CCATCTGATC	AAAGTGACAC	TTCTGAGTCA	GATGTTGACC	TTGGGGATGG
1861	TTATATAATG	AGATGACTCC	AAAACATTTG	AATGAACCTT	GACAGACAAG	CACCTACAGG
1921	GTTCTTTGTC	TCTGCATCCT	CACTTGCTGC	CTTATCGTCT	GCAAGTGTTC	TCCAAGGGAA
1981	GGAGGAGGAA	ACTGTGGTGT	TCCTTTCTTC	CAGGTGACAT	CACCTATGCA	CATTCCCAGT
2041	ATGGGGACCA	TAGTATCATT	CAGTGCATTG	TTTACATATT	CAAAGTGGTG	CACCTTGAAG
2101	GAAGCACATG	TGCACCTTTC	CTTTACACTA	ATGCACTTAG	GATGTTTCTG	CATCATGTCT
2161	ACCAGGGAGC	AGGGTTCCCC	ACAGTTTCAG	AGGTGGTCCA	GGACCCTATG	ATATTTCTCT
2221	TCTTTCGTTC	TTTTTTTTTT	TTTTTTTTTG	GACAGAGTCT	CGTTCTGTCT	CCCAAGCTGG
2281	AGCGCAATGG	TGTGATCTTG	GCTCACTGCA	ACATCCGCCT	CCCAGGTTCA	AGTGATTCTC
2341	CTGCCTCAGC	CTCCCTCGCA	AGTAGCTGGG	ATTACAGGCG	CCTGCCACCA	TGCCTAGCAA

Figure 10 (cont.)

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2401 ATTTTGTAT TTTTAGTAGA GACAGGATTT TACCATGTTG GCCAGGCTGG TCTCAAACCTC
2461 CTGACCTCAA GTGATCTGCC CTCCTCAGCC TCGTAAAGTG CTGGGATTAC AGGGGTGAGC
2521 CGCTGTGCCT GGCTGGCCCT GTGATATTTT TGTGAAATAA ATTGGGCCAG GGTGGGAGCA
2581 GGGAAAGAAA AGGAAAATAG TAGCAAGAGC TGCAAAGCAG GCAGGAAGGG AGGAGGAGAG
2641 CCAGGTGAGC AGTGGAGAGA AGGGGGGCCC TGCACAAGGA AACAGGGAAG AGCCATCGAA
2701 GTTTCAGTCG GTGAGCCTTG GGCACCTCAC CCATGTCACA TCCTGTCTCC TGCAATTGGA
2761 ATTCCACCTT GTCCAGCCCT CCCCAGTTAA AGTGGGGAAG ACAGACTTTA GGATCACGTG
2821 TGTGACTAAT ACAGAAAGGA AACATGGCGT CGGGGAGAGG GATAAACCT GAATGCCATA
2881 TTTTAAGTTA AAAAAAAA

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Figure 11:

(c) Human Interferon receptor 2b (GenBank accession NM_000874)

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MLLSQNAFIFRSLNLVLMVYISLVFGISYDSPDYTDESCTFKIS
LRNFRSILSWELKNHSIVPTHYTLTYIMSKPEDLKVVKNCAN'TTRSFCDLTDEWRS
T
HEAYVTVLEGFSGNTTLFSCSHNFWLAIDMSFEPPEFEIVGFTNHINVMVKFPSIVE
E
ELQFDLSLVIEEQSEGIVKKHKPEIKGNMSGNFTYIIDKLIPNTNYCVSVYLEHSDE
Q
AVIKSPLKCTLLPPGQESESAESA KIGGIITVFLIALVLTSTIVTLKWIGYICLRNS
L
PKVLRQGLAKGWNVAIAIHRC SHNALQSETPELKQSSCLSFPSSWDYKRASLCPSD

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1 GCCCGCGCTT CCGTATCGCT CCTCGTAGGC CGGGGCTCGG CGCGCGCACC CGCACTAAAG
61 ACGCTTCTTC CCGGCGGGTA GGAATCCCGC CGGCGAGCCG AACAGTTCCC CGAGCGCAGC
121 CCGCGGACCA CCACCCGGCC GCACGGGCGC CTTTGTGCCC CCGCCCGCCG CTTCTGTCCG
181 AGAGGCCGCC CGCGAGGCGC ATCCTGACCG CGAGCGTCGG GTCCCAGAGC CGGGCGCGGC
241 TGGGGCCCGA GGCTAGCATC TCTCGGGAGC CGCAAGCGCA GAGCTGCAA GTTTAATTAG
301 ACACCTCAGA ATTTTGATCA CCTAATGTTG ATTTTCAGATG TAAAAGTCAA GAGAAGACTC
361 TAAAAATAGC AAAGATGCTT TTGAGCCAGA ATGCCTTCAT CTTCAGATCA CTTAATTTGG
421 TTCTCATGGT GTATATCAGC CTCGTGTTTG GTATTTTCATA TGATTCGCCT GATTACACAG
481 ATGAATCTTG CACTTTCAG ATATCATTGC GAAATTTCCG GTCCATCTTA TCATGGGAAT
541 TAAAAAACCA CTCCATTGTA CCAACTCACT ATACATTGCT GTATACAATC ATGAGTAAAC
601 CAGAAGATTT GAAGGTGGTT AAGAACTGTG CAAATACCAC AAGATCATTT TGTGACCTCA
661 CAGATGAGTG GAGAAGCACA CACGAGGCC ATGTCAACCG CTTAGAAGGA TTCAGCGGGA
721 ACACAACGTT GTTCAGTTGC TCACACAATT TCTGGCTGGC CATAGACATG TCTTTTGAAC
781 CACCAGAGTT TGAGATTGTT GGTTTTACCA ACCACATTAA TGTGATGGTG AAATTTCCAT
841 CTATTGTTGA GGAAGAATTA CAGTTTGATT TATCTCTCGT CATTGAAGAA CAGTCAGAGG
901 GAATTGTTAA GAAGCATAAA CCCGAAATAA AAGGAAACAT GAGTGGAAT TTCACCTATA
961 TCATTGACAA GTTAATTCCA AACACGAAC ACTGTGTATC TGTTTATTTA GAGCACAGTG
1021 ATGAGCAAGC AGTAATAAAG TCTCCCTTAA AATGCACCT CTTCCACCT GGCCAGGAAT
1081 CAGAATCAGC AGAATCTGCC AAAATAGGAG GAATAATTAC TGTGTTTTTG ATAGCATTGG
1141 TCTTGACAAG CACCATAGTG ACCTGAAAT GGATTGGTTA TATATGCTTA AGAAATAGCC
1201 TCCCCAAGT CTTGAGGCAA GGTCTCGCTA AGGGCTGGAA TGCAGTGGCT ATTCACAGGT
1261 CGAGTCATAA TGCCTACAG TCTGAAACTC CTGAGCTCAA ACAGTCGTCC TGCCTAAGCT
1321 TCCCCAGTAG CTGGGATTAC AAGCGTGCAT CCCTGTGCC CAGTGATTAA GTTTTATTAT
1381 GTAGAAAATA AAGAGCAAAC AGTACAGCTG AAAAAAAAAA AAAAAAAAAA

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Figure 12

(d) Human Interferon receptor 2c (GenBank accession NM_207584)

MLLSQNAFIFRSLNLVLMVYISLVFGISYDSPDYTDESCTFKIS
 LRNFRSILSWELKNHSIVPTHYTLTYTIMSKPEDLKVVKNCANTRSFCDLTDEWRS
 T
 HEAYVTVLEGFSGNTTLFSCSHNFWLAIDMSFEPPEFEIVGFTNHINVMVKFPSIVE
 E
 ELQFDLSLVIEEQSEGIVKKHKPEIKGNMSGNFTYIIDKLIPNTNYCVSVYLEHSDE
 Q
 AVIKSPLKCTLLPPGQESESAESAIGGIITVFLIALVLTSTIVTLKWIGYICLRNS
 L
 PKVLRQGLAKGWNVAIAIHRCSHNALQSETPELKQSSCLSFPSWDYKRASLCPSD

1	GCCCGCGCTT	CCGTATCGCT	CCTCGTAGGC	CGGGGCTCGG	CGCGCGCACC	CGCACTAAAG
61	ACGCTTCTTC	CCGGCGGGTA	GGAATCCCGC	CGGCGAGCCG	AACAGTTCCC	CGAGCGCAGC
121	CCGCGGACCA	CCACCCGGCC	GCACGGGCCG	CTTTTGTTCC	CCGCCCGCCG	CTTCGTGCCG
181	AGAGGCCGCC	CGCGAGGCGC	ATCCTGACCG	CGAGCGTCGG	GTCCCAGAGC	CGGGCGCGGC
241	TGGGGCCCGA	GGCTAGCATC	TCTCGGGAGC	CGCAAGGCGA	GAGCTGCAAA	GATGTAAAAG
301	TCAAGAGAAG	ACTCTAAAAA	TAGCAAAGAT	GCTTTTGAGC	CAGAATGCCT	TCATCTTCAG
361	ATCACTTAAT	TTGGTTCTCA	TGGTGTATAT	CAGCCTCGTG	TTTGGTATTT	CATATGATTC
421	GCCTGATTAC	ACAGATGAAT	CTTGCACTTT	CAAGATATCA	TTGCGAAATT	TCCGGTCCAT
481	CTTATCATGG	GAATTAAAAA	ACCACTCCAT	TGTACCAACT	CACTATACAT	TGCTGTATAC
541	AATCATGAGT	AAACCAGAAG	ATTTGAAGGT	GGTTAAGAAC	TGTGCAAATA	CCACAAGATC
601	ATTTTGTGAC	CTCACAGATG	AGTGGAGAAG	CACACACGAG	GCCTATGTCA	CCGTCCTAGA
661	AGGATTACAG	GGGAACACAA	CGTTGTTTCA	TTGCTCACAC	AATTTCTGGC	TGGCCATAGA
721	CATGTCTTTT	GAACCACCAG	AGTTTGAGAT	TGTTGGTTTT	ACCAACCACA	TTAATGTGAT
781	GGTGAAATTT	CCATCTATTG	TTGAGGAAGA	ATTACAGTTT	GATTTATCTC	TCGTCAATGA
841	AGAACAGTCA	GAGGGAATTG	TTAAGAAGCA	TAAACCCGAA	ATAAAAGGAA	ACATGAGTGG
901	AAATTTACAC	TATATCATTG	ACAAGTTAAT	TCCAAACACG	AACTACTGTG	TATCTGTTTA
961	TTTAGAGCAC	AGTGATGAGC	AAGCAGTAAT	AAAGTCTCCC	TTAAAATGCA	CCCTCCTTCC
1021	ACCTGGCCAG	GAATCAGAAAT	CAGCAGAATC	TGCCAAAATA	GGAGGAATAA	TTACTGTGTT
1081	TTTGATAGCA	TTGGTCTTGA	CAAGCACCAT	AGTGACACTG	AAATGGATTG	GTTATATATG
1141	CTTAAGAAAT	AGCCTCCCCA	AAGTCTTGAG	GCAAGGTCTC	GCTAAGGGCT	GGAATGCAGT
1201	GGCTATTAC	AGGTGCAGTC	ATAATGCACT	ACAGTCTGAA	ACTCCTGAGC	TCAAACAGTC
1261	GTCCTGCCTA	AGCTTCCCCA	GTAGCTGGGA	TTACAAGCGT	GCATCCCTGT	GCCCCAGTGA
1321	TTAAGTTTTA	TTATGTAGAA	AATAAAGAGC	AAACAGTACA	GCTGAAAAAA	AAAAAAAAAA
1381	AA					

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2008/061877

A. CLASSIFICATION OF SUBJECT MATTER

INV. G01N33/576 C12Q1/68 A61P31/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.†
X	TAYLOR MILTON W ET AL: "Changes in gene expression during pegylated interferon and ribavirin therapy of chronic hepatitis C virus distinguish responders from nonresponders to antiviral therapy." JOURNAL OF VIROLOGY APR 2007, vol. 81, no. 7, April 2007 (2007-04), pages 3391-3401, XP002474027 ISSN: 0022-538X the whole document in particular: abstract page 3392, left-hand column, paragraph 3 - paragraph 4 page 3396 - page 3398; table 4 page 3399 - page 3400; table 5 page 3400, left-hand column, line 4 - line 14 figures 1,2 -/--	1-13, 18-20

☒ Further documents are listed in the continuation of Box C.

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Date of the actual completion of the international search

14 January 2009

Date of mailing of the international search report

30/01/2009

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International application No
PCT/EP2008/061877

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	* tables S1 and S2 retrieved from http://jvi.asm.org/cgi/content/full/81/7/3391/DC1 (supplemental material) * ----- MEURS ELIANE F ET AL: "The interferon inducing pathways and the hepatitis C virus." WORLD JOURNAL OF GASTROENTEROLOGY : WJG 7 MAY 2007, vol. 13, no. 17, 7 May 2007 (2007-05-07), pages 2446-2454, XP002474171 ISSN: 1007-9327 the whole document in particular: abstract page 2448; figure 1 ----- CHEN ET AL: "Hepatic Gene Expression Discriminates Responders and Nonresponders in Treatment of Chronic Hepatitis C Viral Infection" GASTROENTEROLOGY, ELSEVIER, PHILADELPHIA, PA, US, vol. 128, no. 5, May 2005 (2005-05), pages 1437-1444, XP005314556 ISSN: 0016-5085 cited in the application the whole document in particular: abstract page 1439; table 3 ----- TATENO MAKOTO ET AL: "Expression profiling of peripheral-blood mononuclear cells from patients with chronic hepatitis C undergoing interferon therapy." THE JOURNAL OF INFECTIOUS DISEASES 15 JAN 2007, vol. 195, no. 2, 15 January 2007 (2007-01-15), pages 255-267, XP002474028 ISSN: 0022-1899 the whole document in particular: page 261; table 4 table 6 -----	1-20 1-13, 18-20 1-13, 18-20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2008/061877

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search reportcovers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-13 and 18-20

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-13 and 18-20

Method for predicting if a subject having a viral infection of the liver will respond to an antiviral therapy that includes stimulation of IFN activity based on gene expression.

2. claims: 14-15

Method for predicting if a subject having a viral infection of the liver will respond to an antiviral therapy that includes stimulation of IFN activity based on the subcellular location of STAT-1 (Signal Transducers and Activators of Transcription 1).

3. claims: 16-17

Agent that reduces the activation of the IFN system and its use for the prevention/treatment of a viral infection of the liver.
