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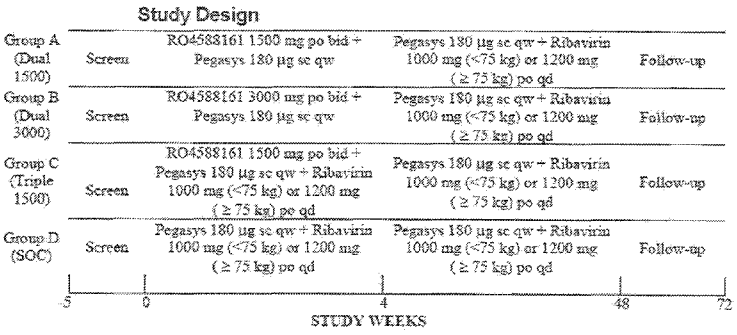


FIG. 1

(57) Abstract: The present invention relates to biomarkers that are useful for predicting the response of hepatitis C virus infected patients to pharmacological treatment.

WO 2011/067195 A1

BIOMARKERS FOR PREDICTING SUSTAINED RESPONSE TO HCV TREATMENT

FIELD OF THE INVENTION

The present invention relates to biomarkers that are useful for predicting the response of hepatitis C virus infected patients to pharmacological treatment.

BACKGROUND OF THE INVENTION

Hepatitis C virus (HCV) is a major health problem and the leading cause of chronic liver disease throughout the world. (Boyer, N. *et al. J. Hepatol.* **2000** 32:98-112). Patients infected with HCV are at risk of developing cirrhosis of the liver and subsequent hepatocellular carcinoma and, hence, HCV is the major indication for liver transplantation.

According to the World Health Organization, there are more than 200 million infected individuals worldwide, with at least 3 to 4 million people being infected each year. Once infected, about 20% of people clear the virus, but the rest can harbor HCV the rest of their lives. Ten to twenty percent of chronically infected individuals eventually develop liver-destroying cirrhosis or cancer. The viral disease is transmitted parenterally by contaminated blood and blood products, contaminated needles, or sexually and vertically from infected mothers or carrier mothers to their offspring. Current treatments for HCV infection, which are restricted to immunotherapy with recombinant interferon-α alone or in combination with the nucleoside analog ribavirin, are of limited clinical benefit as resistance develops rapidly. There is an urgent need for improved therapeutic agents that effectively combat chronic HCV infection

HCV has been classified as a member of the virus family *Flaviviridae* that includes the genera *flaviviruses*, *pestiviruses*, and *hepaciviruses* which includes hepatitis C viruses (Rice, C. M., *Flaviviridae: The viruses and their replication*, in: *Fields Virology*, Editors: Fields, B. N., Knipe, D. M., and Howley, P. M., Lippincott-Raven Publishers, Philadelphia, Pa., Chapter 30, 931-959, 1996). HCV is an enveloped virus containing a positive-sense single-stranded RNA genome of approximately 9.4 kb. The viral genome consists of a 5'-untranslated region (UTR), a long open reading frame (ORF) encoding a polyprotein precursor of-approximately 3011 amino acids, and

a short 3' UTR. The 5' UTR is the most highly conserved part of the HCV genome and is important for the initiation and control of polyprotein translation.

Genetic analysis of HCV has identified six main genotypes showing a >30% divergence in their DNA sequence. Each genotype contains a series of more closely related subtypes which show a 20-25 % divergence in nucleotide sequences (Simmonds, P. **2004** *J. Gen. Virol.* **85:**3173-88). More than 30 subtypes have been distinguished. In the US approximately 70% of infected individuals have Type 1a and 1b infection. Type 1b is the most prevalent subtype in Asia. (X. Forns and J. Bukh, *Clinics in Liver Disease* **1999** 3:693-716; J. Bukh *et al.*, *Semin. Liv. Dis.* **1995** 15:41-63). Unfortunately Type 1 infections are more resistant to therapy than either the type 2 or 3 genotypes (N. N. Zein, *Clin. Microbiol. Rev.*, **2000** 13:223-235).

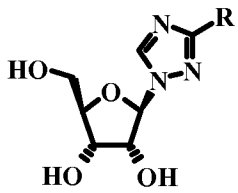
The genetic organization and polyprotein processing of the nonstructural protein portion of the ORF of pestiviruses and hepaciviruses is very similar. These positive stranded RNA viruses possess a single large ORF encoding all the viral proteins necessary for virus replication. These proteins are expressed as a polyprotein that is co- and post-translationally processed by both cellular and virus-encoded proteinases to yield the mature viral proteins. The viral proteins responsible for the replication of the viral genome RNA are located within approximately the carboxy-terminal. Two-thirds of the ORF are termed nonstructural (NS) proteins. For both the pestiviruses and hepaciviruses, the mature nonstructural (NS) proteins, in sequential order from the amino-terminus of the nonstructural protein coding region to the carboxy-terminus of the ORF, consist of p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B.

The NS proteins of pestiviruses and hepaciviruses share sequence domains that are characteristic of specific protein functions. For example, the NS3 proteins of viruses in both groups possess amino acid sequence motifs characteristic of serine proteinases and of helicases (Gorbalenya *et al. Nature* **1988** 333:22; Bazan and Fletterick *Virology* **1989** 171:637-639; Gorbalenya *et al. Nucleic Acid Res.* **1989** 17.3889-3897). Similarly, the NS5B proteins of pestiviruses and hepaciviruses have the motifs characteristic of RNA-directed RNA polymerases (Koonin, E. V. and Dolja, V. V. *Crit. Rev. Biochem. Molec. Biol.* **1993** 28:375-430).

The actual roles and functions of the NS proteins of pestiviruses and hepaciviruses in the lifecycle of the viruses are directly analogous. In both cases, the NS3 serine proteinase is

responsible for all proteolytic processing of polyprotein precursors downstream of its position in the ORF (Wiskerchen and Collett *Virology* **1991** 184:341-350; Bartenschlager *et al.* J. Virol. **1993** 67:3835-3844; Eckart *et al. Biochem. Biophys. Res. Comm.* **1993** 192:399-406; Grakoui *et al. J. Virol.* **1993** 67:2832-2843; Grakoui *et al. Proc. Natl. Acad. Sci. USA* **1993** 90:10583-10587; Ilijikata *et al. J. Virol.* **1993** 67:4665-4675; Tome *et al. J. Virol.* **1993** 67:4017-4026). The NS4A protein, in both cases, acts as a cofactor with the NS3 serine protease (Bartenschlager *et al. J. Virol.* **1994** 68:5045-5055; Failla *et al. J. Virol.* **1994** 68: 3753-3760; Xu *et al. J Virol.* **1997** 71:53 12-5322). The NS3 protein of both viruses also functions as a helicase (Kim *et al. Biochem. Biophys. Res. Comm.* **1995** 215: 160-166; Jin and Peterson *Arch. Biochem. Biophys.* **1995**, 323:47-53; Warrener and Collett *J. Virol.* **1995** 69:1720-1726). Finally, the NS5B proteins of pestiviruses and hepaciviruses have the predicted RNA-directed RNA polymerases activity (Behrens et al. EMBO **1996** 15:12-22; Lechmann *et al. J. Virol.* **1997** 71:8416-8428; Yuan *et al. Biochem. Biophys. Res. Comm.* **1997** 232:231-235; Hagedorn, PCT WO 97/12033; Zhong *et al. J. Virol.* **1998** 72:9365-9369).

Currently there are a limited number of approved therapies are currently available for the treatment of HCV infection. New and existing therapeutic approaches to treating HCV and inhibition of HCV NS5B polymerase have been reviewed: R. G. Gish, *Sem. Liver. Dis.*, **1999** 19:5; Di Besceglie, A. M. and Bacon, B. R., *Scientific American*, October: **1999** 80-85; G. Lake-Bakaar, Current and Future Therapy for Chronic Hepatitis C Virus Liver Disease, *Curr. Drug Targ. Infect Dis.* **2003** 3(3):247-253; P. Hoffmann *et al.*, Recent patents on experimental therapy for hepatitis C virus infection (1999-2002), *Exp. Opin. Ther. Patents* **2003** 13(11):1707-1723; F. F. Poordad *et al.* Developments in Hepatitis C therapy during 2000-2002, *Exp. Opin. Emerging Drugs* **2003** 8(1):9-25; M. P. Walker *et al.*, Promising Candidates for the treatment of chronic hepatitis C, *Exp. Opin. Investig. Drugs* **2003** 12(8):1269-1280; S.-L. Tan *et al.*, Hepatitis C Therapeutics: Current Status and Emerging Strategies, *Nature Rev. Drug Discov.* **2002** 1:867-881; R. De Francesco *et al.* Approaching a new era for hepatitis C virus therapy: inhibitors of the NS3-4A serine protease and the NS5B RNA-dependent RNA polymerase, *Antiviral Res.* **2003** 58:1-16; Q. M. Wang *et al.* Hepatitis C virus encoded proteins: targets for antiviral therapy, *Drugs of the Future* **2000** 25(9):933-8-944; J. A. Wu and Z. Hong, Targeting NS5B-Dependent RNA Polymerase for Anti-HCV Chemotherapy *Cur. Drug Targ.-Inf. Dis* .**2003** 3:207-219. The reviews cite compounds presently in various stages of the development process are hereby incorporated by reference in their entirety.



1a: R = $C(=O)NH_2$
1b: R = $C(=NH^+)NH_2$

Ribavirin (**1a**; 1-((2R,3R,4S,5R)-3,4-Dihydroxy-5-hydroxymethyl-tetrahydro-furan-2-yl)-1H-[1,2,4]triazole-3-carboxylic acid amide; Virazole®) is a synthetic, non-interferon-inducing, broad spectrum antiviral nucleoside analog. Ribavirin has *in vitro* activity against several DNA and RNA viruses including *Flaviviridae* (Gary L. Davis, *Gastroenterology* **2000** 118:S104-S114). In monotherapy ribavirin reduces serum amino transferase levels to normal in 40% of patients, but it does not lower serum levels of HCV-RNA. Ribavirin also exhibits significant toxicity and is known to induce anemia. Ribavirin is an inhibitor of inosine monophosphate dehydrogenase. Ribavirin is not approved in monotherapy against HCV but the compound is approved in combination therapy with interferon α-2a and interferon α-2b. Viramidine **1b** is a prodrug converted to **1a** in hepatocytes.

Interferons (IFNs) have been available for the treatment of chronic hepatitis for nearly a decade. IFNs are glycoproteins produced by immune cells in response to viral infection. Two distinct types of interferon are recognized: Type 1 includes several interferon alphas and one interferon β, type 2 includes interferon γ. Type 1 interferon is produced mainly by infected cells and protects neighboring cells from *de novo* infection. IFNs inhibit viral replication of many viruses, including HCV, and when used as the sole treatment for hepatitis C infection, IFN suppresses serum HCV-RNA to undetectable levels. Additionally, IFN normalizes serum amino transferase levels. Unfortunately, the effects of IFN are temporary. Cessation of therapy results in a 70% relapse rate and only 10-15% exhibit a sustained virological response with normal serum alanine transferase levels. (L.-B. Davis, *supra*)

One limitation of early IFN therapy was rapid clearance of the protein from the blood. Chemical derivatization of IFN with polyethyleneglycol (PEG) has resulted in proteins with substantially improved pharmacokinetic properties. Pegasys® is a conjugate interferon α-2a and a 40 kD branched mono-methoxy PEG and Peg-Intron® is a conjugate of interferon α-2b and a 12 kD

mono-methoxy PEG. (B. A. Luxon *et al.*, *Clin. Therap.* **2002** 24(9):13631383; A. Kozlowski and J. M. Harris, *J. Control. Release*, **2001** 72:217-224).

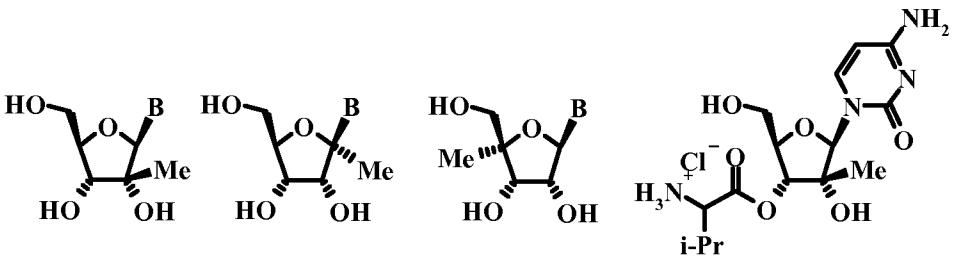
Interferon α-2a and interferon α-2b are currently approved as monotherapy for the treatment of HCV. Roferon-A® (Roche) is the recombinant form of interferon α-2a. Pegasys® (Roche) is the pegylated (i.e. polyethylene glycol modified) form of interferon α-2a. Intron-A® (Schering Corporation) is the recombinant form of Interferon α-2b, and Peg-Intron® (Schering Corporation) is the pegylated form of interferon α-2b.

Other forms of interferon α, as well as interferon β, γ, τ and ω are currently in clinical development for the treatment of HCV. For example, Infergen® (interferon alphacon-1) by InterMune, Omniferon® (natural interferon) by Viragen, Albuferon® by Human Genome Sciences, Rebif® (interferon β-1a) by Ares-Serono, Omega Interferon by BioMedicine, Oral Interferon Alpha by Amarillo Biosciences, pegylated interferon λ1/IL-29 by BMS/Zymogenetics and interferon γ, interferon τ, and interferon γ-1b by InterMune are in development.

Combination therapy of HCV with ribavirin and interferon-α currently represent the optimal therapy. Combining ribavirin and Peg (*infra*) results in a sustained virological response (SVR) in 54-56% of patients. The SVR approaches 80% for type 2 and 3 HCV. (Walker, *supra*) Unfortunately, the combination also produces side effects which pose clinical challenges. Depression, flu-like symptoms and skin reactions are associated with subcutaneous IFN-α and hemolytic anemia is associated with sustained treatment with ribavirin.

A number of potential molecular targets for drug development as anti -HCV therapeutics have now been identified including, but not limited to, the NS2-NS3 autoprotease, the N3 protease, the N3 helicase and the NS5B polymerase. The RNA-dependent RNA polymerase is absolutely essential for replication of the single-stranded, positive sense, RNA genome and this enzyme has elicited significant interest among medicinal chemists.

Nucleoside inhibitors of NS5B polymerase can act either as a non-natural substrate that results in chain termination or as a competitive inhibitor which competes with nucleotide binding to the polymerase. Certain NS5B polymerase nucleoside inhibitors have been disclosed in the following publications, all of which are incorporated by reference in full herein.



2 3 4 5

B= adenine, thymidine, uracil, cytidine, guanine and hypoxanthine

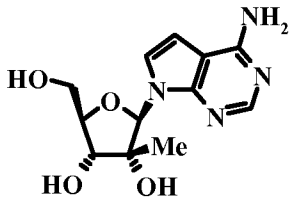
In WO 01 90121 published November 29, 2001, J.-P. Sommadossi and P. Lacolla disclose and exemplify the anti-HCV polymerase activity of 1'-alkyl- and 2'-alkyl nucleosides of formulae **2** and **3.** In WO 01/92282, published December 6, 2001, J.-P. Sommadossi and P. Lacolla disclose and exemplify treating *Flaviviruses* and *Pestiviruses* with 1'-alkyl- and 2'-alkyl nucleosides of formulae **2** and **3**. In WO 03/026675 published April 3, 2003, G. Gosselin discloses 4'-alkyl nucleosides **4** for treating *Flaviviruses* and *Pestiviruses.*

In WO2004003000 published January 8, 2004, J.-P. Sommadossi *et al.*disclose 2'- and 3' prodrugs of 1'-, 2'-, 3'- and 4'-substituted β-D and β-L nucleosides. In WO 2004/002422 published January 8, 2004, 2'-C-methyl-3'-O-valine ester ribofuransyl cytidine for the treatment of *Flaviviridae* infections. Idenix has reported clinical trials for a related compound NM283 which is believed to be the valine ester **5** of the cytidine analog **2** (B = cytosine). In WO 2004/002999 published Jan. 8, 2004, J.-P. Sommadossi *et al.* disclose a series of 2' or 3' prodrugs of 1', 2', 3', or 4' branched nucleosides for the treatment of flavivirus infections including HCV infections.

In WO2004/046331 published June 3, 2004, J.-P. Sommadossi *et al.* disclose 2'-branched nucleosides and *Flaviviridae* mutation. In WO03/026589 published April 3, 2003 G. Gosselin *et al.* disclose methods of treating hepatitis C virus using 4'-modified nucleosides. In WO2005009418 published February 3, 2005, R. Storer *et al.* disclose purine nucleoside analogues for treatment of diseases caused by *Flaviviridae* including HCV.

Other patent applications disclose the use of certain nucleoside analogs to treat hepatitis C virus infection. In WO 01/32153 published May 10, 2001, R. Storer discloses nucleosides derivatives for treating viral diseases. In WO 01/60315 published August 23, 2001, H. Ismaili *et al.*,

disclose methods of treatment or prevention of Flavivirus infections with nucleoside compounds. In WO 02/18404 published March 7, 2002, R. Devos *et al.* disclose 4'-substituted nucleotides for treating HCV virus. In WO 01/79246 published October 25, 2001, K. A. Watanabe disclose 2'- or 3'-hydroxymethyl nucleoside compounds for the treatment of viral diseases. In WO 02/32920 published April 25, 2002 and in WO 02/48 165 published June 20, 2002 L. Stuyver *et al.* disclose nucleoside compounds for the treatment of viral diseases.



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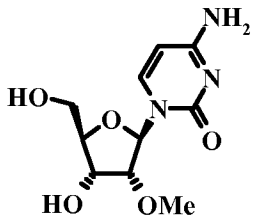


6a

In WO 03/105770 published December 24, 2003, B. Bhat *et al.* disclose a series of carbocyclic nucleoside derivatives that are useful for the treatment of HCV infections. In WO 2004/007512 published January 22, 2003 B. Bhat *et al.* disclose nucleoside compounds that inhibit of RNA-dependent RNA viral polymerase. The nucleosides disclosed in this publication are primarily 2'-methyl-2'-hydroxy substituted nucleosides. In WO 2002/057425 published July 25, 2002 S. S. Carroll *et al.* disclose nucleoside derivatives which inhibitor of RNA-dependent viral polymerase and methods of treating HCV infection. In WO02/057287 published July 25, 2002, S. S. Carroll *et al.* disclose related 2α-methyl and 2β-methylribose derivatives wherein the base is an optionally substituted 7H-pyrrolo[2,3-d]pyrimidine radical **6.** The same application discloses one example of a 3β-methyl nucleoside. S.S. Carroll *et al.* (*J. Biol. Chem.* **2003** 278(14):11979-11984) disclose inhibition of HCV polymerase by 2'-O-methylcytidine (**6a**). In WO 2004/009020 published January 29, 2004, D. B. Olsen *et al.* disclose a series of thionucleoside derivatives as inhibitors of RNA dependent RNA viral polymerase.

PCT Publication No. WO 99/43691 to Emory University, entitled "2'-Fluoronucleosides" discloses the use of certain 2'-fluoronucleosides to treat HCV. U.S. Patent No. 6,348,587 to Emory University entitled "2'-fluoronucleosides" discloses a family of 2'-fluoronucleosides useful for the treatment of hepatitis B, HCV, HIV and abnormal cellular proliferation. Both configurations of the 2' fluoro substituent are disclosed.

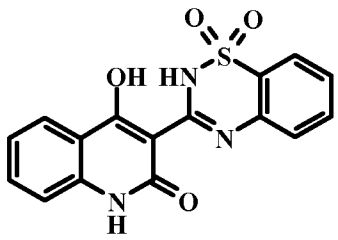
Eldrup *et al.* (Oral Session V, Hepatitis C Virus, Flaviviridae; 16th International

Conference on Antiviral Research (Apr. 27, 2003, Savannah, Ga.)) described the structure activity relationship of 2'-modified nucleosides for inhibition of HCV.

Bhat *et al.* (Oral Session V, Hepatitis C Virus, Flaviviridae; 16th International Conference on Antiviral Research (Apr. 27, 2003, Savannah, Ga.); p A75) describe the synthesis and pharmacokinetic properties of nucleoside analogues as possible inhibitors of HCV RNA replication. The authors report that 2'-modified nucleosides demonstrate potent inhibitory activity in cell-based replicon assays.

Olsen *et al.* (Oral Session V, Hepatitis C Virus, Flaviviridae; 16th International Conference on Antiviral Research (Apr. 27, 2003, Savannah, Ga.) p A76) also described the effects of the 2'-modified nucleosides on HCV RNA replication.

Several classes of non-nucleoside HCV NS5B inhibitors have been described and are incorporated by reference in full herein, including: benzimidazoles, (H. Hashimoto *et al.* WO 01/47833, H. Hashimoto *et al.* WO 03/000254, P. L. Beaulieu *et al.* WO 03/020240 A2; P. L. Beaulieu *et al.* US 6,448,281 B1; P. L. Beaulieu *et al.* WO 03/007945 A1); indoles, (P. L. Beaulieu *et al.* WO 03/0010141 A2); benzothiadiazines, *e.g.*, **7**, (D. Dhanak *et al.* WO 01/85172 A1; D. Dhanak *et al.* WO 03/037262 A2; K. J. Duffy *et al.* WO03/099801 A1, D.Chai *et al.* WO 2004052312, D.Chai *et al.* WO2004052313, D.Chai *et al.* WO02/098424, J. K. Pratt *et al.* WO 2004/041818 A1; J. K. Pratt *et al.* WO 2004/087577 A1), thiophenes, *e.g.*, **8**, (C. K. Chan *et al.* WO 02/100851);



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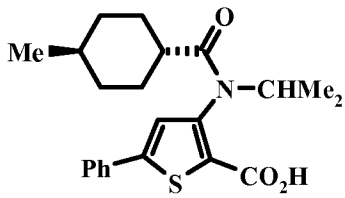

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benzothiophenes (D. C. Young and T. R. Bailey WO 00/18231); β-ketopyruvates (S. Attamura *et al.* US 6,492,423 B1, A. Attamura *et al.* WO 00/06529); pyrimidines (C. Gardelli *et al.* WO 02/06246 A1); pyrimidinediones (T. R. Bailey and D. C. Young WO 00/13708); triazines (K.-H. Chung *et al.* WO 02/079187 A1); rhodanine derivatives (T. R. Bailey and D. C. Young WO 00/10573, J. C. Jean *et al.* WO 01/77091 A2); 2,4-dioxopyrans (R. A. Love *et al.* EP 256628 A2); phenylalanine derivatives (M. Wang *et al. J. Biol. Chem.* **2003** 278:2489-2495).

Nucleoside derivatives often are potent anti-viral (*e.g.,* HIV, HCV, Herpes simplex, CMV) and anti-cancer chemotherapeutic agents. Unfortunately their practical utility is often limited by two factors. Firstly, poor pharmacokinetic properties frequently limit the absorption of the nucleoside from the gut and the intracellular concentration of the nucleoside derivatives and, secondly, suboptimal physical properties restrict formulation options which could be employed to enhance delivery of the active ingredient.

Albert introduced the term prodrug to describe a compound which lacks intrinsic biological activity but which is capable of metabolic transformation to the active drug substance (A. Albert, *Selective Toxicity*, Chapman and Hall, London, 1951). Produgs have been recently reviewed (P. Ettmayer *et al.*, *J. Med Chem.* 2004 47(10):2393-2404; K. Beaumont *et al.*, *Curr. Drug Metab.* 2003 4:461-485; H. Bundgaard, *Design of Prodrugs: Bioreversible derivatives for various functional groups and chemical entities* in *Design of Prodrugs*, H. Bundgaard (ed) Elsevier Science Publishers, Amersterdam 1985; G. M. Pauletti *et al. Adv. Drug Deliv. Rev.* 1997 27:235-256;R. J. Jones and N. Bischofberger, *Antiviral Res.* 1995 27; 1-15 and C. R. Wagner *et al.*, *Med. Res. Rev.* 2000 20:417-45). While the metabolic transformation can catalyzed by specific enzymes, often hydrolases, the active compound can also be regenerated by non-specific chemical processes.

Pharmaceutically acceptable prodrugs refer to a compound that is metabolized, for example hydrolyzed or oxidized, in the host to form the compound of the present invention. The bioconversion should avoid formation fragments with toxicological liabilities. Typical examples of prodrugs include compounds that have biologically labile protecting groups linked to a functional moiety of the active compound. Alkylation, acylation or other lipophilic modification of the hydroxy group(s) on the sugar moiety have been utilized in the design of pronucleotides. These pronucleotides can be hydrolyzed or dealkylated *in vivo* to generate the active compound.

Factors limiting oral bioavailability frequently are absorption from the gastrointestinal tract and first-pass excretion by the gut wall and the liver. Optimization of transcellular absorption through the GI tract requires a $D_{(7.4)}$ greater than zero. Optimization of the distribution coefficient does not, however, insure success. The prodrug may have to avoid active efflux transporters in the enterocyte. Intracellular metabolism in the enterocyte can result in passive

transport or active transport of the metabolite by efflux pumps back into the gut lumen. The prodrug must also resist undesired biotransformations in the blood before reaching the target cells or receptors.

While putative prodrugs sometimes can rationally designed based on the chemical functionality present in the molecule, chemical modification of an active compound produces an entirely new molecular entity which can exhibit undesirable physical, chemical and biological properties absent in the parent compound. Regulatory requirements for identification of metabolites may pose challenges if multiple pathways lead to a plurality of metabolites. Thus, the identification of prodrugs remains an uncertain and challenging exercise. Moreover, evaluating pharmacokinetic properties of potential prodrugs is a challenging and costly endeavor. Pharmacokinetic results from animal models may be difficult to extrapolate to humans.

Recently, it was discovered that in patients infected with Hepatitis C Virus Genotype 1 (HCV-1) or Genotype 4 (HCV-4), a beneficial response to a treatment that includes interferon alpha, ribavirin and a HCV polymerase inhibitor (Triple Therapy) could be predicted if the patient's HCV RNA level becomes undetectable in as short as two weeks post treatment. The correlation between a patient showing Rapid Virologic Response-2 Weeks (RVR2) and achieving Sustained Virologic Response (SVR) at the end of Triple Therapy treatment is disclosed in the commonly owned US patent application USSN 61/138,585, filed December 18, 2008, which is incorporated herein by reference in its entirety.

SUMMARY OF THE INVENTION

The present invention is based on the discovery that in patients infected with Genotype 1 of the Hepatitis C virus (HCV-1) or Genotype 4 HCV (HCV-4) that undergo Triple Therapy treatment of HCV RNA polymerase inhibitor in combination with pegylated IFN and ribavirin, certain biomarkers can be predictive of a patient achieving RVR2, which, in turn, is a positive predictor of the patient showing Sustained Virologic Response at the end of treatment.

In one embodiment, the invention provides for a method for predicting that a human subject infected with HCV-1 or HCV-4 will achieve RVR2 to treatment with interferon, ribavirin and a HCV NS5B polymerase inhibitor comprising:

(i) providing a sample from said subject prior to said treatment (pre-treatment),

(ii) determining the expression level in said sample of at least one protein selected from the group consisting of MDC, Eotaxin, IL10, TARC, and MCP1, and

(iii) comparing the expression level of the at least one protein in said sample to a reference value representative of an expression level of the at least one protein derived from pre-treatment samples of a patient population that did not achieve RVR2 to said treatment;

wherein a statistically significant higher expression level of the at least one protein in said sample is indicative that said subject will achieve RVR2 to said treatment.

In another embodiment, the invention provides for a method for predicting that a human subject infected with HCV-1 or HCV-4 will achieve RVR2 to treatment with interferon, ribavirin and a HCV NS5B polymerase inhibitor comprising:

(i) providing a sample from said subject following one week of said treatment (one-week post treatment),

(ii) determining the expression level in said sample of at least one protein selected from the group consisting of TRAIL and IL12p70, and

(iii) comparing the expression level of the at least one protein in said sample to a reference value representative of an expression level of the at least one protein derived from one-week post treatment samples in a patient population that did not achieve RVR2 to said treatment;

wherein a statistically significant higher expression level of the at least one protein in said sample is indicative that said subject will achieve RVR2 to said treatment.

In yet another embodiment, the invention provides for a method for predicting that a human subject infected with HCV-1 or HCV-4 will achieve RVR2 to treatment with interferon, ribavirin and a HCV NS5B polymerase inhibitor comprising:

(i) providing a sample from said subject prior to said treatment (pre-treatment),

(ii) determining the expression level in said sample of at least one protein selected from the group consisting of TGFbeta1, MIP1b, TRAIL, and MDC,

(iii) providing a sample from said subject following one week of said treatment (one-week post treatment),

(iv) determining the expression level in said sample of at least one protein selected from the group consisting of TGFbeta1, MIP1b, TRAIL, and MDC,

(v) determining a differential expression level of the at least one protein between the pre-treatment sample from said subject and the one-week post treatment sample from said subject,

(vi) comparing said differential expression level of the at least one protein to a reference value representative of a differential expression level of the at least one protein derived from pre-treatment samples and one-week post treatment samples in a patient population that did not achieve RVR2 to said treatment;

wherein a statistically significant change in the differential expression level of the at least one protein is indicative that said subject will achieve RVR2 to said treatment.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the Study Design of the Phase II Clinical Trial for RO4588161.

Figure 2 shows the RVR2 and SVR treatment response of the 31 Group C patients who received Triple Therapy treatment of 1500 mg RO4588161, Pegasys 180 μg, and ribavirin.

Figure 3 shows the expression levels of proteins (in pg/ml) at Week 0 that show a significant difference ($p \leq 0.05$) between patients that achieved SVR (represented by "1") and patients that did not achieve SVR (represented by "0"). ◄ represents the mean value and ► represents the median value. Outliers shown as ■ were not included in the determination of mean and median values.

Figure 4 shows the expression levels of proteins (in pg/ml) at Week 1 that show a significant difference ($p \leq 0.05$) between patients that achieved SVR (represented by "1") and patients that did not achieve SVR (represented by "0"). Symbols have the same meanings as in Figure 3.

Figure 5 shows the differential expression levels of proteins (in Δ pg/ml) between Week 0 and Week 1 that show a significant difference ($p \leq 0.05$) between patients that achieved SVR (represented by "1") and patients that did not achieve SVR (represented by "0"). Symbols have the same meanings as in Figure 3.

Figure 6 shows the performance of four analysis methods for identifying pre-treatment expression levels of proteins that are associated with SVR, including the frequency of being

selected as an important variable (represented by percentage) using each method with 1500 times of simulations, their training error rates, and testing error rates.

DETAILED DESCRIPTION OF THE INVENTION

The term "response" to treatment is a desirable response to the administration of an agent or agents.

The terms "Sustained Virologic Response" ("SVR") and "Complete Response" ("CR") to treatment are herein used interchangeably and refer to the absence of detectable HCV RNA (<15 IU/mL) in the sample of an infected subject by RT-PCR both at the end of treatment and twenty-four weeks after the end of treatment.

The terms "Virologic Non-Response" ("VNR") and "No Response" ("NR") to treatment are herein used interchageably and refer to the presence of detectable HCV RNA (>=15 IU/mL) in the sample of an infected subject by RT-PCR throughout treatment and at the end of treatment.

The term "Rapid Virologic Response-2 Weeks ("RVR2") refers to the absence of detectable HCV RNA (<15 IU/mL) in the sample of an infected subject by RT-PCR after two weeks of treatment.

The terms "sample" or "biological sample" refers to a sample of tissue or fluid isolated from an individual, including, but not limited to, for example, tissue biopsy, plasma, serum, whole blood, spinal fluid, lymph fluid, the external sections of the skin, respiratory, intestinal and genitourinary tracts, tears, saliva, milk, blood cells, tumors, organs. Also included are samples of in vitro cell culture constituents (including, but not limited to, conditioned medium resulting from the growth of cells in culture medium, putatively virally infected cells, recombinant cells, and cell components).

The term "reference value representative of an expression level" refers to an estimate of the mean expression level of a marker protein derived from samples in a HCV patient population that exhibits Virologic Non-Response to a Triple Therapy treatment.

The term "statistically significant" as used herein means that the obtained results are not likely to be due to chance fluctuations at the specified level of probability and as used herein means a level of significance of less than or equal to 0.05 ($p \leq 0.05$), or a probability of error of less than or equal to 5 out of 100.

The terms “interferon” refers to the family of highly homologous species-specific proteins that inhibits viral replication and cellular proliferation and modulate immune response. Typical suitable interferons include, but are not limited to, recombinant interferon alpha-2b such as Intron® A interferon available from Schering Corporation, Kenilworth, N.J., recombinant interferon alpha-2a such as Roferon®-A interferon available from Hoffmann-La Roche, Nutley, N.J., recombinant interferon alpha-2C such as Berofor® alpha 2 interferon available from Boehringer Ingelheim Pharmaceutical, Inc., Ridgefield, Conn., interferon alpha-n1, a purified blend of natural alpha interferons such as Sumiferon® available from Sumitomo, Japan or as Wellferon® interferon alpha-n1 (INS) available from the Glaxo-Wellcome Ltd., London, Great Britain, or a consensus alpha interferon such as those described in U.S. Pat. Nos. 4,897,471 and 4,695,623 (especially Examples 7, 8 or 9 thereof) and the specific product available from Amgen, Inc., Newbury Park, Calif., or interferon alpha-n3 a mixture of natural alpha interferons made by Interferon Sciences and available from the Purdue Frederick Co., Norwalk, Conn., under the Alferon Tradename. “Interferon” may include other forms of interferon alpha, as well as interferon beta, gamma, tau, omega and lambda that are currently in clinical development for the treatment of HCV. For example, Infergen® (interferon alphacon-1) by InterMune, Omniferon® (natural interferon) by Viragen, Albuferon® (Albumin interferon alpha 2b) by Human Genome Sciences, Rebif® (interferon beta-1a) by Ares-Serono, Omega Interferon by BioMedicine, Oral Interferon Alpha by Amarillo Biosciences, and interferon γ, interferon τ, and interferon γ-1b by InterMune, and Glycoferon™ (glycol-engineered consensus interferon). Interferons can include pegylated interferons as defined below.

The terms “pegylated interferon”, “pegylated interferon alpha” and “peginterferon” are used herein interchangeably and means polyethylene glycol modified conjugates of interferon alpha, preferably interferon alpha-2a and alpha-2b. Typical suitable pegylated interferon alpha include, but are not limited to, Pegasys® and Peg-Intron®. Other forms of pegylated interferon may include PEG-Interferon lambda by ZymoGenetics and Bristol-Myers Squibb.

The term "ribavirin" refers to the compound, 1-((2R,3R,4S,5R)-3,4-Dihydroxy-5-hydroxymethyl-tetrahydro-furan-2-yl)-1H-[1,2,4]triazole-3-carboxylic acid amide which is a synthetic, non-interferon-inducing, broad spectrum antiviral nucleoside analog and available under the names, Virazole® and Copegus® .

The term "RO4588161" as used herein refers to the compound, Isobutyric acid (2R,3S,4R,5R)-5-(4-amino-2-oxo-2H-pyrimidin-1-yl)-2-azido-3,4-bis-isobutyryloxy-tetrahydro-furan-2-ylmethyl ester, including pharmaceutically acceptable acid addition salts, and is used interchangeably with the term "R1626" as disclosed in P.J. Pockros *et al.*, *Hepatology*, **2008**, 48: 385-397, which is incorporated by reference in full herein.

The term "RO5024048" as used herein refers to the compound, Isobutyric acid (2R,3R,4R,5R)-5-(4-amino-2-oxo-2H-pyrimidin-1-yl)-4-fluoro-3-isobutyryloxy-4-methyl-tetrahydro-furan-2-ylmethyl ester, including pharmaceutically acceptable acid addition salts, and is used interechangeably with the term "R7128" as disclosed in S. Ali *et al., Antimicrob Agents Chemother.*, **2008** 52(12):4356-4369, which is incorporated by reference in full herein.

The term "around Week 2" refers to a time period of two weeks or fourteen days, plus or minus 1 to 2 days.

The term "CD30" refers to Cytokine receptor CD30, which is also known as Tumor necrosis factor receptor superfamily, member 8 or TNFRSF8, and whose human protein sequence is disclosed in GenBank Accession Number NP_001234.

The term "MIG" refers to Gamma-interferon-induced monokine or Monokine induced by gamma interferon, which is also known as chemokine (C-X-C motif) ligand 9 or CXCL9, and whose human protein sequence is disclosed in GenBank Accession Number NP_002407.

The term "TARC" refers to Thymus and activation-regulated chemokine, which is also known as chemokine (C-C motif) ligand 17 or CCL17, and whose human protein sequence is disclosed in GenBank Accession Number NP_002978.

The term "TFGβ1" "TGFbeta1" refers to Transforming growth factor beta1 (β1), whose human protein sequence is disclosed in GenBank Accession Number NP_000651.

The terms "SDF1b" or "SDF-1b" refers to Stromal cell-derived factor 1 beta, which is also known as chemokine (C-X-C motif) ligand 12 or CXCL12, and whose human protein sequence is disclosed in GenBank Accession Number NP_000600.

The term "Eotaxin-2" refers to Eosinophil chemotactic protein 2, which is also known as chemokine (C-C motif) ligand 24 or CCL24, and whose human protein sequence is disclosed in GenBank Accession Number NP_002982.

The term "TRAIL" refers to TNF-related apoptosis-inducing ligand, which is also known as tumor necrosis factor (ligand) superfamily, member 10 or TNFSF10, and Apo-2L, and whose human protein sequence is disclosed in GenBank Accession Number NP_003801.

The terms "HCC-4" or "HCC4" refers to Human β (CC) chemokine CC-4, which is also known as Monotactin-1 and chemokine (C-C motif) ligand 16 or CCL16, and whose human protein sequence is disclosed in GenBank Accesion Number NP_004581.

The terms "MIP1b" or MIP-1b" refer to Macrophage inflammatory protein 1-beta, which is also known as chemokine (C-C motif) ligand 4 or CCL4, and Lymphocyte-activation gene 1, and whose human protein sequence is disclosed in GenBank Accession Number NP_002975.

The terms "TNFRII" or "TNF-RII" refer to Tumor necrosis factor receptor 2, which is also known as p75 Tumor necrosis factor receptor (p75TNFR) and Tumor necrosis factor receptor superfamily, member 1B or TNFRSF1B, and whose human protein sequence is disclosed under GenBank Accession Number NP_001057.

The terms "ITAC" or "I-TAC" refer to Interferon-inducible T-cell alpha chemoattractant, which is also known as Interferon-gamma-inducible protein 9 or IP9 and chemokine (C-X-C motif) ligand 11 or CXCL11, and whose human protein sequence is disclosed in GenBank Accession Number NP_005400.

The terms "IL2R" or "IL-2R" refer to the high-affinity form of the Interleukin 2 receptor consisting of a heterotrimer amongst Interleukin 2 receptor alpha (IL-2RA), whose human protein sequence is disclosed in GenBank Accession Number NP_000408, Interleukin 2 receptor beta (IL-2RB), whose human protein sequence is disclosed in GenBank Accession Number NP_000869, and Interleukin 2 receptor gamma (IL-2Rγ), also known as the common cytokine receptor gamma chain, whose human protein sequence is disclosed in GenBank Accession Number NP_000197.

The terms "IL-16" or "IL16" refer to Interleukin 16, which is also known as Lymphocyte chemoattractant factor or LCF, and whose human protein sequence is disclosed in GenBank Accession Number NP_004504.

The terms "IP10" or "IP-10" refer to 10 kDa interferon-gamma-induced protein, which is also known as chemokine (C-X-C motif) ligand 10 or CXCL10, and whose human protein sequence is disclosed in GenBank Accession Number NP_001556.

The current recommended first line treatment for patients with chronic hepatitis C is pegylated interferon alpha in combination with ribavirin for 48 weeks in patients carrying genotype 1 or 4 virus and for 24 weeks in patients carrying genotype 2 or 3 virus. Combined treatment with ribavirin was found to be more effective than interferon alpha monotherapy in patients who relapsed after one or more courses of interferon alpha therapy, as well as in previously untreated patients. However, ribavirin exhibits significant side effects including teratogenicity and carcinogenicity. Furthermore, ribavirin causes hemolytic anemia requiring dose reduction or discontinuation of ribavirin therapy in approximately 10 to 20% of patients, which may be related to the accumulation of ribavirin triphosphate in erythrocytes. Therefore, to reduce treatment cost and the incidence of adverse events, it is desirable to tailor the treatment to a shorter duration while not compromising efficacy.

Numerous studies have shown that rapid virological response (RVR) at 4 weeks has been a fairly reliable predictor of a sustained virological response (SVR) for treatment using peginterferon/ribavarin. Some studies have shown that among HCV-1 patients that achieve RVR, the SVR rates were comparable between 24-week and 48-week peginterferon/ribovarin treatment (D.M. Jensen *et al., Hepatology*, **2006**, 43:954-960; S. Zeuzen *et al., J. Hepatol.* **2006**, 44:97-103;

A. Mangia *et al., Hepatology*, **2008**, 47: 43-50), while others demonstrate that even if RVR is attained, 24 weeks of peginterferon/ribavirin is inferior to 48 weeks of treatment in HCV-1 patients (M.-L. Yu *et al., Hepatology*, **2008**, 47:1884-1893.

EXAMPLES

Phase II Clinical Trial involving RO4588161

This was a phase 2A, multi-center, randomized, double-blinded (RO4588161 and ribavirin were double-blinded and Pegasys was open labeled), active-controlled, with a parallel-group study which is ongoing. A screening period (time from the first screening assessment to the first administration of test drug) of 35 days preceded the treatment portion of the trial (Figure 1). The HCV genotype and HCV RNA titer of each patient was confirmed during the screening period and only treatment-naïve patients with HCV genotype-1 and HCV RNA titer ≥ 50,000 IU/mL were eligible for enrollment.

One hundred and seven male and female patients between 18 and 66 years of age were enrolled into the study. Patients were randomized into four treatment groups:

• Group A/Dual 1500 [RO4588161 1500 mg oral, twice daily + Pegasys 180 µg subcutaneous, once weeky] for 4 weeks - 21 patients,

• Group B/Dual 3000 [RO4588161 3000 mg oral, twice daily + Pegasys 180 µg subcutaneous, once weekly] for 4 weeks - 34 patients,

• Group C/Triple 1500 [RO4588161 1500 mg oral, twice daily + Pegasys 180 µg subcutaneous, once weekly + ribavirin 1000 mg (<75 kg) or 1200 mg (≥ 75 kg) oral daily] for 4 weeks – 31 patients or

• Group D/standard of care (SOC) [Pegasys 180 µg subcutaneous, once weekly + ribavirin 1000 mg (<75 kg) or 1200 mg (≥ 75 kg) oral daily] for 4 weeks – 21 patients

From a total of 107 patients, data from 104 patients was evaluable for analysis since 3 patients though randomized did not receive a single dose of study medication. Among the 104 patients there were a total of 43, 4, and 5 patients who prematurely withdrew for safety reasons from RO4588161, Pegasys, and ribavirin treatment, respectively.

Patients meeting all eligibility criteria were randomized to receive RO4588161 in combination with Pegasys with or without ribavirin for 4 weeks or to SOC.

All patients who received at least one dose of study medication would continue to receive open label Pegasys 180 µg sc qw and ribavirin 1000 mg (<75 kg) or 1200 mg (≥ 75 kg) po qd to complete a total treatment period of 48 weeks.

Randomization was stratified by the PK subcohort (sparse PK versus intensive PK) in a 2:3:3:2 ratio into the following treatment groups (Group A/Dual 1500 ~ 20, Group B/Dual 3000 ~ 30, Group C/Triple 1500 ~ 30, Group D/SOC ~ 20).

All patients were to have a safety follow up visit at week 8, 4 weeks after the last dose of the experimental drug combination. Patients were to have this 4 week safety follow up visit during their treatment with the standard of care therapy. Patients who have completed a full 48-week course of therapy were followed for 24 weeks post treatment completion.

Pharmacodynamic analysis included the assessment of serum viral load, and viral response at individual clinical visits and an assessment of antiviral resistance development with RO4588161 given in combination with Pegasys with or without ribavirin in treatment naïve patients with chronic HCV genotype 1 virus infection. Viral response was defined as the percentage of patients with undetectable HCV RNA as measured by the Roche COBAS TaqMan HCV Test (< 15 IU/mL). Pharmacodynamic data were presented by listings, summary statistics (including means, medians, standard errors, confidence intervals for means, ranges, coefficients of variation, proportions of patients with response and confidence intervals for proportions) and plots of means over time.

To identify protein biomarkers predictive for response to the various treatment regimen, plasma samples were collected from each patient at pre-treatment (time point Week 0) and at one-week post treatment (time point Week 1) and tested for the expression levels of various cytokines and chemokines using a customized SearchLight 55-multiplexing sandwich-ELISA system available from Aushon Biosystems (Billerica, MA) by the protocol described in Moody, M.D. et al., "Array-Based ELISAs for High-Throughput Analysis of Human Cytokines", *Biotechniques*, 2001, 31(1): 186-194, which is incorporated herein by reference in its entirety. The human cytokines and chemokines tested in the 55-multiplex assay are listed on Table 1.

TABLE 1

IL-1Ra	IFNg IL	IL-22	IL-8	IL-16	IL-18	IL-4	IL-7
IL-2R	IL-6R	IL-13Ra	MCP1	MCP2	ITAC	MIG	MIP-1a
TNFa	Eotaxin	Exodus-II	IP10	CD30	TARC	IL-15	TRAIL
IL-1β	G-CSF	GM-CSF	MIP-3b	I-309	IL-4R	MIF	HCC-4
IL-5	MDC	Eotaxin-2	MCSF	SDF1b	SCF	RANTES	TNRFII
CD14	IL-10	PARC	IL-12p70	IL-13	IL-17	CD40L	IL-23
IL-6	TGFβ1	MIP-3a	IL-3	MIP-1b	IL-1RII	Lympho-tactin	

Dose-and time-dependent decreases in plasma viral load were observed following treatment with RO4588161, Pegasys and ribavirin. Declines in HCV RNA were observed as early as the first assessment (72 hours) following the first dose. All RO4588161 containing groups had ≥ 3.6 $\log_{10}$ decrease in the mean HCV RNA (IU/mL) from baseline at week 4, all larger than 2.4 $\log_{10}$ with SOC.

Dual 1500 and Dual 3000 revealed dose dependent decreases with a difference in mean change in viral concentrations of minus 0.9 $\log_{10}$ IU/mL (-3.6 vs. -4.5). When comparing Dual 1500 and Triple 1500 (same dose of RO4588161 and Pegasys, but with ribavirin), the difference was even greater at minus 1.6 $\log_{10}$ IU/mL (-5.2 vs. -3.6). In addition, when comparing SOC and Triple 1500 (same dose of Pegasys and ribavirin, but with RO4588161), the difference was the most pronounced at minus 2.8 $\log_{10}$ IU/mL (-5.2 vs. -2.4). In addition, the 95% confidence intervals between Triple 1500 and Dual 1500, and between Triple 1500 and SOC were all non-overlapping, indicating a superior antiviral effect of Triple 1500 over Dual 1500 and SOC.

The treatment outcomes of the 31 Group C patients who underwent Triple Therapy are graphically represented in Figure 2. Out of the 13 patients that were able to show undetectable HCV RNA at two weeks of treatment (i.e. RVR2), eleven were able to achieve SVR at 24 weeks post treatment completion. In contrast, out of the 18 patients that did not exhibit RVR2, only seven achieved SVR.

The expression levels of each of the 55 chemokines and cytokines in pre-treatment plasma samples from patients who achieved SVR were compared to the expression levels of these proteins in pre-treatment plasma samples from patients who did not achieve SVR using the Wilcoxon rank-sum test (a non-parametric method). Similarly, protein expression levels in Week 1 post-treatment samples from SVR patients were compared to protein expression levels in Week1 post-treatment samples from non-SVR patients. Furthermore, differential expression levels of each protein between Week 0 samples and Week 1 samples (delta) were examined and compared between the SVR patients and the non-SVR patients. The statistical significant differences were considered at the critical level of 0.05. The analyses were implemented in the program Spotfire (Spotfire DecisionSite version 9.1.1, 2008, TIBCO, Somerville, MA). The proteins that showed statistically significant differences in expression levels between SVR and non-SVR at Week 0, Week 1 and Week 0-Week 1 differential (delta) are shown on Table 2. The expression level data of each of these proteins for the three test points are shown graphically on Figures 3, 4 and 5.

TABLE 2

WEEK 0		WEEK 1		DELTA	
protein	*p-value*	*protein*	*p-value*	*protein*	*p-value*
CD30	0.0116	CD30	0.01461	HCC-4	0.006044
MIG	0.0213	TRAIL	0.0225	MIP1b	0.006904
TARC	0.02391	TARC	0.04528	SDF1b	0.02683
TGFβ1	0.02683			TNFRII	0.03003
SDF1b	0.04166			ITAC	0.03742
Eotaxin-2	0.0463			MIG	0.04166
				IL2R	0.04627
				IL16	0.0463

In addition to the univariate analyses as described above, multivariate analysis was implemented. The cross validation strategy was applied by randomly selecting 2/3 of patients as the training data set and 1/3 of patients as the test data set. 1500 times of simulations were then run with 4 methods described below:

Method 1. Select best single variable

Method 2. Select up to 2 best variables for Multivariate Logistic Regression Model

Method 3. Select the best 2 variables for Support Vector Machine (SVM)

Method 4. Select the best 5 variables for Random Forest.

The performance of these four methods including the frequency of being selected as an important variable using each method with 1500 times of simulations, their training error rates, and testing error rates were reported in Figure 6. IP10 and MIG both were selected as important variables with more than 40% out of 1500 times of simulations using Multivariate Logistic Regression, SVM and Random Forest methods. Multiple Logistic Regression method appeared to perform better than the other three methods by resulting in a training error rate of 19% and a testing error rate of 39%. All multivariate analyses were implemented in the program R, as described in Gentleman, R. et al. eds, *Bioinformatics and Computational Biology Solutions Using R and Bioconductor*, 2005, Springer, New York.

Multivariate analyses allowed the construction of a multivariate logistic regression equation that can be used to predict the likelihood that a HCV-1 or HCV-4 infected patient would achieve

SVR following Triple Therapy treatment by the measuring the baseline (i.e. pretreatment) expression levels, in picograms per milliliter (pg/ml), of the proteins, IP10, CD30, TGFβ1 and MIG. The equation is: *SVR score = -47.4 – 1.1 x log_2 IP10 + 3.1 x log_2 CD30 + 1.4 x log_2 TGFβ1 + 0.5 x log_2 MIG,* where a SVR score that is greater than or equal to 0.5 would indicate that the patient will achieve SVR to Triple Therapy treatment, and whereas a SVR score that is less than 0.5 would indicate that the patient will not achieve SVR to such treatment.

Claims

1. A method for predicting that a human subject infected with Hepatitis C Virus Genotype 1 (HCV-1) or Hepatitis C Virus Genotype 4 (HCV-4) will achieve Sustained Virologic Response (SVR) to treatment with interferon, ribavirin and a HCV NS5B polymerase inhibitor comprising:
   (i) providing a sample from said subject prior to said treatment (pre-treatment),
   (ii) determining the expression level in said sample of at least one protein selected from the group consisting of CD30, MIG, TARC, TGFβ1, SDF1b, and Eotaxin-2, and
   (iii) comparing the expression level of the at least one protein in said sample to a reference value representative of an expression level of the at least one protein derived from pre-treatment samples of a patient population that did not achieve SVR to said treatment;
   wherein a statistically significant higher expression level of the at least one protein in said sample is indicative that said subject will achieve SVR to said treatment.

2. The method of claim 1 wherein the expression level of at least two proteins is determined.

3. The method of claim 1 or 2 wherein the expression level of at least three proteins is determined.

4. A method for predicting that a human subject infected with Hepatitis C Virus Genotype 1 (HCV-1) or Hepatitis C Virus Genotype 4 (HCV-4) will achieve Sustained Virologic Response (SVR) to treatment with interferon, ribavirin and a HCV NS5B polymerase inhibitor comprising:
   (i) providing a sample from said subject following one week of said treatment (one-week post treatment),
   (ii) determining the expression level in said sample of at least one protein selected from the group consisting of CD30, TRAIL, and TARC, and
   (iii) comparing the expression level of the at least one protein in said sample to a reference value representative of an expression level of the at least one protein

derived from one-week post treatment samples in a patient population that did not achieve SVR to said treatment;

wherein a statistically significant higher expression level of the at least one protein in said sample is indicative that said subject will achieve SVR to said treatment.

5. The method of claim 4 wherein the expression level of at least two proteins is determined.

6. The method of claim 4 or 5 wherein the expression level of at least three proteins is determined.

7. A method for predicting that a human subject infected with Hepatitis C Virus Genotype 1 (HCV-1) or Hepatitis C Virus Genotype 4 (HCV-4) will achieve Sustained Virologic Response (SVR) to treatment with interferon, ribavirin and a HCV NS5B polymerase inhibitor comprising:

(i) providing a sample from said subject prior to said treatment (pre-treatment),

(ii) determining the expression level in said sample of at least one protein selected from the group consisting of HCC4, MIP1b, SDF1b, TNFRII, ITAC, MIG, IL2R, and IL16,

(iii) providing a sample from said subject following one week of said treatment (one-week post treatment),

(iv) determining the expression level in said sample of at least one protein selected from the group consisting of HCC-4, MIP1b, SDF1b, TNFRII, ITAC, MIG, IL2R, and IL16,

(v) determining a differential expression level of the at least one protein between the pre-treatment sample from said subject and the one-week post treatment sample from said subject, and

(vi) comparing said differential expression level of the at least one protein to a reference value representative of a differential expression level of the at least one protein derived from pre-treatment samples and one-week post treatment samples in a patient population that did not achieve SVR to said treatment;

wherein a statistically significant change in the differential expression level of the at least one protein is indicative that said subject will achieve SVR to said treatment.

8. The method of claim 7 wherein the differential expression level of at least two proteins is determined.

9. The method of claim 7 or 8 wherein the differential expression level of at least three proteins is determined.

10. A method for predicting that a human subject infected with Hepatitis C Virus Genotype 1 (HCV-1) or Hepatitis C Virus Genotype 4 (HCV-4) will achieve Sustained Virologic Response (SVR) to treatment with interferon, ribavirin and a HCV NS5B polymerase inhibitor comprising:

(i) providing a sample from said subject prior to said treatment (pre-treatment),

(ii) determining the expression level in picograms per milliliter in said sample of IP10, CD30, TGFβ1 and MIG, and utilizing the equation: SVR score = -47.4 – 1.1 x $\log_2$ IP10 + 3.1 x $\log_2$ CD30 + 1.4 x $\log_2$ TGFβ1 + 0.5 x $\log_2$ MIG,

wherein a SVR score that is greater than or equal to 0.5 is indicative that the subject will achieve SVR to said treatment, and wherein a SVR score that is less than 0.5 is indicative that the subject will not achieve SVR to said treatment.

Figure 1 Study Design

Group A (Dual 1500)	Screen	RO4588161 1500 mg po bid + Pegasys 180 µg sc qw	Pegasys 180 µg sc qw + Ribavirin 1000 mg (<75 kg) or 1200 mg (≥ 75 kg) po qd	Follow-up
Group B (Dual 3000)	Screen	RO4588161 3000 mg po bid + Pegasys 180 µg sc qw	Pegasys 180 µg sc qw + Ribavirin 1000 mg (<75 kg) or 1200 mg (≥ 75 kg) po qd	Follow-up
Group C (Triple 1500)	Screen	RO4588161 1500 mg po bid + Pegasys 180 µg sc qw + Ribavirin 1000 mg (<75 kg) or 1200 mg (≥ 75 kg) po qd	Pegasys 180 µg sc qw + Ribavirin 1000 mg (<75 kg) or 1200 mg (≥ 75 kg) po qd	Follow-up
Group D (SOC)	Screen	Pegasys 180 µg sc qw + Ribavirin 1000 mg (<75 kg) or 1200 mg (≥ 75 kg) po qd	Pegasys 180 µg sc qw + Ribavirin 1000 mg (<75 kg) or 1200 mg (≥ 75 kg) po qd	Follow-up

-5 0 4 48 72

STUDY WEEKS

FIG. 1

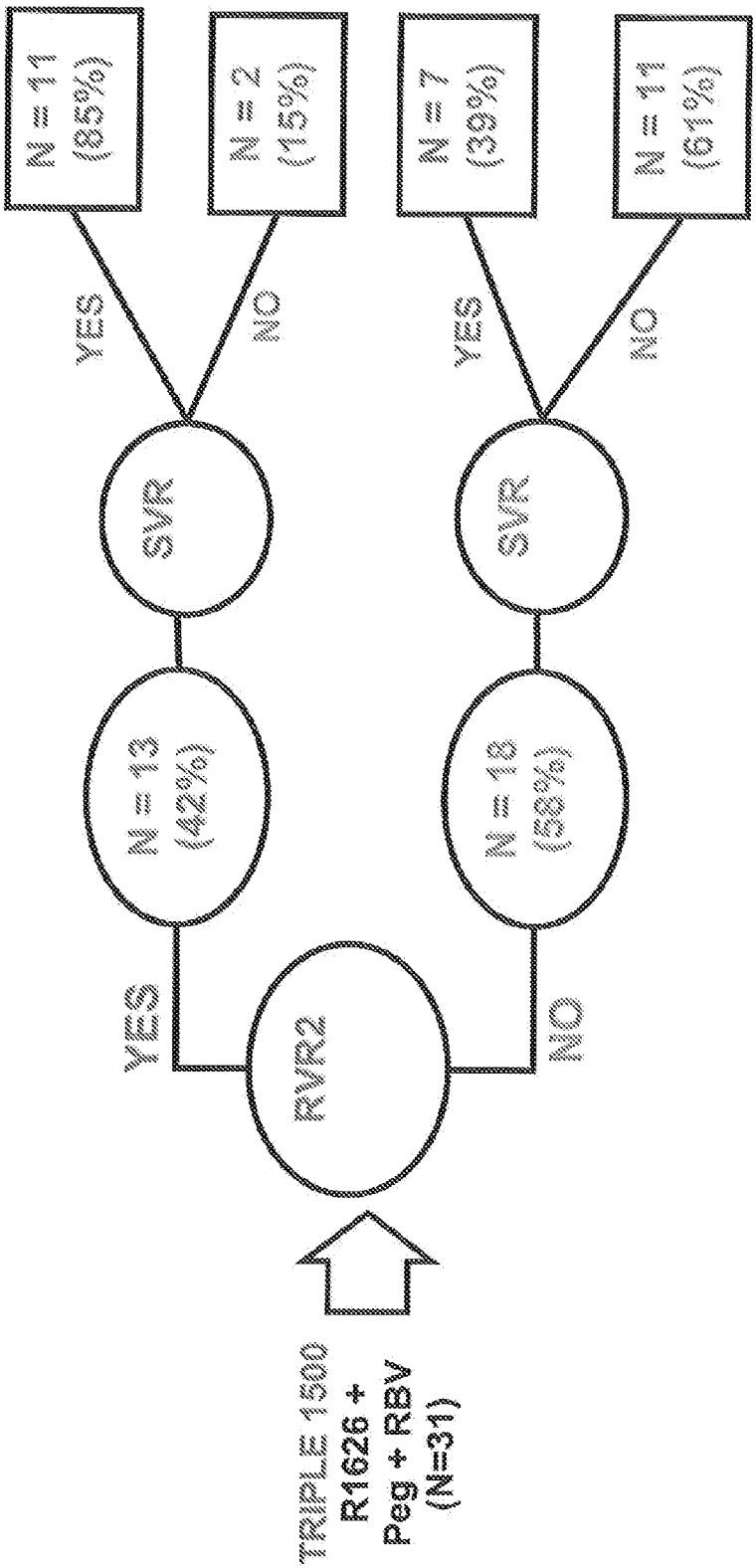
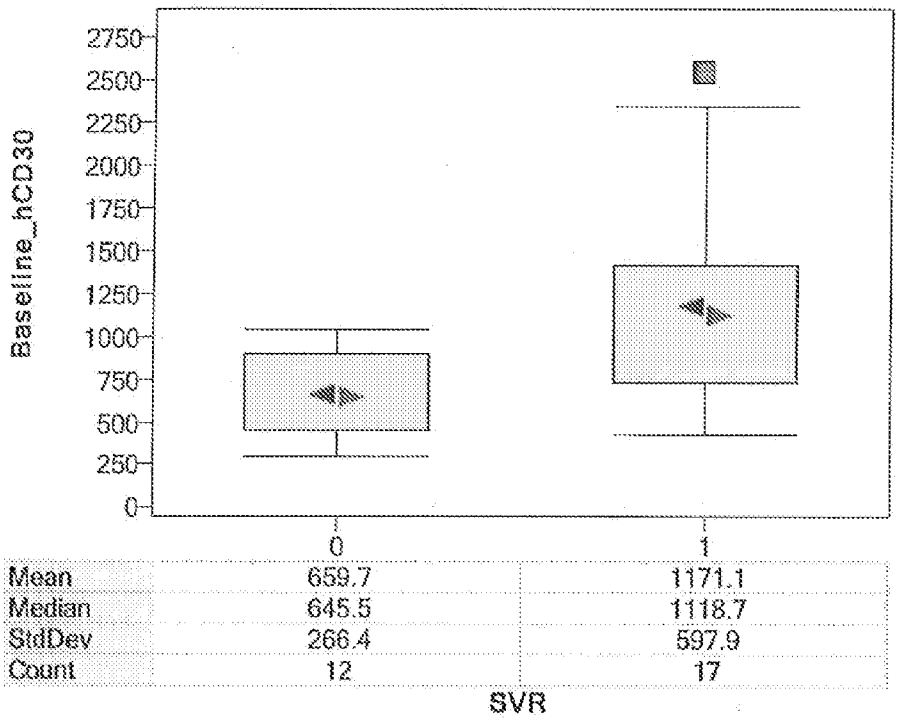
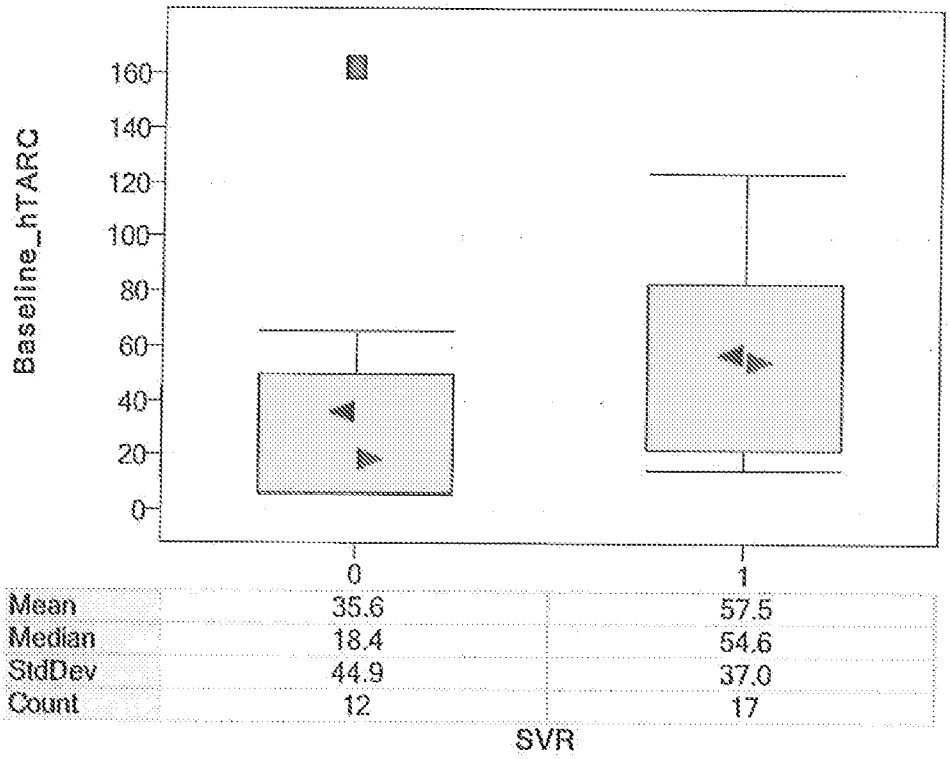

TRIPLE 1500
R1626 +
Peg + RBV
(N=31)
RVR2
YES
NO
N = 13
(42%)
N = 18
(58%)
SVR
SVR
YES
NO
YES
NO
N = 11
(85%)
N = 2
(15%)
N = 7
(39%)
N = 11
(61%)

FIG. 2



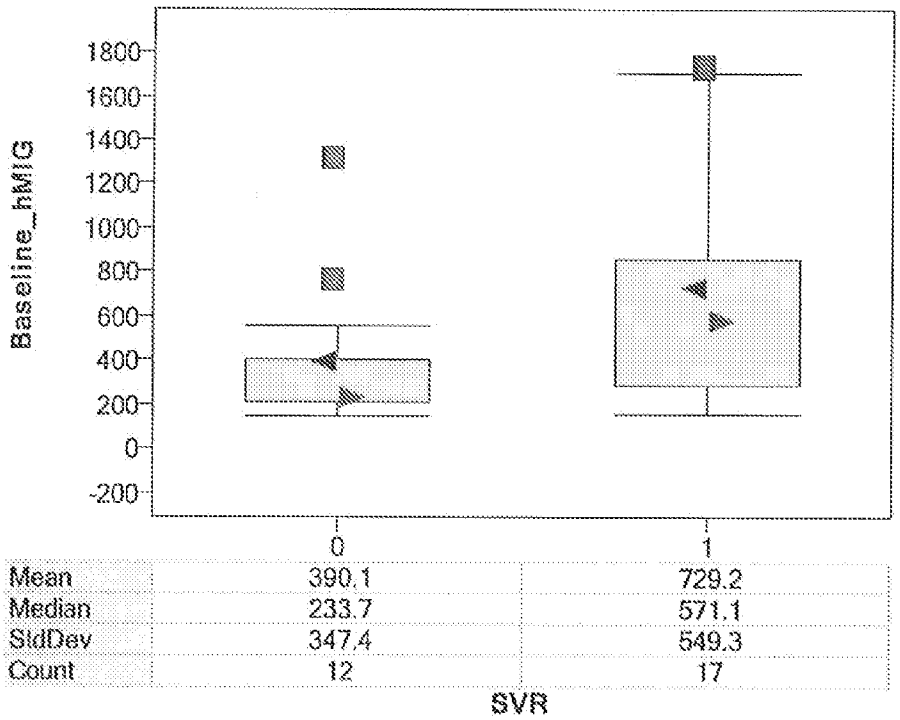
	0	1
Mean	659.7	1171.1
Median	645.5	1118.7
StdDev	266.4	597.9
Count	12	17

FIG. 3A



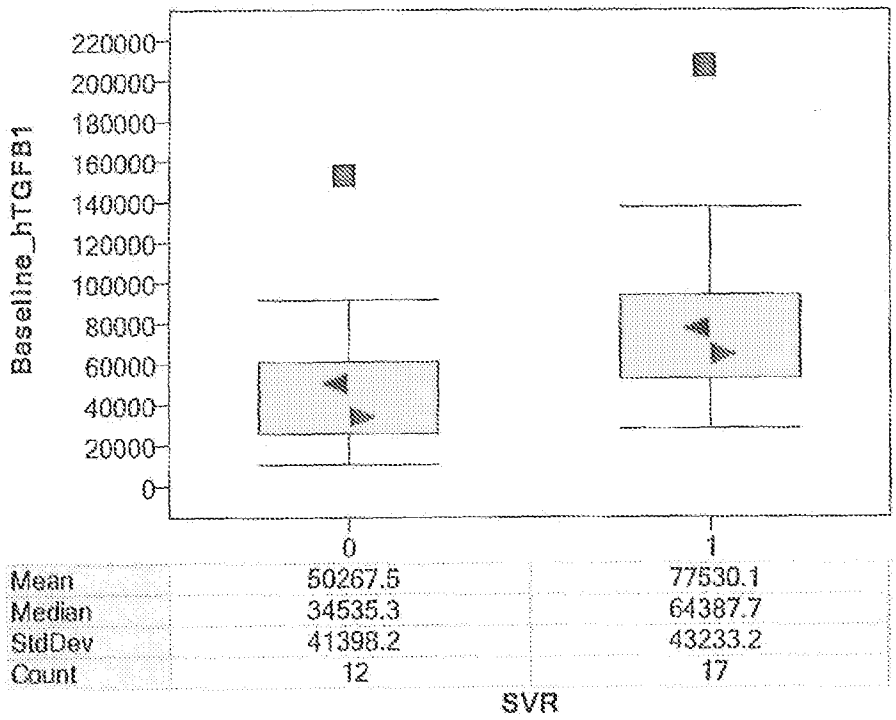
	0	1
Mean	35.6	57.5
Median	18.4	54.6
StdDev	44.9	37.0
Count	12	17

FIG. 3B



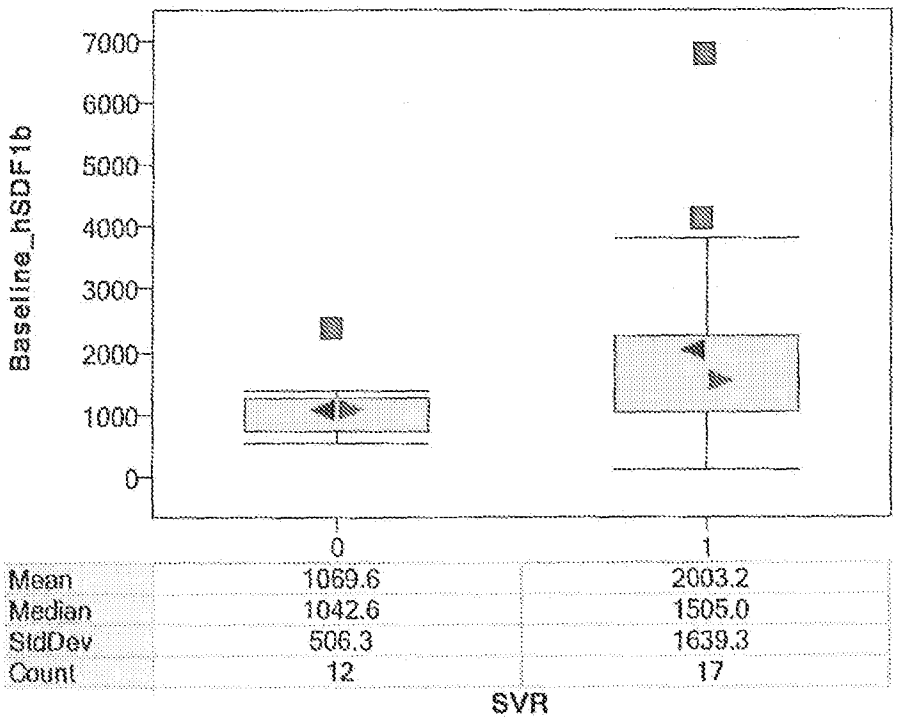
	0	1
Mean	390.1	729.2
Median	233.7	571.1
StdDev	347.4	549.3
Count	12	17

FIG. 3C



	0	1
Mean	50267.5	77530.1
Median	34535.3	64387.7
StdDev	41398.2	43233.2
Count	12	17

FIG. 3D



	0	1
Mean	1069.6	2003.2
Median	1042.6	1505.0
StdDev	506.3	1639.3
Count	12	17

FIG. 3E

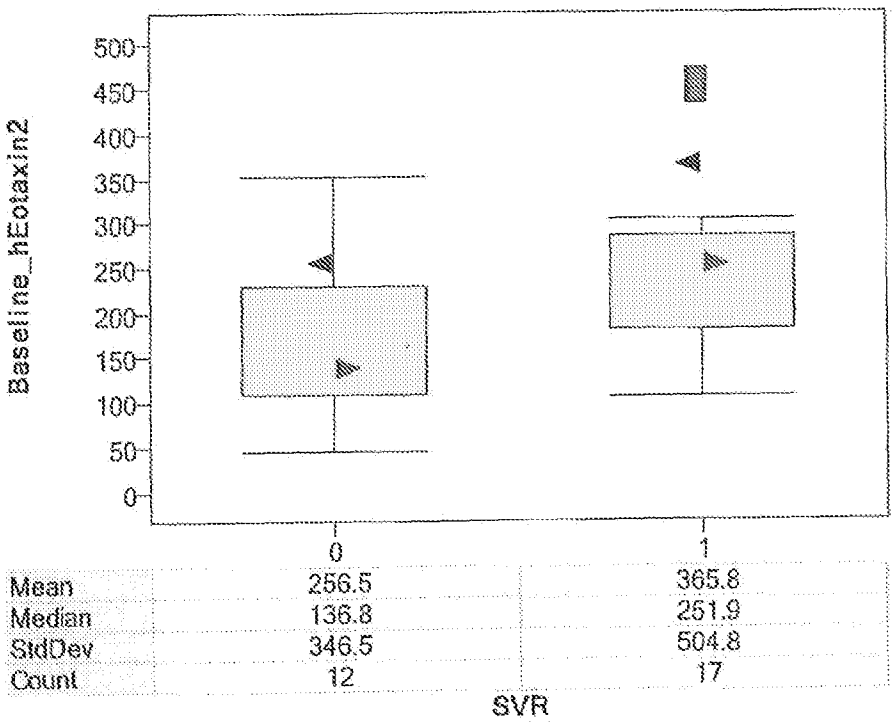
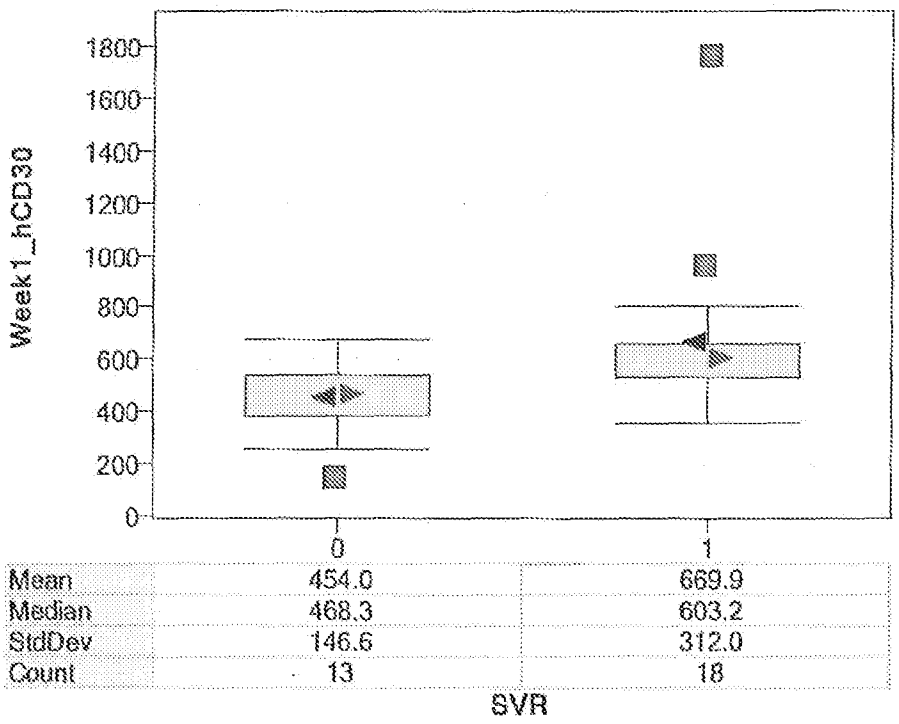
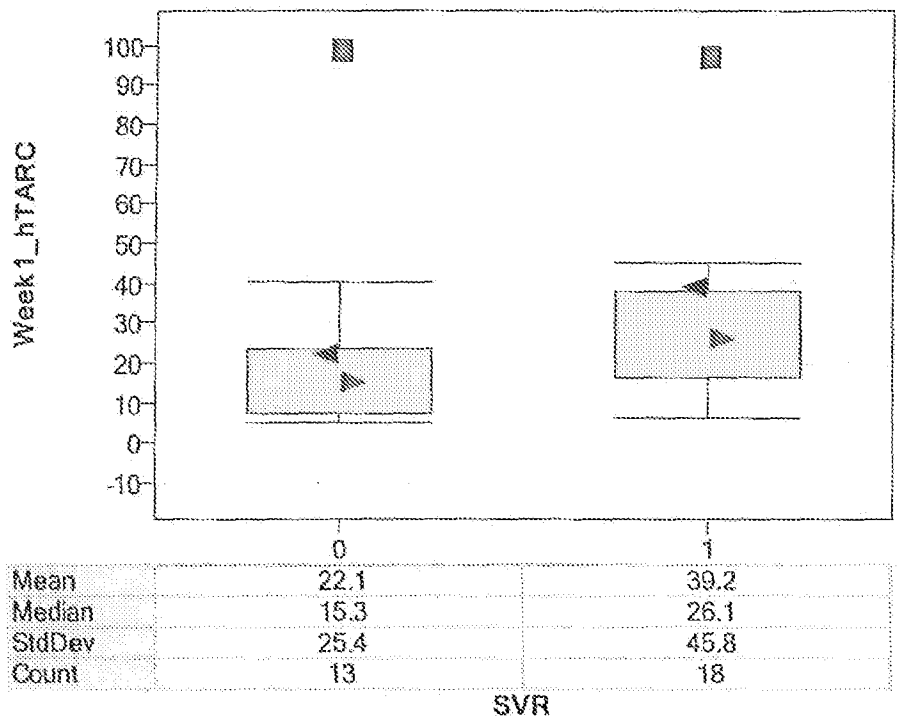

Baseline_hEotaxin2
500
450
400
350
300
250
200
150
100
50
0
0
1
Mean 256.5 365.8
Median 136.8 251.9
StdDev 346.5 504.8
Count 12 17
SVR

FIG. 3 F



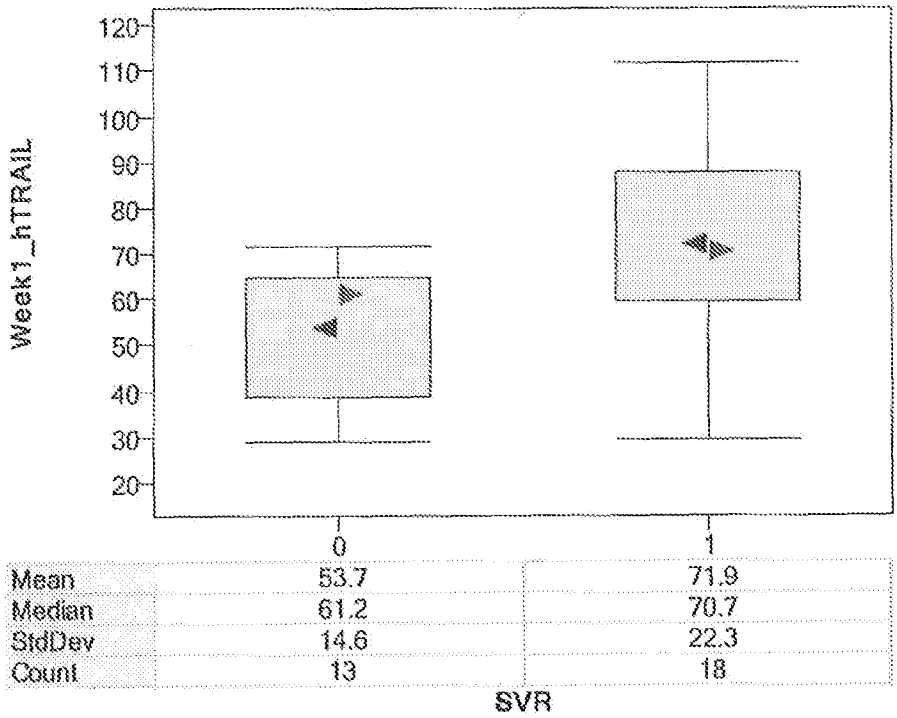
	0	1
Mean	454.0	669.9
Median	468.3	603.2
StdDev	146.6	312.0
Count	13	18

FIG. 4A



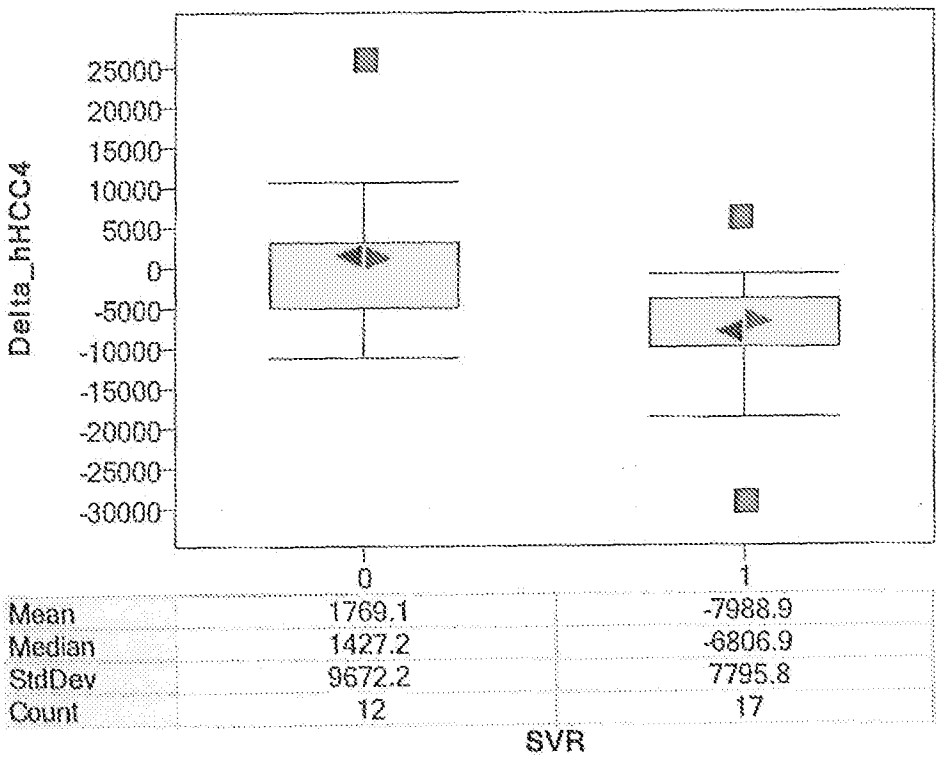
	0	1
Mean	22.1	39.2
Median	15.3	26.1
StdDev	25.4	45.8
Count	13	18

FIG. 43



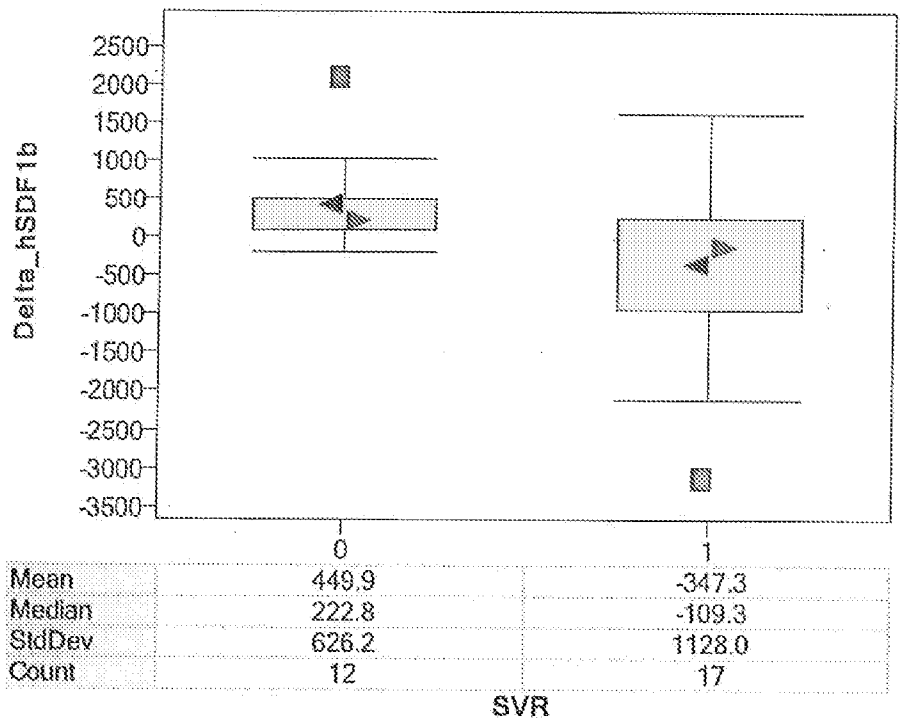
	0	1
Mean	53.7	71.9
Median	61.2	70.7
StdDev	14.6	22.3
Count	13	18

FIG. 4 C



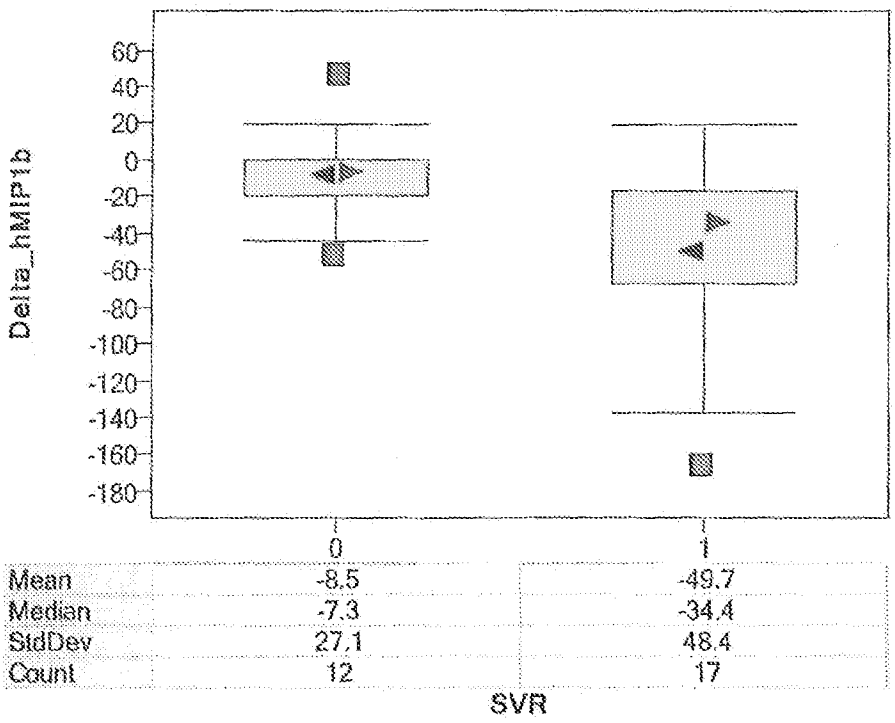
	0	1
Mean	1769.1	-7988.9
Median	1427.2	-6806.9
StdDev	9672.2	7795.8
Count	12	17

FIG. 5A



	0	1
Mean	449.9	-347.3
Median	222.8	-109.3
StdDev	626.2	1128.0
Count	12	17

FIG. 5B



	0	1
Mean	-8.5	-49.7
Median	-7.3	-34.4
StdDev	27.1	48.4
Count	12	17

FIG. 5C

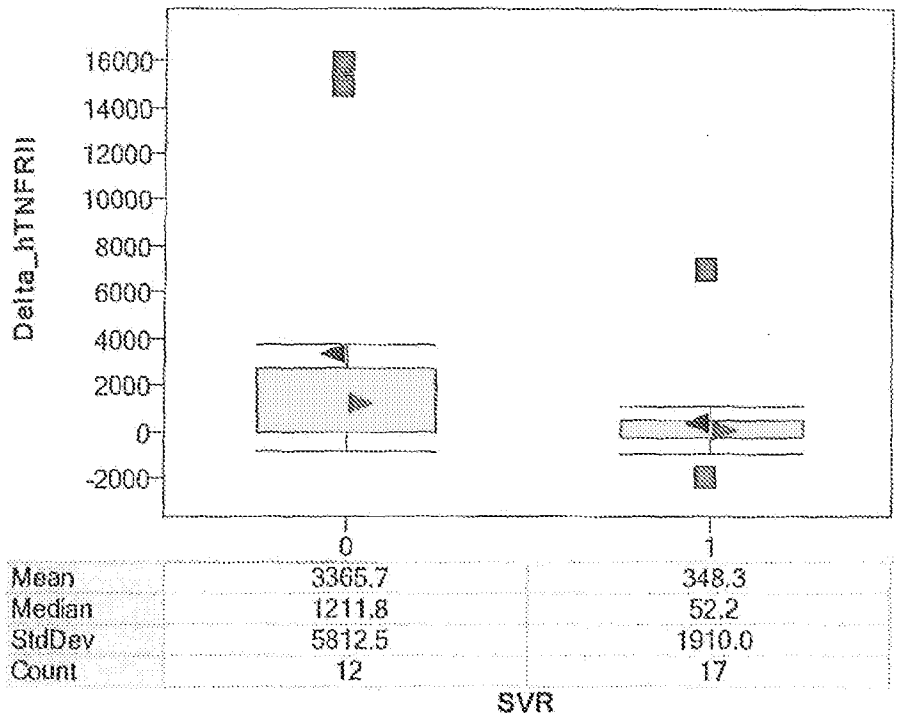
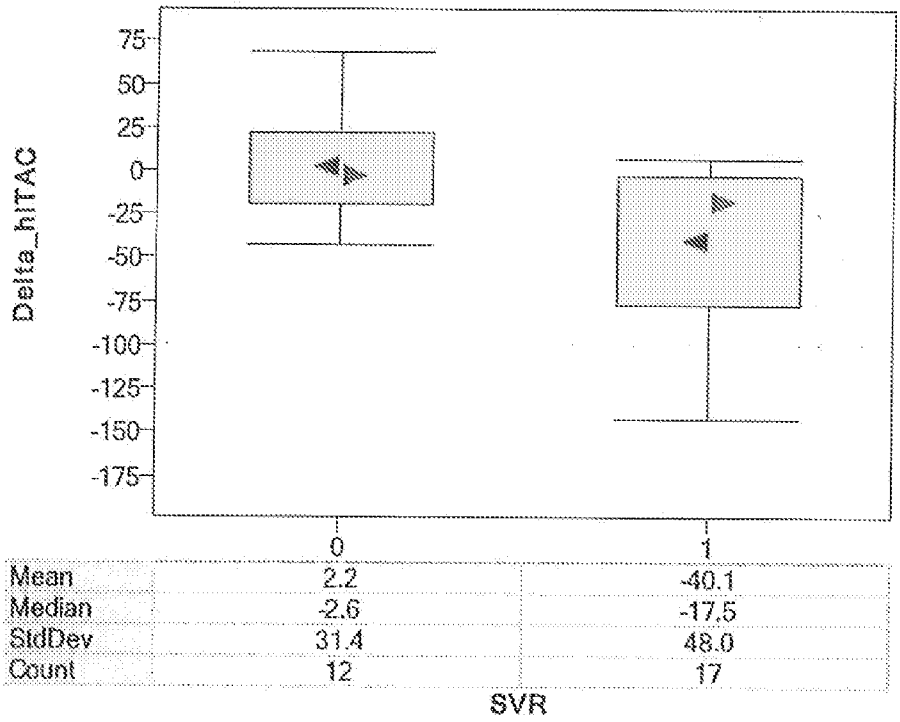
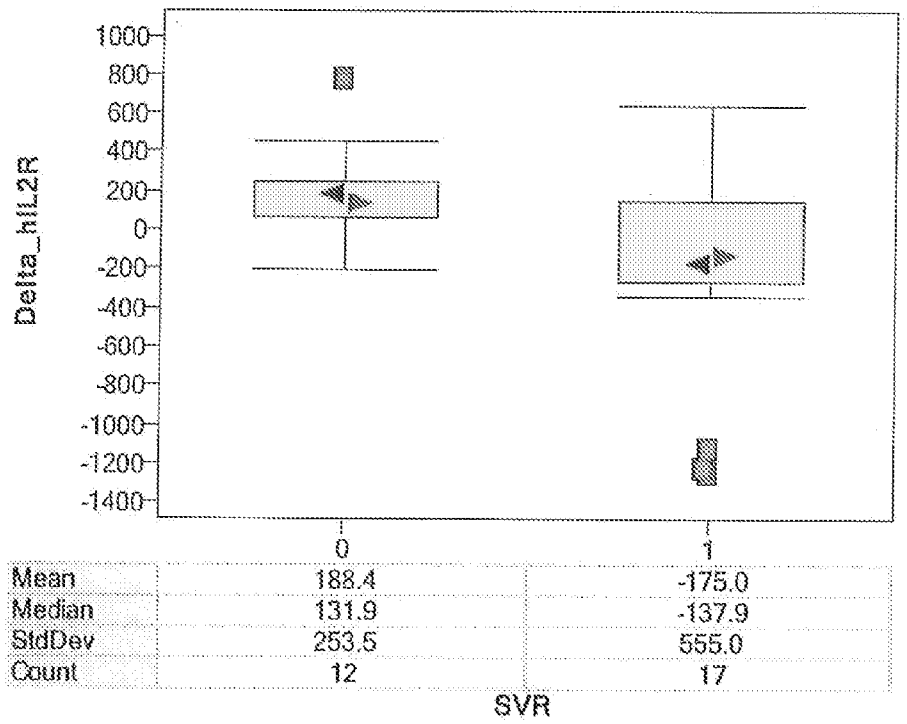

Delta_hTNFRII
16000
14000
12000
10000
8000
6000
4000
2000
0
-2000
0
1
Mean 3365.7 348.3
Median 1211.8 52.2
StdDev 5812.5 1910.0
Count 12 17
SVR

FIG. 5D



	0	1
Mean	2.2	-40.1
Median	-2.6	-17.5
StdDev	31.4	48.0
Count	12	17

FIG. 5E



	0	1
Mean	188.4	-175.0
Median	131.9	-137.9
StdDev	253.5	555.0
Count	12	17

FIG. 5F

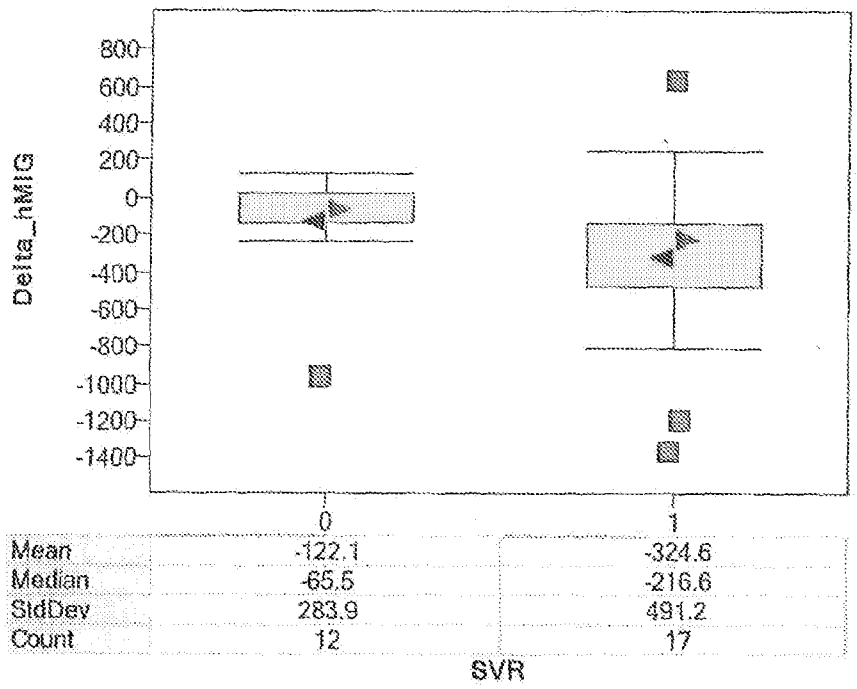
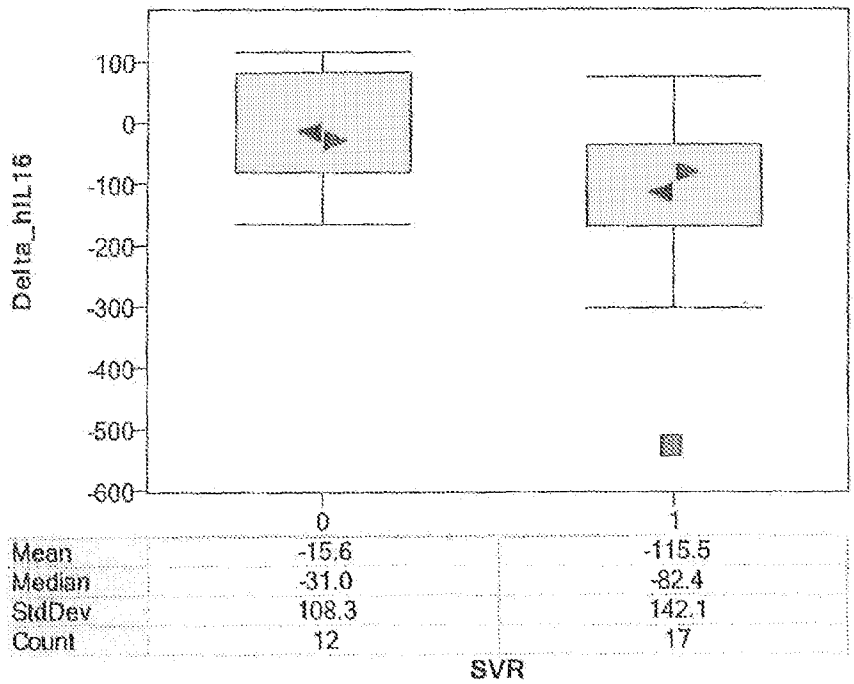
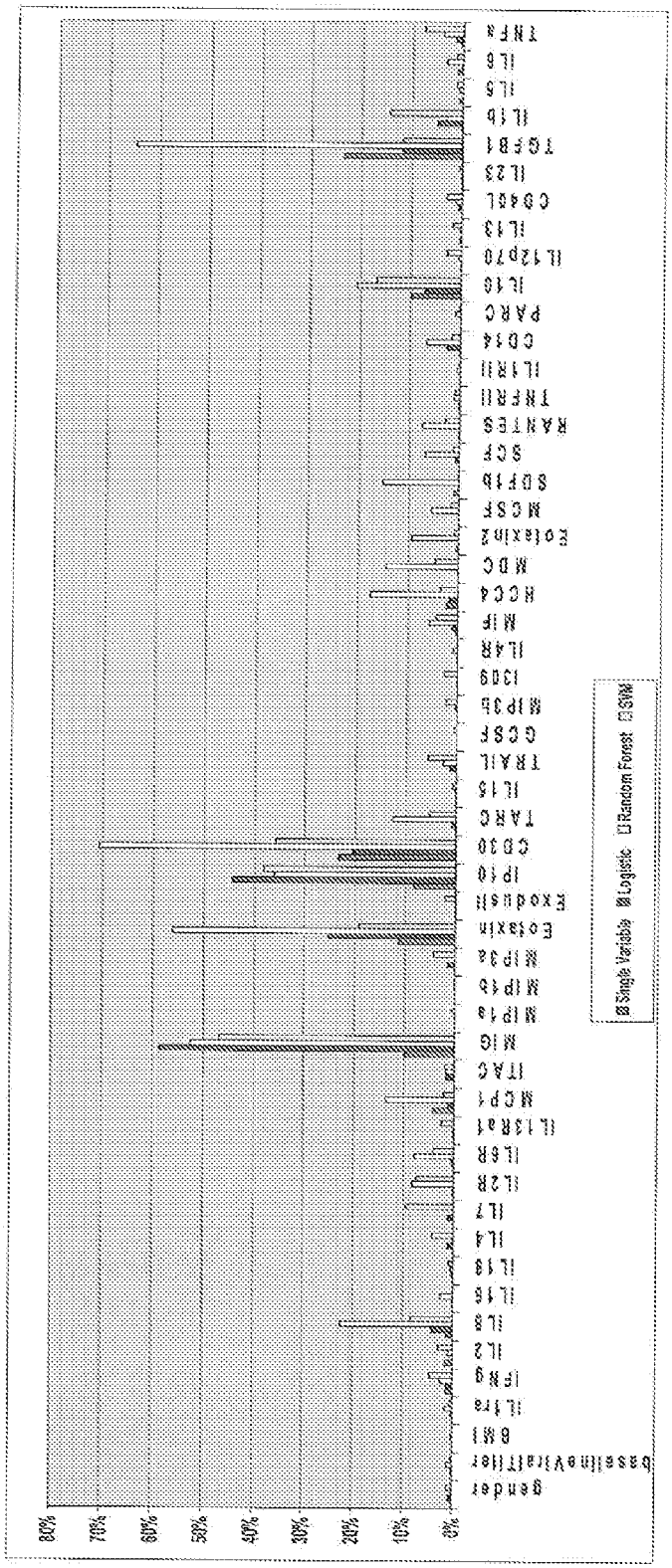

800
600
400
200
0
-200
-400
-600
-800
-1000
-1200
-1400
Delta_hMIG
0
1
Mean -122.1 -324.6
Median -65.5 -216.6
StdDev 283.9 491.2
Count 12 17
SVR

FIG. 5 G



	0	1
Mean	-15.6	-115.5
Median	-31.0	-82.4
StdDev	108.3	142.1
Count	12	17

FIG. 5H



	Single variable	Logistic regression	Random Forest	SVM
Training error	*28%*	*19%*	*26%*	*23%*
Testing error	*46%*	*39%*	*43%*	*41%*

FIG. 6

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2010/068370

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/576
ADD.

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

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, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2009/034055 A1 (NOVARTIS FORSCHUNGSSTIFTUNG [CH]; UNIV HOSPITAL BASEL [CH]; CHRISTEN V) 19 March 2009 (2009-03-19) the whole document claims 1,5	1-10
A	US 2003/013118 A1 (EDGE ALBERT [US] ET AL) 16 January 2003 (2003-01-16) the whole document pages 1,6 paragraph [0021]	1-10

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 January 2011	13/01/2011

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Jenkins, Gareth

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2010/068370

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WU JIM ZHEN ET AL: "TARGETING NS5B RNA-DEPENDENT RNA POLYMERASE FOR ANTI-HCV CHEMOTHERAPY", CURRENT DRUG TARGETS. INFECTIOUS DISORDERS, BENTHAM SCIENCE PUBLISHERS, HILVERSUM, NL, vol. 3, no. 3, 1 September 2003 (2003-09-01), pages 207-219, XP009081028, ISSN: 1568-0053, DOI: DOI:10.2174/1568005033481114 the whole document page 213	1-10
A	DE FRANCESCO RAFFAELE ET AL: "Approaching a new era for hepatitis C virus therapy: Inhibitors of the NS3-4A serine protease and the NS5B RNA-dependent RNA polymerase.", ANTIVIRAL RESEARCH, vol. 58, no. 1, March 2003 (2003-03), pages 1-16, XP002615502, ISSN: 0166-3542 the whole document figure 5A	1-10
A	DATABASE MEDLINE [Online] US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US; July 2006 (2006-07), GATTONI A ET AL: "Interferon-gamma: biologic functions and HCV therapy (type I/II) (1 of 2 parts).", XP002615503, Database accession no. NLM17051976 * abstract & LA CLINICA TERAPEUTICA 2006 JUL-AUG LNKD- PUBMED:17051976, vol. 157, no. 4, July 2006 (2006-07), pages 377-386, ISSN: 0009-9074	1-10
A	POCKROS PAUL J ET AL: "R1626 plus peginterferon alfa-2a provides potent suppression of hepatitis C virus RNA and significant antiviral synergy in combination with ribavirin", HEPATOLOGY, vol. 48, no. 2, August 2008 (2008-08), pages 385-397, XP002615504, ISSN: 0270-9139 the whole document	1-10
A,P	US 2010/158866 A1 (ZHU YONGHONG [US]) 24 June 2010 (2010-06-24) the whole document	1-10

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2010/068370

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		EP 2191274 A1	02-06-2010
		US 2010167302 A1	01-07-2010
US 2003013118 A1	16-01-2003	NONE	
US 2010158866 A1	24-06-2010	WO 2010069809 A1	24-06-2010