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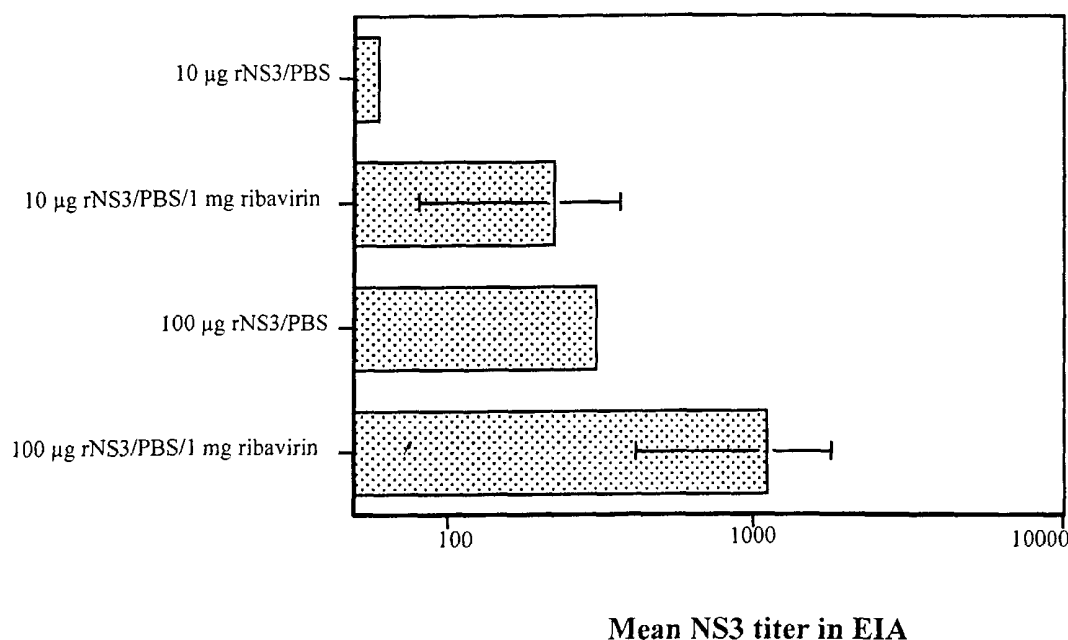
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(54) Title: VACCINES CONTAINING RIBAVIRIN AND METHODS OF USE THEREOF



(57) Abstract: Compositions and methods for enhancing the effect of vaccines in animals, such as domestic, sport, or pet species, and humans are disclosed. More particularly, vaccine compositions comprising ribavirin and an antigen, preferably an antigen that has an epitope present in Hepatitis C virus (HCV), are disclosed for use in treating and preventing disease, preferably HCV infection.

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## VACCINES CONTAINING RIBAVIRIN AND METHODS OF USE THEREOF

### FIELD OF THE INVENTION

The present invention relates to compositions and methods for enhancing the effect of  
5 vaccines in animals, such as domestic, sport, or pet species, and humans. More particularly, preferred embodiments concern the use of ribavirin as an adjuvant and compositions having ribavirin and an antigen.

### BACKGROUND OF THE INVENTION

The use of vaccines to prevent disease in humans, farm livestock, sports animals, and  
10 household pets is a common practice. Frequently, however, the antigen used in a vaccine is not sufficiently immunogenic to raise the antibody titre to levels that are sufficient to provide protection against subsequent challenge or to maintain the potential for mounting these levels over extended time periods. Further, many vaccines are altogether deficient in inducing cell-mediated immunity, which is a primary immune defense against bacterial and viral infection. A  
15 considerable amount of research is currently focussed on the development of more potent vaccines and ways to enhance the immunogenicity of antigen-containing preparations. (See e.g., U.S. Pat. Nos. 6,056,961; 6,060,068; 6,063,380; and Li et al., *Science* 288:2219-2222 (2000)).

Notorious among such "weak" vaccines are hepatitis B vaccines. For example, recombinant vaccines against hepatitis B virus such as Genhevacc (Pasteur Merieux Serums et  
20 Vaccines, 58, Avenue Leclerc 69007 Lyon, France), Engerixb (Smith, Kline and Symbol French), and Recombivaxhb (Merck, Sharp, and Dhome) are effective only after at least three injections at 0, 30, and 60 or 180 days, followed by an obligatory booster after one year. (Chedid et al., U.S. Patent No. 6,063,380). Additionally, many subjects receiving these vaccines respond poorly, if at all. Because many regions of the world are endemic for HBV infection, the poorly immunogenic  
25 character of existing HBV vaccines has become an extremely serious problem.

To obtain a stronger, humoral and/or cellular response, it is common to administer a vaccine in a material that enhances the immune response of the patient to the antigen present in the vaccine. The most commonly used adjuvants for vaccine protocols are oil preparations and alum. (Chedid et al., U.S. Patent No. 6,063,380). A greater repertoire of safe and effective adjuvants is  
30 needed.

Nucleoside analogs have been widely used in anti-viral therapies due to their capacity to reduce viral replication. (Hosoya et al., *J. Inf. Dis.*, 168:641-646 (1993)). ribavirin (1- $\beta$ -D-ribofuranosyl-1,2,4-triazole-3-carboxamide) is a synthetic guanosine analog that has been used to inhibit RNA and DNA virus replication. (Huffman et al., *Antimicrob. Agents. Chemother.*, 3:235  
35 (1973); Sidwell et al., *Science*, 177:705 (1972)). ribavirin has been shown to be a competitive

inhibitor of inositol mono-phosphate (IMP) dehydrogenase (IMPDH), which converts IMP to IMX (which is then converted to GMP). De Clercq, Anti viral Agents: characteristic activity spectrum depending on the molecular target with which they interact, Academic press, Inc., New York N.Y., pp. 1-55 (1993). Intracellular pools of GTP become depleted as a result of long term ribavirin treatment.

In addition to antiviral activity, investigators have observed that some guanosine analogs have an effect on the immune system. (U.S. Patent Nos. 6,063,772 and 4,950,647). ribavirin has been shown to inhibit functional humoral immune responses (Peavy et al., *J. Immunol.*, 126:861-864 (1981); Powers et al., *Antimicrob. Agents. Chemother.*, 22:108-114 (1982)) and IgE-mediated modulation of mast cell secretion. (Marquardt et al., *J. Pharmacol. Exp. Therapeutics*, 240:145-149 (1987)). Some investigators report that a daily oral therapy of ribavirin has an immune modulating effect on humans and mice. (Hultgren et al., *J. Gen. Virol.*, 79:2381-2391 (1998) and Cramp et al., *Gastron. Enterol.*, 118:346-355 (2000)). Nevertheless, the current understanding of the effects of ribavirin on the immune system is in its infancy.

#### SUMMARY OF THE INVENTION

It has been discovered that ribavirin can be used as an adjuvant to enhance or facilitate an immune response to an antigen. Embodiments of the invention described herein include "strong" vaccine preparations that comprise an antigen and ribavirin. Generally, these preparations have an amount of ribavirin that is sufficient to enhance or facilitate an immune response to the antigen. Other aspects of the invention include methods of enhancing or facilitating an immune response of an animal, including a human, to an antigen. By one approach, for example, an animal in need of a potent immune response to an antigen is identified and then is provided an amount of ribavirin together with the antigen. In some methods, the ribavirin and the antigen are provided in combination (e.g., in a single composition) and in others, the ribavirin and the antigen are provided separately. Several embodiments also concern the manufacture and use of compositions having ribavirin and an antigen.

Although the embodied compositions include ribavirin and virtually any antigen or epitope, preferred compositions comprise ribavirin and a hepatitis viral antigen or epitope. The antigen or epitope can be peptide or nucleic acid-based (e.g., a RNA encoding a peptide antigen or a construct that expresses a peptide antigen when introduced to a subject). Compositions having ribavirin and a peptide comprising an antigen or epitope from the hepatitis A virus (HAV) or a nucleic acid encoding said peptide are embodiments. Compositions having ribavirin and a peptide comprising an antigen or epitope from the hepatitis B virus (HBV) or a nucleic acid encoding said peptide are embodiments. HBV antigens that are suitable include, for example, hepatitis B surface antigen (HBsAg), hepatitis core antigen (HBcAg), hepatitis e antigen (HBeAg), and nucleic acids

encoding these molecules. Still further, compositions having ribavirin and a peptide comprising an antigen or epitope from the hepatitis C virus (HCV) or a nucleic acid encoding said peptide are embodiments. Suitable HCV antigens include, but are not limited to, one or more domains of the HCV sequence (e.g., NS3 and/or NS4A) and nucleic acids encoding said molecules.

5           A new HCV sequence was also discovered. A novel NS3/4A fragment of the HCV genome was cloned and sequenced from a patient infected with HCV (SEQ. ID. NO.: 16). This sequence was found to be only 93% homologous to the most closely related HCV sequence. This novel peptide (SEQ. ID. NO.: 17) and fragments thereof at least 3, 4, 6, 8, 10, 12, 15 or 20 amino acids in length, nucleic acids encoding these molecules, vectors having said nucleic acids, and cells  
10   having said vectors, nucleic acids, or peptides are also embodiments of the present invention. A particularly preferred embodiment is a vaccine composition comprising ribavirin and the HCV peptide of SEQ. ID. NO.: 17 or a fragment thereof at least 3, 4, 6, 8, 10, 12, 15 or 20 amino acids in length (e.g., SEQ. ID. NO.: 25) or a nucleic acid encoding said peptide or fragments.

          Additionally, it was discovered that truncated mutants and mutants of the NS3/4A peptide,  
15   which lack a proteolytic cleavage site, are highly immunogenic. These novel peptides (SEQ. ID. NOs.: 29- 32 and 43-49) and fragments thereof at least 3, 4, 6, 8, 10, 12, 15 or 20 amino acids in length (e.g., SEQ. ID. NOs.: 26, 27, and 33-42), nucleic acids encoding these molecules, vectors having said nucleic acids, and cells having said vectors, nucleic acids, or peptides are also embodiments. A particularly preferred embodiment is a vaccine composition comprising ribavirin  
20   and at least one HCV peptide of SEQ. ID. NOs.: 29- 32 and 43-49 or a fragment thereof at least 3, 4, 6, 8, 10, 12, 15 or 20 amino acids in length (e.g., SEQ. ID. NOs.: 26, 27, and 33-42) or a nucleic acid encoding said peptides or fragments.

          Furthermore, compositions having a mixture of the antigens above are embodiments of the invention. For example, some compositions comprise a HBV antigen, a HAV antigen, and  
25   ribavirin or a HBV antigen, a HCV antigen, and ribavirin or a HAV antigen, a HCV antigen, and ribavirin or a HBV antigen, a HAV antigen, a HCV antigen, and ribavirin. Other embodiments comprise ribavirin and a nucleic acid encoding a mixture of the antigens described above. Some embodiments also include other adjuvants, binders, emulsifiers, carriers, and fillers, as known in the art, including, but not limited to, alum, oil, and other compounds that enhance an immune  
30   response.

          Methods of making and using the compositions described herein are also aspects of the invention. Some methods are practiced by mixing ribavirin with a peptide or nucleic acid antigen (e.g., an HAV, HBV, HCV antigen) so as to formulate a single composition (e.g., a vaccine composition). Preferred methods involve the mixing of ribavirin with an HCV antigen that has an  
35   epitope present on one or more domains of HCV (e.g., NS3 and/or NS4A).

Preferred methods of using the compositions described herein involve providing an animal in need with a sufficient amount of ribavirin and a hepatitis viral antigen (e.g., HBV antigen, HAV antigen, HCV antigen a nucleic acid encoding one of these antigens or any combination thereof). By one approach, for example, an animal in need of potent immune response to a hepatitis viral antigen (e.g., an animal at risk or already infected with a hepatitis infection) is identified and said animal is provided an amount of ribavirin and a hepatitis viral antigen (either in a single composition or separately) that is effective to enhance or facilitate an immune response to the hepatitis viral antigen. Preferably, an animal in need of a potent immune response to HCV is identified and said animal is provided a composition comprising ribavirin and a peptide comprising an antigen or epitope present on SEQ. ID. NO.: 1, 6, 7, or 17 or a nucleic acid encoding said peptide. Particularly preferred methods involve the identification of an animal in need of an potent immune response to HCV and providing said animal a composition comprising ribavirin and an amount of an HCV antigen (e.g., NS3/4A (SEQ. ID. NO.: 17), mutant NS3/4A SEQ. ID. NOs.: 29- 32 and 43-49, or a fragment thereof at least 3, 4-10, 10-20, 20-30, or 30-50 amino acids in length (e.g., SEQ. ID. NOs.: 25-27, and 33-42) or a nucleic acid encoding one or more of these molecules) that is sufficient to enhance or facilitate an immune response to said antigen.

#### BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 is a graph showing the humoral response to 10 and 100µg recombinant Hepatitis C virus (HCV) non structural 3 protein (NS3), as determined by mean end point titres, when a single dose of 1mg of ribavirin was co-administered.

FIGURE 2 is a graph showing the humoral response to 20µg recombinant Hepatitis C virus (HCV) non structural 3 protein (NS3), as determined by mean end point titres, when a single dose of 0.1, 1.0, or 10mg of ribavirin was co-administered.

FIGURE 3 is a graph showing the effects of a single dose of 1mg ribavirin on NS3-specific lymph node proliferative responses, as determined by *in vitro* recall responses.

FIGURE 4 is a graph showing the antibody titer in H-2<sup>d</sup> mice against NS3 as a function of time after the first immunization. Diamonds denote antibody titer in mice immunized with NS3/4A-pVAX and squares denote antibody titer in mice immunized with NS3-pVAX.

FIGURE 5A is a graph showing the percentage of specific CTL-mediated lysis of SP2/0 target cells as a function of the effector to target ratio. Phosphate Buffered Saline (PBS) was used as a control immunogen.

FIGURE 5B Is a graph showing the percentage specific CTL-mediated lysis of SP2/0 target cells as a function of the effector to target ratio. Plasmid NS3/4A-pVAX was used as the immunogen.

## DETAILED DESCRIPTION OF THE INVENTION

It has been discovered that compositions comprising ribavirin and an antigen (e.g., a molecule containing an epitope of a pathogen such as a virus, bacteria, mold, yeast, or parasite) enhance and/or facilitate an animal's immune response to the antigen. That is, it was discovered that ribavirin is an effective "adjuvant," which for the purposes of this disclosure, refers to a material that has the ability to enhance or facilitate an immune response to a particular antigen. The adjuvant activity of ribavirin was manifested by a significant increase in immune-mediated protection against the antigen, an increase in the titer of antibody raised to the antigen, and an increase in proliferative T cell responses.

Several compositions (e.g., vaccines) that comprise ribavirin and an antigen or epitope are described herein. Vaccine formulations containing ribavirin, for example, can vary according to the amount of ribavirin, the form of ribavirin, and the type of antigen. The antigen can be a peptide or a nucleic acid (e.g., a RNA encoding a peptide antigen or a construct that expresses a peptide antigen when introduced into a subject). Preferred compositions comprise ribavirin and a hepatitis viral antigen (e.g., HAV antigen, HBV antigen, HCV antigen, a nucleic acid encoding these molecules, or any combination thereof). In particular, at least one HCV antigen or an epitope present on **SEQ. ID. NO.: 1** or a nucleic acid encoding said HCV antigen are desired for mixing with ribavirin to make said compositions. That is, some embodiments include, but are not limited to, compositions comprising ribavirin and a peptide comprising **SEQ. ID. NO.: 1**, or a fragment thereof having at least 2500, 2000, 1600, 1200, 800, 400, 200, 100, 50, 10, or 3 consecutive amino acids of **SEQ. ID. NO.: 1**. Additional embodiments concern compositions comprising ribavirin and a nucleic acid encoding **SEQ. ID. NO.: 13** or a fragment thereof having at least 9, 12, 15, 20, 30, 50, 75, 100, 200, 500 consecutive nucleotides of **SEQ. ID. NO.: 13**.

Other embodiments include a composition (e.g., a vaccine) that comprises ribavirin and a specific fragment of **SEQ. ID. NO.: 1**, wherein said fragment corresponds to a particular domain of HCV. Some embodiments, for example, comprise a fragment of HCV corresponding to amino acids 1-182, 183-379, 380-729, 730-1044, 1045-1657, 1658-1711, 1712-1971, or 1972-3011 of **SEQ. ID. NO.: 1**. Compositions comprising ribavirin and a nucleic acid encoding one or more of these fragments are also embodiments of the invention.

Additionally, a novel HCV sequence was discovered. A novel nucleic acid and protein corresponding to the NS3/4A domain of HCV was cloned from a patient infected with HCV (**SEQ. ID. NO.: 16**). A Genbank search revealed that the cloned sequence had the greatest homology to HCV sequences but was only 93% homologous to the closest HCV relative (accession no AJ 278830). This novel peptide (**SEQ. ID. NO.: 17**) and fragments thereof at least 3, 4, 6, 8, 10, 12, 15 or 20 amino acids in length, nucleic acids encoding these molecules, vectors having said nucleic

acids, and cells having said vectors, nucleic acids, or peptides are also embodiments of the invention. Further, some of the vaccine embodiments described herein comprise ribavirin and this novel NS3/4A peptide or a fragment thereof at least 3, 4, 6, 8, 10, 12, 15 or 20 amino acids in length (e.g., SEQ. ID. NO.: 25) or a nucleic acid encoding one or more of these molecules.

5 Mutants of the novel NS3/4A peptide were also created. It was discovered that truncated mutants (e.g., SEQ. ID. NO.: 29) and mutants, which lack a proteolytic cleavage site, are highly immunogenic. These novel peptides SEQ. ID. NOs.: 29- 32 and 43-49 and fragments thereof at least 3, 4, 6, 8, 10, 12, 15 or 20 amino acids in length (e.g., SEQ. ID. NOs.: 26, 27, and 33-42), nucleic acids encoding these molecules, vectors having said nucleic acids, and cells having said  
10 vectors, nucleic acids, or peptides are also embodiments. Furthermore, some of the compositions described herein comprise ribavirin and at least one of the mutant HCV peptides described above or a fragment thereof at least 3, 4, 6, 8, 10, 12, 15 or 20 amino acids in length. Other vaccine embodiments comprise ribavirin and a nucleic acid (e.g., DNA) encoding one or more of the peptides described above.

15 Methods of making and using the compositions above are also embodiments. For example, the compositions described above can be made by providing ribavirin, providing an antigen (e.g., a peptide comprising an HCV antigen or a nucleic acid encoding said peptide), and mixing said ribavirin and said antigen so as to formulate a composition that can be used to enhance or facilitate an immune response in a subject to said antigen. Preferred methods entail mixing a  
20 preferred antigen or epitope (e.g., a peptide comprising SEQ. ID. NO.: 1, 6, 7, or 17 or specific fragments thereof, such as amino acids 1-182, 183-379, 380-729, 730-1044, 1045-1657, 1658-1711, 1712-1971, 1972-3011 of SEQ. ID. NO.: 1 and nucleic acids encoding these molecules) with ribavirin. Other antigens or epitopes can also be mixed with ribavirin including, but not limited to, fragments of SEQ. ID. NO.: 1 that have at least 2500, 2000, 1600, 1200, 800, 400, 200,  
25 100, 50, 10, or 3 consecutive amino acids and nucleic acids encoding these fragments. Particularly preferred methods concern the making of vaccine compositions comprising the newly discovered NS3/4A fragment or an NS3/4A mutant (e.g., a truncated mutant or a mutant lacking a proteolytic cleavage site), or a fragment thereof of at least four amino acids in length or a nucleic acid encoding one or more of these molecules.

30 Methods of enhancing or facilitating the immune response of an animal, including humans, to an antigen are embodiments of the invention. Such methods can be practiced, for example, by identifying an animal in need of a potent immune response to an antigen/epitope and providing said animal a composition comprising the antigen/epitope and an amount of ribavirin that is effective to enhance or facilitate an immune response to the antigen/epitope. In some  
35 embodiments, the ribavirin and the antigen are administered separately, instead of in a single

mixture. Preferably, in this instance, the ribavirin is administered a short time before or a short time after administering the antigen. Preferred methods involve providing the animal in need with ribavirin and a hepatitis antigen (e.g., HAV antigen, HBV antigen, HCV antigen, a nucleic acid encoding these molecules, or any combination thereof). Some of these methods involve HCV  
 5 antigens, such as a peptide comprising SEQ. ID. NO.: 1, or a fragment thereof having at least 2500, 2000, 1600, 1200, 800, 400, 200, 100, 50, 10, or 3 consecutive amino acids of SEQ. ID. NO.: 1. Additional methods involve compositions comprising ribavirin and a nucleic acid encoding SEQ. ID. NO.: 13 or a nucleic acid encoding one or more of the fragments discussed above.

10 Some preferred methods, for example, concern the use of a composition (e.g., a vaccine) that comprises ribavirin and a peptide comprising SEQ. ID. NO.: 1 or a specific fragment thereof, which corresponds to an HCV domain including, but not limited to, a peptide comprising amino acids 1-182, 183-379, 380-729, 730-1044, 1045-1657, 1658-1711, 1712-1971, or 1972-3011 of  
 15 SEQ. ID. NO.: 1. Particularly preferred methods concern the use of a vaccine composition comprising the NS3/4A fragment of SEQ. ID. NO.: 17 or the mutant NS3/4A (e.g., SEQ. ID. NOs.: 29- 32 and 43-49), which lack a proteolytic cleavage site, or a fragment thereof of at least 3, 4, 6, 8, 10, 12, 15 or 20 amino acids in length (e.g., SEQ. ID. NOs.: 26, 27, and 33-42). Compositions comprising ribavirin and a nucleic acid encoding these fragments can also be used with the methods described herein.

20 Other embodiments concern methods of treating and preventing HCV infection. By one approach, ribavirin and an HCV antigen or epitope are used to prepare a medicament for the treatment and/or prevention of HCV infection. By another approach, an individual in need of a medicament that prevents and/or treats HCV infection is identified and said individual is provided a medicament comprising ribavirin and an HCV antigen or epitope, preferably an epitope present  
 25 on SEQ. ID. NO.: 1, more preferably a fragment of SEQ. ID. NO.: 1 having at least 2500, 2000, 1600, 1200, 800, 400, 200, 100, 50, 10, or 3 consecutive amino acids or most preferably a fragment of SEQ. ID. NO.: 1 such as 1-182, 183-379, 380-729, 730-1044, 1045-1657, 1658-1711, 1712-1971, or 1972-3011 or a nucleic acid encoding SEQ. ID. NO.: 1 or said fragments above. Particularly preferred methods concern the use of a vaccine composition comprising ribavirin and  
 30 the NS3/4A fragment of SEQ. ID. NO.: 17 or the mutant NS3/4A, which lacks a proteolytic cleavage site (e.g., SEQ. ID. NOs.: 29- 32 and 43-49) or a fragment thereof of at least 3, 4, 6, 8, 10, 12, 15 or 20 amino acids in length (e.g., SEQ. ID. NOs.: 25-27, and 33-42) or a nucleic acid encoding one or more of these molecules. The section below discusses the use of ribavirin as an adjuvant in greater detail.

35



*Ribavirin*

The compositions described herein can be manufactured in accordance with conventional methods of galenic pharmacy to produce medicinal agents for administration to animals, e.g., mammals including humans. Ribavirin can be obtained from commercial suppliers (e.g., Sigma and ICN). Ribavirin and/or the antigen can be formulated into the vaccine with and without modification. For example, the ribavirin and/or antigen can be modified or derivatized to make a more stable molecule and/or a more potent adjuvant. By one approach, the stability of ribavirin and/or an antigen can be enhanced by coupling the molecules to a support such as a hydrophilic polymer (e.g., polyethylene glycol).

Many more ribavirin derivatives can be generated using conventional techniques in rational drug design and combinatorial chemistry. For example, Molecular Simulations Inc. (MSI), as well as many other suppliers, provide software that allows one of skill to build a combinatorial library of organic molecules. The C2.Analog Builder program, for example, can be integrated with MSI's suite of Cerius2 molecular diversity software to develop a library of ribavirin derivatives that can be used with the embodiments described herein. (See e.g., <http://msi.com/life/products/cerius2/index.html>).

By one approach, the chemical structure of ribavirin is recorded on a computer readable medium and is accessed by one or more modeling software application programs. The C2.Analog Builder program in conjunction with C2Diversity program allows the user to generate a very large virtual library based on the diversity of R-groups for each substituent position, for example. Compounds having the same structure as the modeled ribavirin derivatives created in the virtual library are then made using conventional chemistry or can be obtained from a commercial source.

The newly manufactured ribavirin derivatives are then screened in assays, which determine the extent of adjuvant activity of the molecule and/or the extent of its ability to modulate of an immune response. Some assays may involve virtual drug screening software, such as C2.Ludi. C2.Ludi is a software program that allows a user to explore databases of molecules (e.g., ribavirin derivatives) for their ability to interact with the active site of a protein of interest (e.g., RAC2 or another GTP binding protein). Based upon predicted interactions discovered with the virtual drug screening software, the ribavirin derivatives can be prioritized for further characterization in conventional assays that determine adjuvant activity and/or the extent of a molecule to modulate an immune response. *Example 1* describes several assays that were used to evaluate the adjuvant activity of ribavirin.

## EXAMPLE 1

This following assays can be used with any ribavirin derivative or combinations of ribavirin derivatives to determine the extent of adjuvant activity of the particular composition. In a

first set of experiments, groups of three to five Balb/c mice (BK Universal, Uppsala, Sweden) were immunized *i.p* or *s.c.* (e.g., at the base of the tail) with 10µg or 100µg of recombinant hepatitis C virus non-structural 3 (rNS3) protein at weeks zero and four. The rNS3 was dissolved in phosphate buffered saline (PBS) alone or PBS containing 1mg ribavirin (obtained from ICN, Costa Mesa, CA). Mice were injected with a total volume of 100µl per injection.

At two, four, and six weeks following first *i.p.* immunization, all mice were bled by retro-orbital sampling. Serum samples were collected and analyzed for the presence of antibodies to rNS3. To determine the antibody titer, an enzyme immunoassay (EIA) was performed. (See e.g., Hultgren et al., *J Gen Virol.* 79:2381-91 (1998) and Hultgren et al., *Clin. Diagn. Lab. Immunol.* 4:630-632 (1997)). The antibody levels were recorded as the highest serum dilution giving an optical density at 405nm more than twice that of non-immunized mice.

Mice that received 10µg or 100µg rNS3 mixed with 1mg ribavirin in PBS displayed consistently higher levels of NS3 antibodies. The antibody titer that was detected by EIA at two weeks post-immunization is shown in **FIGURE 1**. The vaccine formulations having 1mg of ribavirin and either 10µg or 100µg of rNS3 induced a significantly greater antibody titer than the vaccine formulations composed of only rNS3.

In a second set of experiments, groups of eight Balb/c mice were at weeks zero and four immunized intraperitoneally with 10 or 50 µg of rNS3 in 100 µl phosphate buffered saline containing either 0 mg, 1 mg, 3 mg, or 10 mg ribavirin (Sigma). At four, six and eight weeks the mice were bled and serum was separated and frozen. After completion of the study, sera were tested for the levels of antibodies to recombinant NS3, as described above. Mean antibody levels to rNS3 were compared between the groups using Student's t-test (parametric analysis) or Mann-Whitney (non-parametric analysis) and the software package StatView 4.5 (Abacus Concepts, Berkely, CA). The adjuvant effect of ribavirin when added in three doses to 10 µg of rNS3 are provided in **TABLE 1**. The adjuvant effect of ribavirin when added in three doses to 50 µg of rNS3 are provided in **TABLE 2**. Parametrical comparison of the mean rNS3 antibody titres in mice receiving different 10µg or 50 µg of rNS3 and different doses of ribavirin are provided in **TABLES 3 and 4**, respectively. Non-parametrical comparison of mean NS3 antibody titres in mice receiving different 10µg or 50 µg of rNS3 and different doses of ribavirin are provided in **TABLES 5 and 6**, respectively. The values given represent end point titres to recombinant rNS3.

TABLE 1

Amount ribavirin (mg/dose)	Amount immunogen ( $\mu$ g/dose)	Mouse ID	Antibody titre to rNS3 at indicated week		
			Week 4	Week 6	Week 8
None	10	5:1	300	1500	1500
None	10	5:2	<60	7500	1500
None	10	5:3	<60	1500	300
None	10	5:4	60	1500	1500
None	10	5:5	<60	1500	nt
None	10	5:6	60	1500	1500
None	10	5:7	<60	7500	7500
None	10	5:8	300	37500	7500
Group mean titre (mean $\pm$ SD)			180 $\pm$ 139	7500 $\pm$ 12421	3042 $\pm$ 3076
1	10	6:1	300	37500	37500
1	10	6:2	<60	1500	1500
1	10	6:3	300	37500	187500
1	10	6:4	300	37500	7500
1	10	6:5	60	nt	nt
1	10	6:6	<60	37500	7500
1	10	6:7	<60	37500	7500
1	10	6:8	300	7500	7500
Group mean titre (mean $\pm$ SD)			252 $\pm$ 107	28071 $\pm$ 16195	36642 $\pm$ 67565
3	10	7:1	60	37500	7500
3	10	7:2	60	37500	37500
3	10	7:3	300	7500	7500
3	10	7:4	300	37500	7500
3	10	7:5	300	37500	37500
3	10	7:6	300	37500	37500
3	10	7:7	60	7500	7500
3	10	7:8	60	37500	37500
Group mean titre (mean $\pm$ SD)			180 $\pm$ 128	30000 $\pm$ 13887	22500 $\pm$ 34637
10	10	8:1	300	37500	37500
10	10	8:2	300	37500	37500
10	10	8:3	<60	300	300
10	10	8:4	60	7500	7500
10	10	8:5	<60	300	300
10	10	8:6	<60	37500	37500
10	10	8:7	<60	7500	7500
10	10	8:8	<60	nt	nt
Group mean titre (mean $\pm$ SD)			220 $\pm$ 139	18300 $\pm$ 18199	18300 $\pm$ 18199

TABLE 2

Amount ribavirin (mg/dose)	Amount immunogen (µg/dose)	Mouse ID	Antibody titre to rNS3 at indicated week		
			Week 4	Week 6	Week 8
None	50	1:1	60	7500	7500
None	50	1:2	60	7500	7500
None	50	1:3	60	7500	7500
None	50	1:4	<60	1500	300
None	50	1:5	300	37500	37500
None	50	1:6	60	7500	7500
None	50	1:7	60	37500	7500
None	50	1:8	.	.	.
Group mean titre (mean±SD)			100 ±98	15214 ±15380	10757 ±12094
1	50	2:1	60	7500	7500
1	50	2:2	300	37500	7500
1	50	2:3	60	187500	7500
1	50	2:4	60	37500	187500
1	50	2:5	60	37500	7500
1	50	2:6	60	37500	37500
1	50	2:7	300	37500	7500
1	50	2:8	300	37500	37500
Group mean titre (mean±SD)			150 ±124	52500 ±55549	37500 ±62105
3	50	3:1	60	37500	7500
3	50	3:2	300	37500	37500
3	50	3:3	300	37500	7500
3	50	3:4	60	37500	7500
3	50	3:5	300	37500	7500
3	50	3:6	60	37500	7500
3	50	3:7	-	7500	37500
3	50	3:8	1500	7500	37500
Group mean titre (mean±SD)			387 ±513	30000 ±13887	18750 ±15526
10	50	4:1	300	7500	7500
10	50	4:2	300	37500	37500
10	50	4:3	60	7500	7500
10	50	4:4	60	7500	7500
10	50	4:5	60	1500	1500
10	50	4:6	60	7500	37500
10	50	4:7	-	7500	7500
10	50	8:8	60	37500	7500
Group mean titre (mean±SD)			140 ±124	10929 ±11928	15214 ±15380

TABLE 3

Group	Week	Mean±SD	Group	Mean±SD	analysis	p-value
10µg NS3/no ribavirin	4	180 ±139	10 µg NS3/ 1 mg ribavirin	252 ±107	Students t-test	0.4071
	6	7500 ±12421		28071 ±16195	Students t-test	0.0156
	8	3042 ±3076		36642 ±67565	Students t-test	0.2133
10µg NS3/no ribavirin	4	180 ±139	10 µg NS3/ 3 mg ribavirin	180± 128	Students t-test	1.000
	6	7500 ±12421		30000± 13887	Students t-test	0.0042
	8	3042 ±3076		22500± 34637	Students t-test	0.0077
10µg NS3/no ribavirin	4	180 ±139	10 µg NS3/ 10mg ribavirin	220± 139	Students t-test	0.7210
	6	7500 ±12421		18300± 18199	Students t-test	0.1974
	8	3042 ±3076		18300± 18199	Students t-test	0.0493

5

TABLE4

Group	Week	Mean±SD	Group	Mean±SD	analysis	p-value
50µg NS3/no ribavirin	4	100 ±98	50 µg NS3/ 1 mg ribavirin	150 ±124	Students t-test	0.4326
	6	15214 ±15380		52500 ±55549	Students t-test	0.1106
	8	10757 ±12094		37500 ±62105	Students t-test	0.2847
50µg NS3/no ribavirin	4	100 ±98	50 µg NS3/ 3 mg ribavirin	387 ±513	Students t-test	0.2355
	6	15214 ±15380		30000 ±13887	Students t-test	0.0721
	8	10757 ±12094		18750 ±15526	Students t-test	0.2915
50µg NS3/no ribavirin	4	100 ±98	50 µg NS3/ 10mg ribavirin	140 ±124	Students t-test	0.5490
	6	15214 ±15380		10929 ±11928	Students t-test	0.5710
	8	10757 ±12094		15214 ±15380	Students t-test	0.5579

Significance levels: NS = not significant; \* = p<0.05; \*\* = p<0.01; \*\*\* = p<0.001

TABLE 5

Group	Week	Mean±SD	Group	Mean±SD	analysis	p-value
10µg NS3/no ribavirin	4	180 ±139	10 µg NS3/ 1 mg ribavirin	252 ±107	Mann-Whitney	0.4280
	6	7500 ±12421		28071 ±16195	Mann-Whitney	0.0253
	8	3042 ±3076		36642 ±67565	Mann-Whitney	0.0245
10µg NS3/no ribavirin	4	180 ±139	10 µg NS3/ 3 mg ribavirin	180± 128	Mann-Whitney	0.0736
	6	7500 ±12421		30000± 13887	Mann-Whitney	0.0050
	8	3042 ±3076		22500± 34637	Mann-Whitney	0.0034
10µg NS3/no ribavirin	4	180 ±139	10 µg NS3/ 10mg ribavirin	220± 139	Mann-Whitney	0.8986
	6	7500 ±12421		18300± 18199	Mann-Whitney	0.4346
	8	3042 ±3076		18300± 18199	Mann-Whitney	0.2102

5

TABLE 6

Group	Week	Mean±SD	Group	Mean±SD	analysis	p-value
50µg NS3/no ribavirin	4	100 ±98	50 µg NS3/ 1 mg ribavirin	150 ±124	Mann-Whitney	0.1128
	6	15214 ±15380		52500 ±55549	Mann-Whitney	0.0210
	8	10757 ±12094		37500 ±62105	Mann-Whitney	0.1883
50µg NS3/no ribavirin	4	100 ±98	50 µg NS3/ 3 mg ribavirin	387 ±513	Mann-Whitney	0.1400
	6	15214 ±15380		30000 ±13887	Mann-Whitney	0.0679
	8	10757 ±12094		18750 ±15526	Mann-Whitney	0.2091
50µg NS3/no ribavirin	4	100 ±98	50 µg NS3/ 10 mg ribavirin	140 ±124	Mann-Whitney	0.4292
	6	15214 ±15380		10929 ±11928	Mann-Whitney	0.9473
	8	10757 ±12094		15214 ±15380	Mann-Whitney	0.6279

Significance levels: NS = not significant; \* = p<0.05; \*\* = p<0.01; \*\*\* = p<0.001

The data above demonstrate that ribavirin facilitates or enhances an immune response to an HCV antigen or HCV epitopes. A potent immune response to rNS3 was elicited after immunization with a vaccine composition comprising as little as 1 mg ribavirin and 10 µg of rNS3 antigen. The data above also provide evidence that the amount of ribavirin that is sufficient to facilitate an immune response to an antigen is between 1 and 3 mg per injection for a 25-30g Balb/c mouse. It should be realized, however, that these amounts are intended for guidance only and should not be interpreted to limit the scope of the invention in any way. Nevertheless, the data shows that vaccine compositions comprising approximately 1 to 3 mg doses of ribavirin induce an immune response that is more than 12 times higher than the immune response elicited in the absence of ribavirin (TABLES 3 and 4). Thus, ribavirin has a significant adjuvant effect on the humoral immune response of an animal and thereby, enhances or facilitates the immune response to the antigen. The example below describes experiments that were performed to better understand the amount of ribavirin needed to enhance or facilitate an immune response to an antigen.

#### EXAMPLE 2

To determine a dose of ribavirin that is sufficient to provide an adjuvant effect, the following experiments were performed. In a first set of experiments, groups of mice (three per group) were immunized with a 20µg rNS3 alone or a mixture of 20µg rNS3 and 0.1mg, 1mg, or 10mg ribavirin. The levels of antibody to the antigen were then determined by EIA. The mean endpoint titers at weeks 1 and 3 were plotted and are shown in FIGURE 2. It was discovered that the adjuvant effect provided by ribavirin had different kinetics depending on the dose of ribavirin provided. For example, even low doses (<1mg) of ribavirin were found to enhance antibody levels at week one but not at week three, whereas, higher doses (1-10mg) were found to enhance antibody levels at week three.

A second set of experiments was also performed. In these experiments, groups of mice were injected with vaccine compositions comprising various amounts of ribavirin and rNS3 and the IgG response in these animals was monitored. The vaccine compositions comprised approximately 100 µl phosphate buffered saline and 20 µg rNS3 with or without 0.1 mg, 1.0 mg, or 10 mg ribavirin (Sigma). The mice were bled at week six and rNS3-specific IgG levels were determined by EIA as described previously. As shown in TABLE 7, the adjuvant effects on the sustained antibody levels were most obvious in the dose range of 1 to 10 mg per injection for a 25-30g mouse.

TABLE 7

Immunogen	Amount (mg) ribavirin mixed with the immunogen	Mouse ID	Endpoint titre of rNS3 IgG at indicated week		
			Week 1	Week 2	Week 3
20 µg rNS3	None	1	60	360	360
20 µg rNS3	None	2	360	360	2160
20 µg rNS3	None	3	360	2160	2160
		Mean	260±173	960±1039	1560±1039
20 µg rNS3	0.1	4	2160	12960	2160
20 µg rNS3	0.1	5	60	60	60
20 µg rNS3	0.1	6	<60	2160	2160
			1110±1484	5060±6921	1460±1212
20 µg rNS3	1.0	7	<60	60	12960
20 µg rNS3	1.0	8	<60	2160	2160
20 µg rNS3	1.0	9	360	2160	2160
		Mean	360	1460±1212	5760±6235
20 µg rNS3	10.0	10	360	12960	77760
20 µg rNS3	10.0	11	<60	2160	12960
20 µg rNS3	10.0	12	360	2160	2160
		Mean	360	5760±6235	30960±40888

- 5 In a third set of experiments, the adjuvant effect of ribavirin after primary and booster injections was investigated. In these experiments, mice were given two intraperitoneal injections of a vaccine composition comprising 10 µg rNS3 with or without ribavirin and the IgG subclass responses to the antigen was monitored, as before. Accordingly, mice were immunized with 100 µl phosphate buffered containing 10 µg recombinant NS3 alone, with or without 0.1 or 1.0 mg
- 10 ribavirin (Sigma) at weeks 0 and 4. The mice were bled at week six and NS3-specific IgG subclasses were determined by EIA as described previously. As shown in **TABLE 8**, the addition of ribavirin to the immunogen prior to the injection does not change the IgG subclass response in the NS3-specific immune response. Thus, the adjuvant effect of a vaccine composition comprising ribavirin and an antigen can not be explained by a shift in the Th1/Th2-balance. It appears that
- 15 another mechanism may be responsible for the adjuvant effect of ribavirin.



TABLE 8

Immunogen	Amount (mg) ribavirin mixed with the immunogen	Mouse ID	Endpoint titre of indicated NS3 IgG subclass			
			IgG1	IgG2a	IgG2b	IgG3
10 µg rNS3	None	1	360	60	<60	60
10 µg rNS3	None	2	360	<60	<60	60
10 µg rNS3	None	3	2160	60	<60	360
		Mean	960±1039	60	-	160±173
10 µg rNS3	0.1	4	360	<60	<60	60
10 µg rNS3	0.1	5	60	<60	<60	<60
10 µg rNS3	0.1	6	2160	60	60	360
			860±1136	60	60	210±212
10 µg rNS3	1.0	7	2160	<60	<60	60
10 µg rNS3	1.0	8	360	<60	<60	<60
10 µg rNS3	1.0	9	2160	<60	<60	60
		Mean	1560±1039	-	-	60

- 5           The data presented in this example further verify that ribavirin can be administered as an adjuvant and establish that the dose of ribavirin can modulate the kinetics of the adjuvant effect. The example below describes another assay that was performed to evaluate the ability of ribavirin to enhance or facilitate an immune response to an antigen.

## EXAMPLE 3

- 10           This assay can be used with any ribavirin derivative or combinations of ribavirin derivatives to determine the extent that a particular vaccine formulation modulates a cellular immune response. To determine CD4<sup>+</sup> T cell responses to a ribavirin-containing vaccine, groups of mice were immunized *s.c.* with either 100µg rNS3 in PBS or 100µg rNS3 and 1mg ribavirin in PBS. The mice were sacrificed ten days post-immunization and their lymph nodes were harvested
- 15           and drained. *In vitro* recall assays were then performed. (See e.g., Hultgren et al., *J Gen Virol.* 79:2381-91 (1998) and Hultgren et al., *Clin. Diagn. Lab. Immunol.* 4:630-632 (1997)). The amount of CD4<sup>+</sup> T cell proliferation was determined at 96 h of culture by the incorporation of [<sup>3</sup>H] thymidine.

- 20           As shown in **FIGURE 3**, mice that were immunized with 100µg rNS3 mixed with 1mg ribavirin had a much greater T cell proliferative response than mice that were immunized with 100µg rNS3 in PBS. These data provide additional evidence that ribavirin enhances or facilitates a cellular immune response (e.g., by promoting the effective priming of T cells). The section

below discusses some of the antigens and epitopes that can be used with the embodiments described herein.

*Antigens and epitopes*

Virtually any antigen that can be used to generate an immune response in an animal can be combined with ribavirin so as to prepare the compositions described herein. That is, antigens that can be incorporated into such compositions (e.g., vaccines) comprise bacterial antigens or epitopes, fungal antigens or epitopes, plant antigens or epitopes, mold antigens or epitopes, viral antigens or epitopes, cancer cell antigens or epitopes, toxin antigens or epitopes, chemical antigens or epitopes, and self-antigens or epitopes. Although many of these molecules induce a significant immune response without an adjuvant, ribavirin can be administered in conjunction with or combined with “strong” or “weak” antigens or epitopes to enhance or facilitate the immune response to said antigen or epitope. In addition, the use of ribavirin as an adjuvant may allow for the use of lesser amounts of antigens while retaining immunogenicity.

In addition to peptide antigens, nucleic acid-based antigens can be used in the vaccine compositions described herein. Various nucleic acid-based vaccines are known and it is contemplated that these compositions and approaches to immunotherapy can be augmented by reformulation with ribavirin (See e.g., U.S. Pat. No. 5,589,466 and 6,235,888). By one approach, for example, a gene encoding a polypeptide antigen of interest is cloned into an expression vector capable of expressing the polypeptide when introduced into a subject. The expression construct is introduced into the subject in a mixture of ribavirin or in conjunction with ribavirin (e.g., ribavirin is administered shortly after the expression construct at the same site). Alternatively, RNA encoding a polypeptide antigen of interest is provided to the subject in a mixture with ribavirin or in conjunction with ribavirin.

Where the antigen is to be DNA (e.g., preparation of a DNA vaccine composition), suitable promoters include Simian Virus 40 (SV40), Mouse Mammary Tumor Virus (MMTV) promoter, Human Immunodeficiency Virus (HIV) such as the HIV Long Terminal Repeat (LTR) promoter, Moloney virus, ALV, Cytomegalovirus (CMV) such as the CMV immediate early promoter, Epstein Barr Virus (EBV), Rous Sarcoma Virus (RSV) as well as promoters from human genes such as human actin, human myosin, human hemoglobin, human muscle creatine and human metallothionein can be used. Examples of polyadenylation signals useful with some embodiments, especially in the production of a genetic vaccine for humans, include but are not limited to, SV40 polyadenylation signals and LTR polyadenylation signals. In particular, the SV40 polyadenylation signal, which is in pCEP4 plasmid (Invitrogen, San Diego Calif.), referred to as the SV40 polyadenylation signal, is used.

In addition to the regulatory elements required for gene expression, other elements may also be included in a gene construct. Such additional elements include enhancers. The enhancer may be selected from the group including but not limited to: human actin, human myosin, human hemoglobin, human muscle creatine and viral enhancers such as those from CMV, RSV and EBV.

5 Gene constructs can be provided with mammalian origin of replication in order to maintain the construct extrachromosomally and produce multiple copies of the construct in the cell. Plasmids pCEP4 and pREP4 from Invitrogen (San Diego, CA) contain the Epstein Barr virus origin of replication and nuclear antigen EBNA-1 coding region, which produces high copy episomal replication without integration. All forms of DNA, whether replicating or non-replicating, which

10 do not become integrated into the genome, and which are expressible, can be used. The example below describes the use of a composition comprising a nucleic acid-based antigen and ribavirin.

#### EXAMPLE 4

The following describes the immunization of an animal with a vaccine comprising a nucleic acid-based antigen and ribavirin. Five to six week old female and male Balb/C mice are

15 anesthetized by intraperitoneal injection with 0.3ml of 2.5% Avertin. A 1.5cm incision is made on the anterior thigh, and the quadriceps muscle is directly visualized. One group of mice are injected with approximately 20:g of an expression construct having the gp-120 gene, driven by a cytomegalovirus (CMV) promotor and second group of mice are injected with approximately 5:g of capped *in vitro* transcribed RNA (e.g., SP6, T7, or T3 (Ambion)) encoding gp-120. These two

20 groups are controls. A third group of mice is injected with approximately 20:g of the expression vector having the gp-120 gene and the CMV promoter mixed with 1mg of ribavirin and a fourth group of mice is injected with approximately 5:g of capped *in vitro* transcribed RNA mixed with 1mg ribavirin. The vaccines are injected in 0.1ml of solution (PBS) in a 1 cc syringe through a 27 gauge needle over one minute, approximately 0.5cm from the distal insertion site of the muscle

25 into the knee and about 0.2cm deep. A suture is placed over the injection site for future localization, and the skin is then closed with stainless steel clips.

Blood samples are obtained prior to the injection (Day 0) and up to more than 40 days post injection. The serum from each sample is serially diluted and assayed in a standard ELISA technique assay for the detection of antibody, using recombinant gp-120 protein made in yeast as

30 the antigen. Both IgG and IgM antibodies specific for gp-120 will be detected in all samples, however, groups three and four, which contained the ribavirin, will exhibit a greater immune response to the gp-120 as measured by the amount and/or titer of antibody detected in the sera.

Preferred embodiments of the invention comprise ribavirin and a viral antigen or an epitope present on a virus, preferably a hepatitis virus. Compositions comprise, for example,

35 ribavirin and an HAV antigen, HBV antigen, HCV antigen or any combination of these antigens or

epitopes present on one or more of these viruses. The hepatitis antigens can be peptides or nucleic acids. Compositions that can be used to vaccinate against HAV infection, for example, comprise ribavirin and an HAV peptide with a length of at least 3-10 consecutive amino acids, 10-50 consecutive amino acids, 50-100 consecutive amino acids, 100-200 consecutive amino acids, 200-400 consecutive amino acids, 400-800 consecutive amino acids, 800-1200 consecutive amino acids, 1200-1600 consecutive amino acids, 1600-2000 consecutive amino acids, and 2000-2227 consecutive amino acids of **SEQ ID. NO.: 12**.

Additionally, compositions comprising ribavirin and a nucleic acid encoding one or more of the HAV peptides, described above, can be used to treat or prevent HAV infection. Preferred nucleic acid-based antigens include a nucleotide sequence of at least 9 consecutive nucleotides of an HAV sequence (e.g., **SEQ. ID. NO.: 15**). That is, a nucleic acid based antigen can comprise at least 9-25 consecutive nucleotides, 25-50 consecutive nucleotides, 50-100 consecutive nucleotides, 100-200 consecutive nucleotides, 200-500 consecutive nucleotides, 500-1000 consecutive nucleotides, 1000-2000 consecutive nucleotides, 2000-4000 consecutive nucleotides, 4000-8000 consecutive nucleotides, and 8000-9416 consecutive nucleotides of **SEQ. ID. NO.: 15** or an RNA that corresponds to these sequences.

Similarly, preferred HBV vaccine embodiments comprise ribavirin and a HBV peptide of at least 3 consecutive amino acids of HBsAg (**SEQ. ID. NO.: 10**) or HBcAg and HBeAg (**SEQ. ID. NO.: 11**). That is, some embodiments have ribavirin and a HBV peptide with a length of at least 3-10 consecutive amino acids, 10-50 consecutive amino acids, 50-100 consecutive amino acids, 100-150 consecutive amino acids, 150-200 consecutive amino acids, and 200-226 consecutive amino acids of either **SEQ. ID. NO.: 10** or **SEQ. ID. NO.: 11**.

Additionally, compositions comprising ribavirin and a nucleic acid encoding one or more of the HBV peptides, described above, can be used to treat or prevent HBV infection. Preferred nucleic acid-based antigens include a nucleotide sequence of at least 9 consecutive nucleotides of an HBV (e.g., **SEQ. ID. NO.: 14**). That is, a nucleic acid based antigen can comprise at least 9-25 consecutive nucleotides, 25-50 consecutive nucleotides, 50-100 consecutive nucleotides, 100-200 consecutive nucleotides, 200-500 consecutive nucleotides, 500-1000 consecutive nucleotides, 1000-2000 consecutive nucleotides, 2000-4000 consecutive nucleotides, 4000-8000 consecutive nucleotides, and 8000-9416 consecutive nucleotides of **SEQ. ID. NO.: 14** or an RNA that corresponds to these sequences. The example below describes the use of ribavirin in conjunction with a commercial HBV vaccine preparation.

#### EXAMPLE 5

The adjuvant effect of ribavirin was tested when mixed with two doses of a commercially available vaccine containing HBsAg and alum. (Engerix, SKB). Approximately 0.2 $\mu$ g or 2 $\mu$ g of

Engerix vaccine was mixed with either PBS or 1mg ribavirin in PBS and the mixtures were injected intra peritoneally into groups of mice (three per group). A booster containing the same mixture was given on week four and all mice were bled on week six. The serum samples were diluted from 1:60 to 1:37500 and the dilutions were tested by EIA, as described above, except that purified human HBsAg was used as the solid phase antigen. As shown in **TABLE 9**, vaccine formulations having ribavirin enhanced the response to 2µg of an existing vaccine despite the fact that the vaccine already contained alum. That is, by adding ribavirin to a suboptimal vaccine dose (i.e., one that does not induce detectable antibodies alone) antibodies became detectable, providing evidence that the addition of ribavirin allows for the use of lower antigen amounts in a vaccine formulation without compromising the immune response.

TABLE 9

Week	Endpoint antibody titer to HBsAg in EIA											
	0.02µg Engerix						0.2µg Engerix					
	No ribavirin			1mg ribavirin			No ribavirin			1mg ribavirin		
	#1	#2	#3	#1	#2	#3	#1	#2	#3	#1	#2	#3
6	<60	<60	<60	<60	<60	<60	<60	<60	<60	300	60	<60

15

Some HCV vaccine compositions comprise ribavirin and a HCV peptide of at least 3 consecutive amino acids of **SEQ. ID. NO.: 1** or a nucleic acid encoding said HCV peptide. That is, a vaccine composition can comprise ribavirin and one or more HCV peptides with a length of at least 3-10 consecutive amino acids, 10-50 consecutive amino acids, 50-100 consecutive amino acids, 100-200 consecutive amino acids, 200-400 consecutive amino acids, 400-800 consecutive amino acids, 800-1200 consecutive amino acids, 1200-1600 consecutive amino acids, 1600-2000 consecutive amino acids, 2000-2500 consecutive amino acids, and 2500-3011 consecutive amino acids of **SEQ. ID. NO.: 1** or a nucleic acid encoding one or more of said fragments.

Preferred HCV compositions comprise ribavirin and a peptide of at least 3 consecutive amino acids of HCV core protein (**SEQ. ID. NO.: 2**), HCV E1 protein (**SEQ. ID. NO.: 3**), HCV E2 protein (**SEQ. ID. NO.: 4**), HCV NS2 (**SEQ. ID. NO.: 5**), HCV NS3 (**SEQ. ID. NO.: 6**), HCV NS4A (**SEQ. ID. NO.: 7**), HCV NS4B (**SEQ. ID. NO.: 8**), or HCV NS5A/B (**SEQ. ID. NO.: 9**) or peptides consisting of combinations of these domains. That is, preferred HCV vaccines comprise

ribavirin and a peptide with a length of at least 3-10 consecutive amino acids, 10-50 consecutive amino acids, 50-100 consecutive amino acids, 100-200 consecutive amino acids, 200-400 consecutive amino acids, 400-800 consecutive amino acids, and 800-1040 consecutive amino acids of any one or more of (SEQ. ID. NOs.: 2-9). These domains correspond to amino acid residues 1-182, 183-379, 380-729, 730-1044, 1045-1657, 1658-1711, 1712-1971, or 1972-3011 of SEQ. ID. NO.: 1. Thus, preferred embodiments also include one or more of 1-182, 183-379, 380-729, 730-1044, 1045-1657, 1658-1711, 1712-1971, or 1972-3011 of SEQ. ID. NO.: 1 or fragments thereof.

Vaccine compositions comprising ribavirin and a nucleic acid encoding one or more of the peptides described above are also embodiments. Preferred nucleic acid-based antigens include a nucleotide sequence of at least 9 consecutive nucleotides of HCV (SEQ. ID. NO.: 13). That is, a nucleic acid based antigen can comprise at least 9-25 consecutive nucleotides, 25-50 consecutive nucleotides, 50-100 consecutive nucleotides, 100-200 consecutive nucleotides, 200-500 consecutive nucleotides, 500-1000 consecutive nucleotides, 1000-2000 consecutive nucleotides, 2000-4000 consecutive nucleotides, 4000-8000 consecutive nucleotides, and 8000-9416 consecutive nucleotides of any one of SEQ. ID. NOs.: 13 or an RNA that corresponds to these sequences. The section below discusses some of the compositions containing ribavirin and an antigen.

*Compositions containing ribavirin and an antigen*

Compositions (e.g., vaccines) that comprise ribavirin and an antigen or epitope of a pathogen (e.g., virus, bacteria, mold, yeast, and parasite) may contain other ingredients including, but not limited to, adjuvants, binding agents, excipients such as stabilizers (to promote long term storage), emulsifiers, thickening agents, salts, preservatives, solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. These compositions are suitable for treatment of animals either as a preventive measure to avoid a disease or condition or as a therapeutic to treat animals already afflicted with a disease or condition.

Many other ingredients can be present in the vaccine. For example, the ribavirin and antigen can be employed in admixture with conventional excipients (e.g., pharmaceutically acceptable organic or inorganic carrier substances suitable for parenteral, enteral (e.g., oral) or topical application that do not deleteriously react with the ribavirin and/or antigen). Suitable pharmaceutically acceptable carriers include, but are not limited to, water, salt solutions, alcohols, gum arabic, vegetable oils, benzyl alcohols, polyethylene glycols, gelatine, carbohydrates such as lactose, amylose or starch, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid monoglycerides and diglycerides, pentaerythritol fatty acid esters, hydroxy methylcellulose, polyvinyl pyrrolidone, etc. Many more suitable carriers are described in *Remington's Pharmaceutical Sciences*, 15th Edition, Easton: Mack Publishing Company, pages

1405-1412 and 1461-1487(1975) and The National *Formulary* XIV, 14th Edition, Washington, American Pharmaceutical Association (1975).

The gene constructs described herein may be formulated with or administered in conjunction with agents that increase uptake and/or expression of the gene construct by the cells  
5 relative to uptake and/or expression of the gene construct by the cells that occurs when the identical genetic vaccine is administered in the absence of such agents. Such agents and the protocols for administering them in conjunction with gene constructs are described in U.S. Ser. No. 08/008,342 filed Jan. 26, 1993, U.S. Ser. No. 08/029,336 filed Mar. 11, 1993, U.S. Ser. No. 08/125,012 filed Sep. 21, 1993, PCT Patent Application Serial Number PCT/US94/00899 filed Jan.  
10 26, 1994, and U.S. Ser. No. 08/221,579 filed Apr. 1, 1994. Examples of such agents include: CaPO<sub>4</sub>, DEAE dextran, anionic lipids; extracellular matrix-active enzymes; saponins; lectins; estrogenic compounds and steroidal hormones; hydroxylated lower alkyls; dimethyl sulfoxide (DMSO); urea; and benzoic acid esters anilides, amidines, urethanes and the hydrochloride salts thereof such as those of the family of local anesthetics. In addition, the gene constructs are  
15 encapsulated within/administered in conjunction with lipids/polycationic complexes.

Vaccines can be sterilized and if desired mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or aromatic substances and the like that do not deleteriously react with ribavirin or the antigen.

20 The effective dose and method of administration of a particular vaccine formulation can vary based on the individual patient and the type and stage of the disease, as well as other factors known to those of skill in the art. Therapeutic efficacy and toxicity of the vaccines can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED<sub>50</sub> (the dose therapeutically effective in 50% of the population). The data obtained from cell  
25 culture assays and animal studies can be used to formulate a range of dosage for human use. The dosage of the vaccines lies preferably within a range of circulating concentrations that include the ED<sub>50</sub> with no toxicity. The dosage varies within this range depending upon the type of ribavirin derivative and antigen, the dosage form employed, the sensitivity of the patient, and the route of administration.

30 Since ribavirin has been on the market for several years, many dosage forms and routes of administration are known. All known dosage forms and routes of administration can be provided within the context of the embodiments described herein. Preferably, an amount of ribavirin that is effective to enhance an immune response to an antigen in an animal can be considered to be an amount that is sufficient to achieve a blood serum level of antigen approximately 0.25 - 12.5 µg/ml  
35 in the animal, preferably, about 2.5 µg/ml. In some embodiments, the amount of ribavirin is

determined according to the body weight of the animal to be given the vaccine. Accordingly, the amount of ribavirin in a vaccine formulation can be from about 0.1 - 6.0mg/kg body weight. That is, some embodiments have an amount of ribavirin that corresponds to approximately 0.1 - 1.0mg/kg, 1.1 - 2.0mg/kg, 2.1 - 3.0mg/kg, 3.1 - 4.0mg/kg, 4.1 - 5.0mg/kg, 5.1, and 6.0mg/kg body weight of an animal. More conventionally, the vaccines contain approximately 0.25mg - 2000mg of ribavirin. That is, some embodiments have approximately 250µg, 500µg, 1mg, 25mg, 50mg, 100mg, 150mg, 200mg, 250mg, 300mg, 350mg, 400mg, 450mg, 500mg, 550mg, 600mg, 650mg, 700mg, 750mg, 800mg, 850mg, 900mg, 1g, 1.1g, 1.2g, 1.3g, 1.4g, 1.5g, 1.6g, 1.7g, 1.8g, 1.9g, and 2g of ribavirin.

Conventional vaccine preparations can be modified by adding an amount of ribavirin that is sufficient to enhance an immune response to the antigen. That is, existing conventional vaccine formulations can be modified by simply adding ribavirin to the preparation or by administering the conventional vaccine in conjunction with ribavirin (e.g., shortly before or after providing the antigen). As one of skill in the art will appreciate, the amount of antigens in a vaccine can vary depending on the type of antigen and its immunogenicity. The amount of antigens in the vaccines can vary accordingly. Nevertheless, as a general guide, the vaccines can have approximately 0.25mg - 5mg, 5-10mg, 10-100mg, 100-500mg, and upwards of 2000mg of an antigen (e.g., a hepatitis viral antigen).

In some approaches described herein, the exact amount of ribavirin and/or antigen is chosen by the individual physician in view of the patient to be treated. Further, the amounts of ribavirin can be added in combination with or separately from the same or equivalent amount of antigen and these amounts can be adjusted during a particular vaccination protocol so as to provide sufficient levels in light of patient-specific or antigen-specific considerations. In this vein, patient-specific and antigen-specific factors that can be taken into account include, but are not limited to, the severity of the disease state of the patient, age, and weight of the patient, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. The next section describes the discovery of a novel HCV gene and the creation of mutant HCV sequences, which can be used with the embodiments described herein.

#### *Novel NS3/4A and mutant NS3/4A sequences*

A novel nucleic acid and protein corresponding to the NS3/4A domain of HCV was cloned from a patient infected with HCV (SEQ. ID. NOs.: 16 and 17). A Genebank search revealed that the cloned sequence had the greatest homology to HCV sequences but was only 93% homologous to the closest HCV relative (accession no AJ 278830). A truncated mutant of the novel NS3/4A peptide and NS3/4A mutants, which lack a proteolytic cleavage site, were also created. It was discovered that these novel peptides and nucleic acids encoding said peptides were potent



immunogens that can be mixed with fibavirin so as to make a composition that provides a recipient with a potent immune response to HCV. The cloning of the novel NS3/4A domain and the creation of the various NS3/4A mutants is described in the following example.

#### EXAMPLE 6

5 The NS3/4A sequence was amplified from the serum of an HCV-infected patient (HCV genotype 1a) using the Polymerase Chain Reaction (PCR). Total RNA was extracted from serum, cDNA synthesis, and PCR was performed according to standard protocols (Chen M et al., *J. Med. Virol.* 43:223-226 (1995)). The cDNA synthesis was initiated using the antisense primer "NS4KR" (5'-CCG TCT AGA TCA GCA CTC TTC CAT TTC ATC-3' (SEQ. ID. NO.: 18)). From this  
10 cDNA, a 2079 base pair DNA fragment of HCV, corresponding to amino acids 1007 to 1711, which encompasses the NS3 and NS4A genes, was amplified. A high fidelity polymerase (Expand High Fidelity PCR, Boehringer-Mannheim, Mannheim, Germany) was used with the "NS3KF" primer (5'-CCT GAA TTC ATG GCG CCT ATC ACG GCC TAT-3' (SEQ. ID. NO.: 19) and the NS4KR primer. The NS3KF primer contained a *EcoRI* restriction enzyme cleavage site and a start  
15 codon and the primer NS4KR contained a *XbaI* restriction enzyme cleavage site and a stop codon.

The amplified fragment was then sequenced SEQ. ID. NO.: 16. Sequence comparison analysis revealed that the gene fragment was indeed amplified from a viral strain of genotype 1a. A computerized BLAST search against the Genbank database using the NCBI website revealed that the closest HCV homologue was 93% identical in nucleotide sequence.

20 The amplified DNA fragment was then digested with *EcoRI* and *XbaI*, and was inserted into a pcDNA3.1/His plasmid (Invitrogen) digested with the same enzymes. The NS3/4A-pcDNA3.1 plasmid was then digested with *EcoRI* and *XbaI* and the insert was purified using the QiaQuick kit (Qiagen, Hamburg, Germany) and was ligated to a *EcoRI/XbaI* digested pVAX vector (Invitrogen) so as to generate the NS3/4A-pVAX plasmid.

25 The rNS3 truncated mutant was obtained by deleting NS4A sequence from the NS3/4A DNA. Accordingly, the NS3 gene sequence of NS3/4A-pVAX was PCR amplified using the primers NS3KF and 3'*NotI* (5'-CCA CGC GGC CGC GAC GAC CTA CAG-3' (SEQ. ID. NO.: 20)) containing *EcoRI* and *NotI* restriction sites, respectively. The NS3 fragment (1850 bp) was then ligated to a *EcoRI* and *NotI* digested pVAX plasmid to generate the NS3-pVAX vector.  
30 Plasmids were grown in BL21 *E.coli* cells. The plasmids were sequenced and were verified by restriction cleavage and the results were as to be expected based on the original sequence.

To change the proteolytic cleavage site between NS3 and NS4A, the NS3/4A-pVAX plasmid was mutagenized using the QUICKCHANGE™ mutagenesis kit (Stratagene), following the manufacturer's recommendations. To generate the "TPT" mutation, the plasmid was amplified  
35 using the primers 5'-CTGGAGGTCGTCACGCCTACCTGGGTGCTCGTT-3' (SEQ. ID. NO.:

21) and 5'-ACCGAGCACCCAGGTAGGCGTGACGACCTCCAG-3' (SEQ. ID. NO.: 22) resulting in NS3/4A-TPT-pVAX. To generate the "RGT" mutation, the plasmid was amplified using the primers 5'-CTGGAGGTCGTCGCGGTACCTGGGTGCTCGTT-3' (SEQ. ID. NO.: 23) and 5'-ACCGAGCACCCAGGTACC-GCGGACGACCTCCAG-3' (SEQ. ID. NO.: 24) resulting in NS3/4A-RGT-pVAX.

All mutagenized constructs were sequenced to verify that the mutations had been correctly made. Plasmids were grown in competent BL21 E. coli. The plasmid DNA used for *in vivo* injection was purified using Qiagen DNA purification columns, according to the manufacturers instructions (Qiagen GmbH, Hilden, FRG). The concentration of the resulting plasmid DNA was determined spectrophotometrically (Dynaquant, Pharmacia Biotech, Uppsala, Sweden) and the purified DNA was dissolved in sterile phosphate buffer saline (PBS) at concentrations of 1 mg/ml. The amino acid sequences of the wild-type and mutated junctions are shown in TABLE 10. The section below describes several nucleic acids that encode HCV peptides.

TABLE 10

Plasmid	Deduced amino acid sequence
*NS3/4A-pVAX	TKYMTCMSADLEVVTSTWVLVGGVL (SEQ. ID. NO.: 25)
NS3/4A-TGT-pVAX	TKYMTCMSADLEVVTGTWVLVGGVL (SEQ. ID. NO.: 26)
20 NS3/4A-RGT-pVAX	TKYMTCMSADLEVVRGTWVLVGGVL (SEQ. ID. NO.: 27)
NS3/4A-TPT-pVAX	TKYMTCMSADLEVVTPTWVLVGGVL (SEQ. ID. NO.: 33)
NS3/4A-RPT-pVAX	TKYMTCMSADLEVVRPTWVLVGGVL (SEQ. ID. NO.: 34)
NS3/4A-RPA-pVAX	TKYMTCMSADLEVVRPAWVLVGGVL (SEQ. ID. NO.: 35)
NS3/4A-CST-pVAX	TKYMTCMSADLEVVCSTWVLVGGVL (SEQ. ID. NO.: 36)
25 NS3/4A-CCST-pVAX	TKYMTCMSADLEVCCSTWVLVGGVL (SEQ. ID. NO.: 37)
NS3/4A-SSST-pVAX	TKYMTCMSADLEVSSSTWVLVGGVL (SEQ. ID. NO.: 38)
NS3/4A-SSSSCST-pVAX	TKYMTCMSADSSSSCSTWVLVGGVL (SEQ. ID. NO.: 39)
NS3A/4A-VVVVTST-pVAX	TKYMTCMSADVVVVTSTWVLVGGVL (SEQ. ID. NO.: 40)
NS5-pVAX	ASDEVVCCSMSTWTG (SEQ. ID. NO.: 41)
30 NS5A/B-pVAX	SSDEVVCCSMWVLVGGVL (SEQ. ID. NO.: 42)

\*The wild type sequence for the NS3/4A fragment is NS3/4A-pVAX. The NS3/4A breakpoint is identified by underline, wherein the P1 position corresponds to the first Thr (T) and the P1' position corresponds to the next following amino acid the NS3/4A-pVAX sequence. In the wild type NS3/4A sequence the NS3 protease cleaves between the P1 and P1' positions.

#### Nucleic acids encoding HCV peptides

40 The nucleic acid embodiments include nucleotides encoding the HCV peptides described herein (e.g., SEQ. ID. NO.: 17, 29, 31, 32, and 43-49) or fragments thereof at least 4, 6, 8, 10, 12, 15, or 20 amino acids in length (e.g., SEQ. ID. NOs.: 25-27, and 33-42). Some embodiments for

example, include genomic DNA, RNA, and cDNA encoding these HCV peptides. The HCV nucleotide embodiments not only include the DNA sequences shown in the sequence listing (e.g., **SEQ. ID. NO.: 16**) but also include nucleotide sequences encoding the amino acid sequences shown in the sequence listing (e.g., **SEQ. ID. NO.: 17**) and any nucleotide sequence that hybridizes to the DNA sequences shown in the sequence listing under stringent conditions (e.g., hybridization to filter-bound DNA in 0.5 M NaHPO<sub>4</sub>, 7.0% sodium dodecyl sulfate (SDS), 1 mM EDTA at 50°C) and washing in 0.2 X SSC/0.2% SDS at 50°C and any nucleotide sequence that hybridizes to the DNA sequences that encode an amino acid sequence provided in the sequence listing (**SEQ. ID. NOS.: 17**) under less stringent conditions (e.g., hybridization in 0.5 M NaHPO<sub>4</sub>, 7.0% sodium dodecyl sulfate (SDS), 1 mM EDTA at 37°C and washing in 0.2X SSC/0.2% SDS at 37°C).

The nucleic acid embodiments also include fragments, modifications, derivatives, and variants of the sequences described above. Desired embodiments, for example, include nucleic acids having at least 12 consecutive bases of one of the novel HCV sequences or a sequence complementary thereto and preferred fragments include at least 12 consecutive bases of a nucleic acid encoding the NS3/4A molecule of **SEQ. ID. NO.: 17** or a sequence complementary thereto.

In this regard, the nucleic acid embodiments of the invention can have from 12 to approximately 2079 consecutive nucleotides. Some DNA fragments of the invention, for example, include nucleic acids having at least 12-15, 15-20, 20-30, 30-50, 50-100, 100-200, 200-500, 500-1000, 1000-1500, 1500-2079 consecutive nucleotides of **SEQ. ID. NO.: 16** or a complement thereof. The nucleic acid embodiments can also be altered by mutation such as substitutions, additions, or deletions. Due to the degeneracy of nucleotide coding sequences, for example, other DNA sequences that encode substantially the same HCV amino acid sequence as depicted in **SEQ. ID. NOS: 17** can be used in some embodiments. These include, but are not limited to, nucleic acid sequences encoding all or portions of NS3/4A (**SEQ. ID. NO.: 16**) or nucleic acids that complement all or part of this sequence that have been altered by the substitution of different codons that encode a functionally equivalent amino acid residue within the sequence, thus producing a silent change, or a functionally non-equivalent amino acid residue within the sequence, thus producing a detectable change.

By using the nucleic acid sequences described above, probes that complement these molecules can be designed and manufactured by oligonucleotide synthesis. Desirable probes comprise a nucleic acid sequence of (**SEQ. ID. NO.: 16**) that is unique to this HCV isolate. These probes can be used to screen cDNA from patients so as to isolate natural sources of HCV, some of which may be novel HCV sequences in themselves. Screening can be by filter hybridization or by PCR, for example. By filter hybridization, the labeled probe preferably contains at least 15-30

base pairs of the nucleic acid sequence of (SEQ. ID. NO.: 16) that is unique to this NS3/4A peptide. The hybridization washing conditions used are preferably of a medium to high stringency. The hybridization can be performed in 0.5M NaHPO<sub>4</sub>, 7.0% sodium dodecyl sulfate (SDS), 1 mM EDTA at 42°C overnight and washing can be performed in 0.2X SSC/0.2% SDS at 42°C. For  
5 guidance regarding such conditions see, for example, Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Springs Harbor Press, N.Y.; and Ausubel et al., 1989, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y.

HCV nucleic acids can also be isolated from patients infected with HCV using the nucleic acids described herein. (See also *Example 6*). Accordingly, RNA obtained from a patient infected  
10 with HCV is reverse transcribed and the resultant cDNA is amplified using PCR or another amplification technique. The primers are preferably obtained from the NS3/4A sequence (SEQ. ID. NO.: 16).

For a review of PCR technology, see *Molecular Cloning to Genetic Engineering*, White, B.A. Ed. in Methods in Molecular Biology 67: Humana Press, Totowa (1997) and the publication entitled  
15 "PCR Methods and Applications" (1991, Cold Spring Harbor Laboratory Press). For amplification of mRNAs, it is within the scope of the invention to reverse transcribe mRNA into cDNA followed by PCR (RT-PCR); or, to use a single enzyme for both steps as described in U.S. Patent No. 5,322,770. Another technique involves the use of Reverse Transcriptase Asymmetric Gap Ligase Chain Reaction (RT-AGLCR), as described by Marshall R.L. et al. (*PCR Methods and*  
20 *Applications* 4:80-84, 1994).

Briefly, RNA is isolated, following standard procedures. A reverse transcription reaction is performed on the RNA using an oligonucleotide primer specific for the most 5' end of the amplified fragment as a primer of first strand synthesis. The resulting RNA/DNA hybrid is then  
"tailed" with guanines using a standard terminal transferase reaction. The hybrid is then digested  
25 with RNase H, and second strand synthesis is primed with a poly-C primer. Thus, cDNA sequences upstream of the amplified fragment are easily isolated. For a review of cloning strategies which can be used, see e.g., Sambrook et al., 1989, *supra*.

In each of these amplification procedures, primers on either side of the sequence to be amplified are added to a suitably prepared nucleic acid sample along with dNTPs and a thermostable  
30 polymerase, such as Taq polymerase, Pfu polymerase, or Vent polymerase. The nucleic acid in the sample is denatured and the primers are specifically hybridized to complementary nucleic acid sequences in the sample. The hybridized primers are then extended. Thereafter, another cycle of denaturation, hybridization, and extension is initiated. The cycles are repeated multiple times to produce an amplified fragment containing the nucleic acid sequence between the primer sites. PCR

has further been described in several patents including US Patents 4,683,195, 4,683,202 and 4,965,188.

The primers are selected to be substantially complementary to a portion of the nucleic acid sequence of (SEQ. ID. NO.: 16) that is unique to this NS3/4A molecule, thereby allowing the sequences between the primers to be amplified. Preferably, primers are at least 16-20, 20-25, or 25-30 nucleotides in length. The formation of stable hybrids depends on the melting temperature (T<sub>m</sub>) of the DNA. The T<sub>m</sub> depends on the length of the primer, the ionic strength of the solution and the G+C content. The higher the G+C content of the primer, the higher is the melting temperature because G:C pairs are held by three H bonds whereas A:T pairs have only two. The G+C content of the amplification primers described herein preferably range between 10 and 75 %, more preferably between 35 and 60 %, and most preferably between 40 and 55 %. The appropriate length for primers under a particular set of assay conditions can be empirically determined by one of skill in the art.

The spacing of the primers relates to the length of the segment to be amplified. In the context of the embodiments described herein, amplified segments carrying nucleic acid sequence encoding HCV peptides can range in size from at least about 25 bp to the entire length of the HCV genome. Amplification fragments from 25-1000 bp are typical, fragments from 50-1000 bp are preferred and fragments from 100-600 bp are highly preferred. It will be appreciated that amplification primers can be of any sequence that allows for specific amplification of the NS3/4A region and can, for example, include modifications such as restriction sites to facilitate cloning.

The PCR product can be subcloned and sequenced to ensure that the amplified sequences represent the sequences of an HCV peptide. The PCR fragment can then be used to isolate a full length cDNA clone by a variety of methods. For example, the amplified fragment can be labeled and used to screen a cDNA library, such as a bacteriophage cDNA library. Alternatively, the labeled fragment can be used to isolate genomic clones via the screening of a genomic library. Additionally, an expression library can be constructed utilizing cDNA synthesized from, for example, RNA isolated from an infected patient. In this manner, HCV gene products can be isolated using standard antibody screening techniques in conjunction with antibodies raised against the HCV gene product. (For screening techniques, see, for example, Harlow, E. and Lane, eds., 1988, Antibodies: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor.)

Embodiments also include (a) DNA vectors that contain any of the foregoing nucleic acid sequences and/or their complements (i.e., antisense); (b) DNA expression vectors that contain any of the foregoing nucleic acid sequences operatively associated with a regulatory element that directs the expression of the nucleic acid; and (c) genetically engineered host cells that contain any of the foregoing nucleic acid sequences operatively associated with a regulatory element that

directs the expression of the coding sequences in the host cell. These recombinant constructs are capable of replicating autonomously in a host cell. Alternatively, the recombinant constructs can become integrated into the chromosomal DNA of a host cell. Such recombinant polynucleotides typically comprise an HCV genomic or cDNA polynucleotide of semi-synthetic or synthetic origin  
5 by virtue of human manipulation. Therefore, recombinant nucleic acids comprising these sequences and complements thereof that are not naturally occurring are provided.

Although nucleic acids encoding an HCV peptide or nucleic acids having sequences that complement an HCV gene as they appear in nature can be employed, they will often be altered, e.g., by deletion, substitution, or insertion and can be accompanied by sequence not present in  
10 humans. As used herein, regulatory elements include, but are not limited to, inducible and non-inducible promoters, enhancers, operators and other elements known to those skilled in the art that drive and regulate expression. Such regulatory elements include, but are not limited to, the cytomegalovirus hCMV immediate early gene, the early or late promoters of SV40 adenovirus, the lac system, the trp system, the TAC system, the TRC system, the major operator and promoter  
15 regions of phage A, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase, the promoters of acid phosphatase, and the promoters of the yeast  $\alpha$ -mating factors.

In addition, recombinant HCV peptide-encoding nucleic acid sequences and their complementary sequences can be engineered so as to modify their processing or expression. For example, and not by way of limitation, the HCV nucleic acids described herein can be combined  
20 with a promoter sequence and/or ribosome binding site, or a signal sequence can be inserted upstream of HCV peptide-encoding sequences so as to permit secretion of the peptide and thereby facilitate harvesting or bioavailability. Additionally, a given HCV nucleic acid can be mutated *in vitro* or *in vivo*, to create and/or destroy translation, initiation, and/or termination sequences, or to create variations in coding regions and/or form new restriction sites or destroy preexisting ones, or  
25 to facilitate further *in vitro* modification. (See Example 6). Any technique for mutagenesis known in the art can be used, including but not limited to, *in vitro* site-directed mutagenesis. (Hutchinson et al., *J. Biol. Chem.*, 253:6551 (1978)).

Further, nucleic acids encoding other proteins or domains of other proteins can be joined to nucleic acids encoding an HCV peptide so as to create a fusion protein. Nucleotides encoding  
30 fusion proteins can include, but are not limited to, a full length NS3/4A sequence (SEQ. ID. NO.: 16), a truncated NS3/4A sequence or a peptide fragment of an NS3/4A sequence fused to an unrelated protein or peptide, such as for example, poly histidine, hemagglutinin, an enzyme, fluorescent protein, or luminescent protein, as discussed below.

Surprisingly, it was discovered that the NS3-pVAX and NS3/4A-pVAX vectors were capable of eliciting a potent immune response when injected into an immunocompetent mammal. The example below describes these experiments in greater detail.

#### EXAMPLE 7

5 To determine whether a humoral immune response was elicited by the NS3-pVAX and NS3/4A-pVAX vectors, the expression constructs described in *Example 6* were purified using the Qiagen DNA purification system, according to the manufacturer's instructions and the purified DNA vectors were used to immunize groups of four to ten Balb/c mice. The plasmids were injected directly into regenerating tibialis anterior (TA) muscles as previously described (Davis et al.,  
10 *Human Gene Therapy* 4(6):733 (1993)). In brief, mice were injected intramuscularly with 50  $\mu$ l/TA of 0.01mM cardiotoxin (Latoxan, Rosans, France) in 0.9% sterile NaCl. Five days later, each TA muscle was injected with 50  $\mu$ l PBS containing either rNS3 or DNA.

Inbred mouse strains C57/BL6 (H-2b) Balb/C (H-2d), and CBA (H-2k) were obtained from the breeding facility at Møllegaard Denmark, Charles River Uppsala, Sweden, or B&K Sollentuna  
15 Sweden. All mice were female and were used at 4-8 weeks of age. For monitoring of humoral responses, all mice received a booster injection of 50  $\mu$ l /TA of plasmid DNA every fourth week. In addition, some mice were given recombinant NS3 (rNS3) protein, which was purified as described herein. The mice receiving rNS3 were immunized no more than twice. All mice were bled twice a month.

20 Enzyme immunosorbent assays (EIAs) were used to detect the presence of murine NS3 antibodies. These assays were performed essentially as described in (Chen et al., *Hepatology* 28(1): 219 (1998)). Briefly, rNS3 was passively adsorbed overnight at 4°C to 96-well microtiter plates (Nunc, Copenhagen, Denmark) at 1  $\mu$ g/ml in 50 mM sodium carbonate buffer (pH 9.6). The plates were then blocked by incubation with dilution buffer containing PBS, 2% goat serum, and  
25 1% bovine serum albumin for one hour at 37°C. Serial dilutions of mouse sera starting at 1:60 were then incubated on the plates for one hour. Bound murine serum antibodies were detected by an alkaline phosphatase conjugated goat anti-mouse IgG (Sigma Cell Products, Saint Louis, MO) followed by addition of the substrate pNPP (1 tablet/5ml of 1M Diethanol amine buffer with 0.5 mM MgCl<sub>2</sub>). The reaction was stopped by addition of 1M NaOH and absorbency was read at 405  
30 nm.

After four weeks, four out of five mice immunized with NS3/4A-pVAX had developed NS3 antibodies, whereas one out of five immunized with NS3-pVAX had developed antibodies (**FIGURE 4**). After six weeks, four out of five mice immunized with NS3/4A-pVAX had developed high levels ( $>10^4$ ) of NS3 antibodies (mean levels  $10800 \pm 4830$ ) and one had a titer of  
35 2160. Although all mice immunized with NS3-pVAX developed NS3 antibodies, none of them

developed levels as high as that produced by the NS3/4A-pVAX construct (mean levels  $1800 \pm 805$ ). The antibody levels elicited by the NS3/4A fusion construct were significantly higher than those induced by NS3-pVAX at six weeks (mean ranks 7.6 v.s 3.4,  $p < 0.05$ , Mann-Whitney rank sum test, and  $p < 0.01$ , Students t-test). Thus, immunization with either NS3-pVAX or NS3/4A-pVAX resulted in the production of anti-NS3 antibodies, but the NS3/4A fusion gene was a more potent immunogen. The example below describes experiments that were performed to determine if the NS3/4A-TPT-pVAX construct could elicit a potent immune response.

#### EXAMPLE 8

To test if the enhanced immunogenicity of NS3/4A could be solely attributed to the presence of NS4A, or if the NS3/4A fusion protein in addition had to be cleaved at the NS3/4A junction, new experiments were performed. In a first experiment, the immunogenicity of the NS3-pVAX, NS3/4A-pVAX, and NS3/4A-TPT-pVAX vectors were compared in Balb/c mice. Mice were immunised on week 0 as described above, and, after two weeks, all mice were bled and the presence of antibodies to NS3 at a serum dilution of 1:60 was determined (TABLE 11). Mice were bled again on week 4. Although, the NS3/4A-TPT-pVAX vector was comparable to the NS3-pVAX vector (4/10 vs. 0/10; NS, Fisher's exact test), the NS3/4A-pVAX vector continued to be the most potent immunogen. Thus, all of the HCV constructs that were introduced into mice were capable of eliciting an immune response against NS3, however, the NS4A sequence and a functional proteolytic cleavage site between the NS3 and NS4A sequences provided for a more potent immune response.

TABLE 11

Weeks from 1 <sup>st</sup> immunization	No. of antibody responders to the respective immunogen after one 100 $\mu$ g <i>i.m</i> immunization		
	NS3-pVAX	NS3/4A-pVAX	NS3/4A-TPT-pVAX
2	0/10	17/20	4/10
4	0/10 ( $<60$ )	20/20 ( $2415 \pm 3715$ ) 55% $> 10^3$ 10% $> 10^4$	10/10 ( $390 \pm 639$ ) 50% $> 10^2$ 10% $> 10^3$

During the chronic phase of infection, HCV replicates in hepatocytes, and spreads within the liver. A major factor in combating chronic and persistent viral infections is the cell-mediated immune defense system. CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes infiltrate the liver during the chronic



phase of HCV infection, but they are incapable of clearing the virus or preventing liver damage. In addition, persistent HCV infection is associated with the onset of hepatocellular carcinoma (HCC). The examples below describe experiments that were performed to determine whether the NS3 and NS3/4A construct were capable of eliciting a T-cell mediated immune response against NS3.

5

## EXAMPLE 9

To study whether the constructs described above were capable of eliciting a cell-mediated response against NS3, an *in vivo* tumor growth assay was performed. To this end, an SP2/0 tumor cell line stably transfected with the NS3/4A gene was made. The pcDNA3.1 plasmid containing the NS3/4A gene was linearized by BglII digestion. A total of 5µg linearized plasmid DNA was mixed with 60µg transfection reagent (Superfect, Qiagen, Germany) and the mixture was added to a 50% confluent layer of SP2/0 cells in a 35 mm dish. The transfected SP2/0 cells (NS3/4A-SP2/0) were grown for 14 days in the presence of 800µg/ml geneticin and individual clones were isolated. A stable NS3/4A-expressing SP2/0 clone was identified using PCR and RTPCR. The cloned cell line was maintained in DMEM containing 10% fetal bovine serum, L-glutamine, and penicillin-streptomycin.

The *in vivo* growth kinetics of the SP2/0 and the NS3/4A-SP2/0 cell lines were then evaluated in Balb/c mice. Mice were injected subcutaneously with  $2 \times 10^6$  tumor cells in the right flank. Each day the size of the tumor was determined through the skin. The growth kinetics of the two cell lines was comparable. For example, the mean tumor sizes did not differ between the two cell lines at any time point. (See TABLE 12). The example below describes experiments that were performed to determine whether mice immunized with the NS3/4A constructs had developed a T-cell response against NS3.

25

TABLE 12

Mouse ID	Tumor cell line	Maximum <i>in vivo</i> tumor size at indicated time point								
		5	6	7	8	11	12	13	14	15
1	SP2/0	1.6	2.5	4.5	6.0	10.0	10.5	11.0	12.0	12.0
2	SP2/0	1.0	1.0	2.0	3.0	7.5	7.5	8.0	11.5	11.5
3	SP2/0	2.0	5.0	7.5	8.0	11.0	11.5	12.0	12.0	13.0
4	SP2/0	4.0	7.0	8.0	10.0	13.0	15.0	16.5	16.5	17.0
5	SP2/0	1.0	1.0	3.0	4.0	5.0	6.0	6.0	6.0	7.0

Mouse ID	Tumor cell line	Maximum <i>in vivo</i> tumor size at indicated time point								
		5	6	7	8	11	12	13	14	15
Group mean		1,92	3.3	5.0	6.2	9.3	10.1	10.7	11.6	12.1
6	NS3/4A-SP2/0	1.0	2.0	3.0	3.5	4.0	5.5	6.0	7.0	8.0
7	NS3/4A-SP2/0	2.0	2.5	3.0	5.0	7.0	9.0	9.5	9.5	11.0
8	NS3/4A-SP2/0	1.0	2.0	3.5	3.5	9.5	11.0	12.0	14.0	14.0
9	NS3/4A-SP2/0	1.0	1.0	2.0	6.0	11.5	13.0	14.5	16.0	18.0
10	NS3/4A-SP2/0	3.5	6.0	7.0	10.5	15.0	15.0	15.0	15.5	20.0
Group mean		1,7	2.7	3.7	5.7	9.4	10.7	11.4	12.4	14.2
p-value of student's t-test comparison between group means		0,7736	0.6918	0.4027	0.7903	0.9670	0.7986	0.7927	0.7508	0.4623

## EXAMPLE 10

To examine whether a T-cell response is elicited by the NS3/4A immunization, the capacity of an immunized mouse's immune defense system to attack the NS3-expressing tumor cell line was assayed. The protocol for testing for *in vivo* inhibition of tumor growth of the SP2/0 myeloma cell line in Balb/c mice has been described in detail previously (Encke et al., *J. Immunol.* 161:4917 (1998)). Inhibition of tumor growth in this model is dependent on the priming of cytotoxic T lymphocytes (CTLs). Briefly, groups of ten mice were immunized *i.m.* five times with one month intervals with either 100µg NS3-pVAX or 100 µg NS3/4A-pVAX. Two weeks after the last immunization 2 x 10<sup>6</sup> SP2/0 or NS3/4A-SP2/0 cells were injected into the right flank of each mouse. Two weeks later the mice were sacrificed and the maximum tumor sizes were measured. There was no difference between the mean SP2/0 and NS3/4A-SP2/0 tumor sizes in the NS3-pVAX immunized mice (See TABLE 13).

TABLE 13

Mouse ID	Immunogen	Dose (µg)	Tumor cell line	Tumor growth	Maximum tumor size (mm)
1	NS3-pVAX	100	SP2/0	Yes	5
2	NS3-pVAX	100	SP2/0	Yes	15
3	NS3-pVAX	100	SP2/0	No	-
4	NS3-pVAX	100	SP2/0	Yes	6
5	NS3-pVAX	100	SP2/0	Yes	13
Group total				4/5	9.75±4.992
6	NS3-pVAX	100	NS3/4A-SP2/0	Yes	9
7	NS3-pVAX	100	NS3/4A-SP2/0	Yes	8
8	NS3-pVAX	100	NS3/4A-SP2/0	Yes	7
9	NS3-pVAX	100	NS3/4A-SP2/0	No	-
10	NS3-pVAX	100	NS3/4A-SP2/0	No	-
				3/5	8.00±1.00

Note: Statistical analysis (StatView): Student's t-test on maximum tumor size. P-values < 0.05 are considered significant.

5

**Unpaired t-test for Max diam**  
**Grouping Variable: Column 1**  
**Hypothesized Difference = 0**  
**Row exclusion: NS3DNA-Tumor-001213**

10

	Mean Diff.	DF	t-Value	P-Value
NS3-sp2, NS3-spNS3	1.750	5	0.58	0.584

15

**Group Info for Max diam**  
**Grouping Variable: Column 1**  
**Row exclusion: NS3DNA-Tumor-001213**

	Count	Mean	Variance	Std. Dev.	Std. Err
NS3-sp2	4	9.750	24.917	4.992	2.496
NS3-spNS3	3	8.000	1.000	1.000	0.57

20

In the next set of experiments, the inhibition of SP2/0 or NS3/4A-SP2/0 tumor growth was evaluated in NS3/4A-pVAX immunized Balb/c mice. In mice immunized with the NS3/4A-pVAX plasmid the growth of NS3/4A-SP2/0 tumor cells was significantly inhibited as compared to growth of the non-transfected SP2/0 cells. (See TABLE 14). Thus, NS3/4A-pVAX immunization elicits CTLs that inhibit growth of cells expressing NS3/4A *in vivo*. The example below describes experiments that were performed to analyze the efficiency of various NS3 containing compositions in eliciting a cell-mediated response to NS3.

25

TABLE 14

Mouse ID	Immunogen	Dose (μg)	Tumor cell line	Tumor growth	Maximum tumor size (mm)
11	NS3/4A-pVAX	100	SP2/0	No	-
12	NS3/4A-pVAX	100	SP2/0	Yes	24
13	NS3/4A-pVAX	100	SP2/0	Yes	9
14	NS3/4A-pVAX	100	SP2/0	Yes	11
15	NS3/4A-pVAX	100	SP2/0	Yes	25
				4/5	17.25±8.421
16	NS3/4A-pVAX	100	NS3/4A-SP2/0	No	-
17	NS3/4A-pVAX	100	NS3/4A-SP2/0	Yes	9
18	NS3/4A-pVAX	100	NS3/4A-SP2/0	Yes	7
19	NS3/4A-pVAX	100	NS3/4A-SP2/0	Yes	5
20	NS3/4A-pVAX	100	NS3/4A-SP2/0	Yes	4
				4/5	6.25±2.217

Note: Statistical analysis (StatView): Student's t-test on maximum tumor size. P-values < 0.05 are considered significant.

5

#### Unpaired t-test for Max diam

Grouping Variable: Column 1

Hypothesized Difference = 0

10

Row exclusion: NS3DNA-Tumor-001213

	Mean Diff.	DF	t-Value	P-Value
NS3/4-sp2, NS3/4-spNS3	11.000	6	2.526	0.044

#### Group Info for Max diam

15

Grouping Variable: Column 1

Row exclusion: NS3DNA-Tumor-001213

	Count	Mean	Variance	Std. Dev.	Std. Err
NS3/4-sp2	4	17.250	70.917	8.421	4.211
NS3/4-spNS3	4	6.250	4.917	2.217	1.109

20

#### EXAMPLE 11

To analyze whether administration of different NS3 containing compositions affected the elicitation of a cell-mediated immune response, mice were immunized with PBS, rNS3, irrelevant DNA or the NS3/4A construct, and tumor sizes were determined, as described above. Only the NS3/4A construct was able to elicit a T-cell response sufficient to cause a statistically significant reduction in tumor size (See TABLE 15). The example below describes experiments that were performed to determine whether the reduction in tumor size can be attributed to the generation of NS3-specific T-lymphocytes.

25

TABLE 15

Mouse ID	Immunogen	Dose (µg)	Tumor cell line	Anti-NS3	Tumor growth	Maximum tumor size (mm)
1	NS3-pVAX	10	NS3/4A-SP2/0	<60	+	12.0
2	NS3-pVAX	10	NS3/4A-SP2/0	<60	+	20.0
3	NS3-pVAX	10	NS3/4A-SP2/0	60	+	18.0
4	NS3-pVAX	10	NS3/4A-SP2/0	<60	+	13.0
5	NS3-pVAX	10	NS3/4A-SP2/0	<60	+	17.0
Group mean				60	5/5	16.0±3.391
6	NS3-pVAX	100	NS3/4A-SP2/0	2160	+	10.0
7	NS3-pVAX	100	NS3/4A-SP2/0	<60	-	-
8	NS3-pVAX	100	NS3/4A-SP2/0	<60	-	-
9	NS3-pVAX	100	NS3/4A-SP2/0	360	-	-
10	NS3-pVAX	100	NS3/4A-SP2/0	<60	+	12.5
Group mean				1260	2/5	11.25±1.768
11	NS3/4A-pVAX	10	NS3/4A-SP2/0	<60	+	10.0
12	NS3/4A-pVAX	10	NS3/4A-SP2/0	<60	-	-
13	NS3/4A-pVAX	10	NS3/4A-SP2/0	<60	-	-
14	NS3/4A-pVAX	10	NS3/4A-SP2/0	<60	+	13.0
15	NS3/4A-pVAX	10	NS3/4A-SP2/0	<60	+	13.5
Group mean				<60	3/5	12.167±1.893
16	NS3/4A-pVAX	100	NS3/4A-SP2/0	60	+	10.0
17	NS3/4A-pVAX	100	NS3/4A-SP2/0	360	-	-
18	NS3/4A-pVAX	100	NS3/4A-SP2/0	2160	+	8.0
19	NS3/4A-pVAX	100	NS3/4A-SP2/0	2160	+	12.0
20	NS3/4A-pVAX	100	NS3/4A-SP2/0	2160	+	7.0
Group mean				1380	4/5	9.25±2.217
36	p17-pcDNA3	100	NS3/4A-SP2/0	<60	+	20.0
37	p17-pcDNA3	100	NS3/4A-SP2/0	<60	+	7.0
38	p17-pcDNA3	100	NS3/4A-SP2/0	<60	+	11.0
39	p17-pcDNA3	100	NS3/4A-SP2/0	<60	+	15.0
40	p17-pcDNA3	100	NS3/4A-SP2/0	<60	+	18.0
Group mean				<60	5/5	14.20±5.263
41	rNS3/CFA	20	NS3/4A-SP2/0	>466560	+	13.0
42	rNS3/CFA	20	NS3/4A-SP2/0	>466560	-	-
43	rNS3/CFA	20	NS3/4A-SP2/0	>466560	+	3.5
44	rNS3/CFA	20	NS3/4A-SP2/0	>466560	+	22.0
45	rNS3/CFA	20	NS3/4A-SP2/0	>466560	+	17.0
Group mean				466560	4/5	17.333±4.509
46	PBS	-	NS3/4A-SP2/0	<60	+	10.0
47	PBS	-	NS3/4A-SP2/0	<60	+	16.5
48	PBS	-	NS3/4A-SP2/0	60	+	15.0
49	PBS	-	NS3/4A-SP2/0	<60	+	21.0
50	PBS	-	NS3/4A-SP2/0	<60	+	15.0
51	PBS	-	NS3/4A-SP2/0	<60	-	-
Group mean				60	5/6	15.50±3.937

Note: Statistical analysis (StatView): Student's t-test on maximum tumor size. P-values < 0.05 are considered as significant.

Unpaired t-test for Largest Tumor size  
 Grouping Variable: group  
 Hypothesized Difference = 0

	Mean Diff.	DF	t-Value	P-Value
p17-sp3-4, NS3-100-sp3-4	2.950	5	.739	.4933
p17-sp3-4, NS3/4-10-sp3-4	2.033	6	.628	.5532
p17-sp3-4, NS3-10-sp3-4	-1.800	8	-.643	.5383
p17-sp3-4, NS3/4-100-sp3-4	4.950	7	1.742	.1250
p17-sp3-4, PBS-sp3-4	-1.300	8	-.442	.6700
p17-sp3-4, rNS3-sp3-4	-3.133	6	-.854	.4259
NS3-100-sp3-4, NS3/4-10-sp3-4	-.917	3	-.542	.6254
NS3-100-sp3-4, NS3-10-sp3-4	-4.750	5	-1.811	.1299
NS3-100-sp3-4, NS3/4-100-sp3-4	2.000	4	1.092	.3360
NS3-100-sp3-4, PBS-sp3-4	-4.250	5	-1.408	.2183
NS3-100-sp3-4, rNS3-sp3-4	-6.083	3	-1.744	.1795
NS3/4-10-sp3-4, NS3-10-sp3-4	-3.833	6	-1.763	.1283
NS3/4-10-sp3-4, NS3/4-100-sp3-4	2.917	5	1.824	.1277
NS3/4-10-sp3-4, PBS-sp3-4	-3.333	6	-1.344	.2274
NS3/4-10-sp3-4, rNS3-sp3-4	-5.167	4	-1.830	.1412
NS3-10-sp3-4, NS3/4-100-sp3-4	6.750	7	3.416	.0112
NS3-10-sp3-4, PBS-sp3-4	.500	8	.215	.8350
NS3-10-sp3-4, rNS3-sp3-4	-1.333	6	-.480	.6480
NS3/4-100-sp3-4, PBS-sp3-4	-6.250	7	-2.814	.0260
NS3/4-100-sp3-4, rNS3-sp3-4	-8.083	5	-3.179	.0246
PBS-sp3-4, rNS3-sp3-4	-1.833	6	-.607	.5662

#### EXAMPLE 12

- 5 To determine whether NS3-specific T-cells were elicited by the NS3/4A immunizations, an *in vitro* T-cell mediated tumor cell lysis assay was employed. The assay has been described in detail previously (Townsend et al., *J. Virol.* 71:3365 (1997)). Briefly, groups of five Balb/c mice were immunized three times with 100µg NS3/4A-pVAX *i.m.* Two weeks after the last injection the mice were sacrificed and splenocytes were harvested. Re-stimulation cultures with 3 x 10<sup>6</sup>
- 10 splenocytes and 3 x 10<sup>6</sup> NS3/4A-SP2/0 cells were set. After five days, a standard Cr<sup>51</sup>-release assay was performed using NS3/4A-SP2/0 or SP2/0 cells as targets. Percent specific lysis was calculated as the ratio between lysis of NS3/4A-SP2/0 cells and lysis of SP2/0 cells. Only mice immunized with NS3/4A-pVAX displayed specific lysis over 10% in four out of five tested mice, using an effector to target ratio of 20:1 (See FIGURES 5A and B). Accordingly, mice immunized
- 15 with NS3/4A exhibited a reduction in cancer cell proliferation and/or NS3/4A caused the lysis of cancer cells. The section below describes several of the embodied HCV polypeptides in greater detail.

*HCV peptides*

The nucleic acids encoding the HCV peptides, described in the previous section, can be manipulated using conventional techniques in molecular biology so as to create recombinant constructs that express the HCV peptides. The embodied HCV peptides or derivatives thereof, include but are not limited to, those containing as a primary amino acid sequence all of the amino acid sequence substantially as depicted in the Sequence Listing (SEQ. ID. NOs.: 17, 29- 32 and 43-49) and fragments thereof at least four amino acids in length (e.g., SEQ. ID. NOs.: 25-27, and 33-42) including altered sequences in which functionally equivalent amino acid residues are substituted for residues within the sequence resulting in a silent change. Preferred fragments of a sequence of SEQ. ID. NOs.: 17, 29- 32 and 43-49 are at least four amino acids and comprise amino acid sequence unique to the discovered NS3/4A peptide (SEQ. ID. NO.: 17) including altered sequences in which functionally equivalent amino acid residues are substituted for residues within the sequence resulting in a silent change. The HCV peptides can be, for example, at least 12-15, 15-20, 20-25, 25-50, 50-100, 100-150, 150-250, 250-500 or 500-704 amino acids in length. Other fragments (e.g., SEQ. ID. NOs.: 25-27, and 33-42) are also aspects of the invention.

Embodiments of the invention also include HCV peptides that are substantially identical to those described above. That is, HCV peptides that have one or more amino acid residues within SEQ. ID. NO.: 17 and fragments thereof that are substituted by another amino acid of a similar polarity that acts as a functional equivalent, resulting in a silent alteration. Substitutes for an amino acid within the sequence can be selected from other members of the class to which the amino acid belongs. For example, the non-polar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine and glutamine. The positively charged (basic) amino acids include arginine, lysine, and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. The aromatic amino acids include phenylalanine, tryptophan, and tyrosine.

The HCV peptides described herein can be prepared by chemical synthesis methods (such as solid phase peptide synthesis) using techniques known in the art such as those set forth by Merrifield et al., *J. Am. Chem. Soc.* 85:2149 (1964), Houghten et al., *Proc. Natl. Acad. Sci. USA*, 82:51:32 (1985), Stewart and Young (Solid phase peptide synthesis, Pierce Chem Co., Rockford, IL (1984), and Creighton, 1983, Proteins: Structures and Molecular Principles, W. H. Freeman & Co., N.Y. Such polypeptides can be synthesized with or without a methionine on the amino terminus. Chemically synthesized HCV peptides can be oxidized using methods set forth in these references to form disulfide bridges.

While the HCV peptides described herein can be chemically synthesized, it can be more effective to produce these polypeptides by recombinant DNA technology. Such methods can be used to construct expression vectors containing the HCV nucleotide sequences described above, for example, and appropriate transcriptional and translational control signals. These methods include, 5 for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Alternatively, RNA capable of encoding HCV nucleotide sequences can be chemically synthesized using, for example, synthesizers. See, for example, the techniques described in Oligonucleotide Synthesis, 1984, Gait, M. J. ed., IRL Press, Oxford. Accordingly, several embodiments concern cell lines that have been engineered to express the embodied HCV 10 peptides. For example, some cells are made to express the HCV peptides of (SEQ. ID. NOs.: 17, 29- 32 and 43-49) or fragments of these molecules.

A variety of host-expression vector systems can be utilized to express the embodied HCV peptides. Suitable expression systems include, but are not limited to, microorganisms such as bacteria (e.g., *E. coli* or *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid 15 DNA or cosmid DNA expression vectors containing HCV nucleotide sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing the HCV nucleotide sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing the HCV sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or 20 transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing HCV sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter).

25 In bacterial systems, a number of expression vectors can be advantageously selected depending upon the use intended for the HCV gene product being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of HCV peptide or for raising antibodies to the HCV peptide, for example, vectors which direct the expression of high levels of fusion protein products that are readily purified can be 30 desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., *EMBO J.*, 2:1791 (1983), in which the HCV coding sequence can be ligated individually into the vector in frame with the lacZ coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, *Nucleic Acids Res.*, 13:3101-3109 (1985); Van Heeke & Schuster, *J. Biol. Chem.*, 264:5503-5509 (1989)); and the like. pGEX vectors can also be used to 35 express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general,



such fusion proteins are soluble and can be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The PGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

5 In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The HCV coding sequence can be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of an HCV gene coding sequence will result in inactivation of the  
10 polyhedrin gene and production of non-occluded recombinant virus, (i.e., virus lacking the proteinaceous coat coded for by the polyhedrin gene). These recombinant viruses are then used to infect *Spodoptera frugiperda* cells in which the inserted gene is expressed. (See e.g., Smith et al., *J. Virol.* 46: 584 (1983); and Smith, U.S. Pat. No. 4,215,051).

In mammalian host cells, a number of viral-based expression systems can be utilized. In  
15 cases where an adenovirus is used as an expression vector, the HCV nucleotide sequence of interest can be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene can then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing  
20 the HCV gene product in infected hosts. (See e.g., Logan & Shenk, *Proc. Natl. Acad. Sci. USA* 81:3655-3659 (1984)). Specific initiation signals can also be required for efficient translation of inserted HCV nucleotide sequences. These signals include the ATG initiation codon and adjacent sequences.

However, in cases where only a portion of the HCV coding sequence is inserted,  
25 exogenous translational control signals, including, perhaps, the ATG initiation codon, can be provided. Furthermore, the initiation codon can be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of appropriate transcription enhancer  
30 elements, transcription terminators, etc. (See Bittner et al., *Methods in Enzymol.*, 153:516-544 (1987)).

In addition, a host cell strain can be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products are important  
35 for the function of the protein. Different host cells have characteristic and specific mechanisms for

the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells that possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product  
5 can be used. Such mammalian host cells include, but are not limited to, CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, and WI38.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines that stably express the HCV peptides described above can be engineered. Rather than using expression vectors that contain viral origins of replication, host cells  
10 can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells are allowed to grow for 1-2 days in an enriched media; and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably  
15 integrate the plasmid into their chromosomes and grow to form foci which in turn are cloned and expanded into cell lines. This method is advantageously used to engineer cell lines which express the HCV gene product.

A number of selection systems can be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler, et al., *Cell* 11:223 (1977)), hypoxanthine-guanine  
20 phosphoribosyltransferase (Szybalska & Szybalski, *Proc. Natl. Acad. Sci. USA* 48:2026 (1962)), and adenine phosphoribosyltransferase (Lowy, et al., *Cell* 22:817 (1980)) genes can be employed in tk<sup>-</sup>, hgp<sup>r</sup>t<sup>-</sup> or ap<sup>r</sup>t<sup>-</sup> cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler, et al.,  
25 *Proc. Natl. Acad. Sci. USA* 77:3567 (1980); O'Hare, et al., *Proc. Natl. Acad. Sci. USA* 78:1527 (1981); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, *Proc. Natl. Acad. Sci. USA* 78:2072 (1981); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin, et al., *J. Mol. Biol.* 150:1 (1981); and hyg<sup>r</sup>, which confers resistance to hygromycin (Santerre, et al., *Gene* 30:147 (1984)).

Alternatively, any fusion protein can be readily purified by utilizing an antibody specific  
30 for the fusion protein being expressed. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines. (Janknecht, et al., *Proc. Natl. Acad. Sci. USA* 88: 8972-8976 (1991)). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the gene's open reading frame is translationally fused to an amino-terminal tag consisting of six histidine residues. Extracts from  
35 cells infected with recombinant vaccinia virus are loaded onto Ni<sup>2+</sup>-nitriloacetic acid-agarose

columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers. The example below describes a method that was used to express the HCV peptides encoded by the embodied nucleic acids.

#### EXAMPLE 13

5 To characterize the NS3/4A fusion protein, and the truncated and mutated versions thereof, the vector constructs, described in *Example 6*, were transcribed and translated *in vitro*, and the resulting polypeptides were visualized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). *In vitro* transcription and translation were performed using the T7 coupled reticulocyte lysate system (Promega, Madison, WI) according to the manufacturer's  
10 instructions. All *in vitro* translation reactions of the expression constructs were carried out at 30°C with <sup>35</sup>S-labeled methionine (Amersham International, Plc, Buckinghamshire, UK). The labeled proteins were separated on 12% SDS-PAGE gels and visualized by exposure to X-ray film (Hyper Film-MP, Amersham) for 6-18 hours.

The *in vitro* analysis revealed that all proteins were expressed to high amounts from their  
15 respective expression constructs. The rNS3 construct (NS3-pVAX vector) produced a single peptide of approximately 61kDa, whereas, the TPT construct (NS3/4A-TPT-pVAX) and the RGT construct (NS3/4A-RGT-pVAX) produced a single polypeptide of approximately 67 kDa, which is identical to the molecular weight of the uncleaved NS3/4A peptide produced from the NS3/4A-pVAX construct. The cleaved product produced from the expressed NS3/4A peptide was  
20 approximately 61 kDa, which was identical in size to the rNS3 produced from the NS3-pVAX vector. These results demonstrated that the expression constructs were functional, the NS3/4A construct was enzymatically active, the rNS3 produced a peptide of the predicted size, and the TPT and RGT mutations completely abolished cleavage at the NS3-NS4A junction.

25 The sequences, constructs, vectors, clones, and other materials comprising the embodied HCV nucleic acids and peptides can be in enriched or isolated form. As used herein, "enriched" means that the concentration of the material is at least about 2, 5, 10, 100, or 1000 times its natural concentration (for example), advantageously 0.01%, by weight, preferably at least about 0.1% by weight. Enriched preparations from about 0.5%, 1%, 5%, 10%, and 20% by weight are also  
30 contemplated. The term "isolated" requires that the material be removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide present in a living animal is not isolated, but the same polynucleotide, separated from some or all of the coexisting materials in the natural system, is isolated. It is also advantageous that the sequences be in purified form. The term "purified" does not require absolute  
35 purity; rather, it is intended as a relative definition. Isolated proteins have been conventionally

purified to electrophoretic homogeneity by Coomassie staining, for example. Purification of starting material or natural material to at least one order of magnitude, preferably two or three orders, and more preferably four or five orders of magnitude is expressly contemplated.

The HCV gene products described herein can also be expressed in plants, insects, and animals so as to create a transgenic organism. Desirable transgenic plant systems having an HCV peptide include *Arabidopsis*, maize, and *Chlamydomonas*. Desirable insect systems having an HCV peptide include, but are not limited to, *D. melanogaster* and *C. elegans*. Animals of any species, including, but not limited to, amphibians, reptiles, birds, mice, hamsters, rats, rabbits, guinea pigs, pigs, micro-pigs, goats, dogs, cats, and non-human primates, e.g., baboons, monkeys, and chimpanzees can be used to generate transgenic animals having an embodied HCV molecule. These transgenic organisms desirably exhibit germline transfer of HCV peptides described herein.

Any technique known in the art is preferably used to introduce the HCV transgene into animals to produce the founder lines of transgenic animals or to knock out or replace existing HCV genes. Such techniques include, but are not limited to pronuclear microinjection (Hoppe, P. C. and Wagner, T. E., 1989, U.S. Pat. No. 4,873,191); retrovirus mediated gene transfer into germ lines (Van der Putten et al., *Proc. Natl. Acad. Sci., USA* 82:6148-6152 (1985); gene targeting in embryonic stem cells (Thompson et al., *Cell* 56:313-321 (1989); electroporation of embryos (Lo, *Mol Cell. Biol.* 3:1803-1814 (1983); and sperm-mediated gene transfer (Lavitrano et al., *Cell* 57:717-723 (1989); see also Gordon, *Transgenic Animals, Intl. Rev. Cytol.* 115:171-229 (1989). The section below describes the manufacture of antibodies that interact with the HCV peptides described herein.

#### *Anti-HCV antibodies*

Following synthesis or expression and isolation or purification of the HCV peptides, the isolated or purified peptide can be used to generate antibodies. Depending on the context, the term "antibodies" can encompass polyclonal, monoclonal, chimeric, single chain, Fab fragments and fragments produced by a Fab expression library. Antibodies that recognize the HCV peptides have many uses including, but not limited to, biotechnological applications, therapeutic/prophylactic applications, and diagnostic applications.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, and humans etc. can be immunized by injection with an HCV peptide. Depending on the host species, various adjuvants can be used to increase immunological response. Such adjuvants include, but are not limited to, ribavirin, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. BCG (Bacillus Calmette-Guerin) and *Corynebacterium parvum* are also potentially useful adjuvants.

Peptides used to induce specific antibodies can have an amino acid sequence consisting of at least four amino acids, and preferably at least 10 to 15 amino acids. By one approach, short stretches of amino acids encoding fragments of NS3/4A are fused with those of another protein such as keyhole limpet hemocyanin such that an antibody is produced against the chimeric molecule. Additionally, a composition comprising ribavirin and NS3/4A (SEQ. ID. NO.: 17), a fragment thereof at least 4, 6, 8, 10, 12, 15, or 20 amino acids in length, or a nucleic acid encoding one or more of these molecules is administered to an animal. While antibodies capable of specifically recognizing HCV can be generated by injecting synthetic 3-mer, 10-mer, and 15-mer peptides that correspond to an HCV peptide into mice, a more diverse set of antibodies can be generated by using recombinant HCV peptides, prepared as described above.

To generate antibodies to an HCV peptide, substantially pure peptide is isolated from a transfected or transformed cell. The concentration of the peptide in the final preparation is adjusted, for example, by concentration on an Amicon filter device, to the level of a few micrograms/ml. Monoclonal or polyclonal antibody to the peptide of interest can then be prepared as follows:

Monoclonal antibodies to an HCV peptide can be prepared using any technique that provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique originally described by Koehler and Milstein (*Nature* 256:495-497 (1975)), the human B-cell hybridoma technique (Kosbor et al. *Immunol Today* 4:72 (1983); Cote et al *Proc Natl Acad Sci* 80:2026-2030 (1983)), and the EBV-hybridoma technique Cole et al. Monoclonal Antibodies and Cancer Therapy, Alan R. Liss Inc, New York N.Y., pp 77-96 (1985). In addition, techniques developed for the production of "chimeric antibodies", the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity can be used. (Morrison et al. *Proc Natl Acad Sci* 81:6851-6855 (1984); Neuberger et al. *Nature* 312:604-608(1984); Takeda et al. *Nature* 314:452-454(1985). Alternatively, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,946,778) can be adapted to produce HCV-specific single chain antibodies. Antibodies can also be produced by inducing *in vivo* production in the lymphocyte population or by screening recombinant immunoglobulin libraries or panels of highly specific binding reagents as disclosed in Orlandi et al., *Proc Natl Acad Sci* 86: 3833-3837 (1989), and Winter G. and Milstein C; *Nature* 349:293-299 (1991).

Antibody fragments that contain specific binding sites for an HCV peptide can also be generated. For example, such fragments include, but are not limited to, the F(ab')<sub>2</sub> fragments that can be produced by pepsin digestion of the antibody molecule and the Fab fragments that can be generated by reducing the disulfide bridges of the F(ab')<sub>2</sub> fragments. Alternatively, Fab expression

libraries can be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. (Huse W. D. et al. *Science* 256:1275-1281 (1989)).

By one approach, monoclonal antibodies to an HCV peptide are made as follows. Briefly, a mouse is repetitively inoculated with a few micrograms of the selected protein or peptides derived therefrom over a period of a few weeks. The mouse is then sacrificed, and the antibody producing cells of the spleen isolated. The spleen cells are fused in the presence of polyethylene glycol with mouse myeloma cells, and the excess unfused cells destroyed by growth of the system on selective media comprising aminopterin (HAT media). The successfully fused cells are diluted and aliquots of the dilution placed in wells of a microtiter plate where growth of the culture is continued. Antibody-producing clones are identified by detection of antibody in the supernatant fluid of the wells by immunoassay procedures, such as ELISA, as originally described by Engvall, E., *Meth. Enzymol.* 70:419 (1980), and derivative methods thereof. Selected positive clones can be expanded and their monoclonal antibody product harvested for use. Detailed procedures for monoclonal antibody production are described in Davis, L. et al. Basic Methods in Molecular Biology Elsevier, New York. Section 21-2.

Polyclonal antiserum containing antibodies to heterogenous epitopes of a single protein can be prepared by immunizing suitable animals with the expressed protein or peptides derived therefrom described above, which can be unmodified or modified to enhance immunogenicity. Effective polyclonal antibody production is affected by many factors related both to the antigen and the host species. For example, small molecules tend to be less immunogenic than others and can require the use of carriers and adjuvant. Also, host animals vary in response to site of inoculations and dose, with both inadequate or excessive doses of antigen resulting in low titer antisera. Small doses (ng level) of antigen administered at multiple intradermal sites appears to be most reliable. An effective immunization protocol for rabbits can be found in Vaitukaitis, J. et al. *J. Clin. Endocrinol. Metab.* 33:988-991 (1971).

Booster injections are given at regular intervals, and antiserum harvested when antibody titer thereof, as determined semi-quantitatively, for example, by double immunodiffusion in agar against known concentrations of the antigen, begins to fall. See, for example, Ouchterlony, O. et al., Chap. 19 in: Handbook of Experimental Immunology D. Wier (ed) Blackwell (1973). Plateau concentration of antibody is usually in the range of 0.1 to 0.2 mg/ml of serum (about 12 $\mu$ M). Affinity of the antisera for the antigen is determined by preparing competitive binding curves, as described, for example, by Fisher, D., Chap. 42 in: Manual of Clinical Immunology, 2d Ed. (Rose and Friedman, Eds.) Amer. Soc. For Microbiol., Washington, D.C. (1980). Antibody preparations prepared according to either protocol are useful in quantitative immunoassays that determine concentrations of antigen-bearing substances in biological samples; they are also used semi-quantitatively or

qualitatively (e.g., in diagnostic embodiments that identify the presence of HCV in biological samples). The section below describes some of the diagnostic embodiments in greater detail.

*Diagnostic embodiments*

Generally, the embodied diagnostics are classified according to whether a nucleic acid or protein-based assay is used. Some diagnostic assays detect the presence or absence of an embodied HCV nucleic acid sequence in a sample obtained from a patient, whereas, other assays seek to identify whether an embodied HCV peptide is present in a biological sample obtained from a patient. Additionally, the manufacture of kits that incorporate the reagents and methods described herein that allow for the rapid detection and identification of HCV are also embodied. These diagnostic kits can include, for example, an embodied nucleic acid probe or antibody, which specifically detects HCV. The detection component of these kits will typically be supplied in combination with one or more of the following reagents. A support capable of absorbing or otherwise binding DNA, RNA, or protein will often be supplied. Available supports include membranes of nitrocellulose, nylon or derivatized nylon that can be characterized by bearing an array of positively charged substituents. One or more restriction enzymes, control reagents, buffers, amplification enzymes, and non-human polynucleotides like calf-thymus or salmon-sperm DNA can be supplied in these kits.

Useful nucleic acid-based diagnostics include, but are not limited to, direct DNA sequencing, Southern Blot analysis, dot blot analysis, nucleic acid amplification, and combinations of these approaches. The starting point for these analysis is isolated or purified nucleic acid from a biological sample obtained from a patient suspected of contracting HCV or a patient at risk of contracting HCV. The nucleic acid is extracted from the sample and can be amplified by RT-PCR and/or DNA amplification using primers that correspond to regions flanking the embodied HCV nucleic acid sequences (e.g., NS3/4A (SEQ. ID. NO.: 16)).

In some embodiments, nucleic acid probes that specifically hybridize with HCV sequences are attached to a support in an ordered array, wherein the nucleic acid probes are attached to distinct regions of the support that do not overlap with each other. Preferably, such an ordered array is designed to be "addressable" where the distinct locations of the probe are recorded and can be accessed as part of an assay procedure. These probes are joined to a support in different known locations. The knowledge of the precise location of each nucleic acid probe makes these "addressable" arrays particularly useful in binding assays. The nucleic acids from a preparation of several biological samples are then labeled by conventional approaches (e.g., radioactivity or fluorescence) and the labeled samples are applied to the array under conditions that permit hybridization.

If a nucleic acid in the samples hybridizes to a probe on the array, then a signal will be detected at a position on the support that corresponds to the location of the hybrid. Since the

identity of each labeled sample is known and the region of the support on which the labeled sample was applied is known, an identification of the presence of the polymorphic variant can be rapidly determined. These approaches are easily automated using technology known to those of skill in the art of high throughput diagnostic or detection analysis.

5           Additionally, an opposite approach to that presented above can be employed. Nucleic acids present in biological samples can be disposed on a support so as to create an addressable array. Preferably, the samples are disposed on the support at known positions that do not overlap. The presence of HCV nucleic acids in each sample is determined by applying labeled nucleic acid probes that complement nucleic acids, which encode HCV peptides, at locations on the array that  
10       correspond to the positions at which the biological samples were disposed. Because the identity of the biological sample and its position on the array is known, the identification of a patient that has been infected with HCV can be rapidly determined. These approaches are also easily automated using technology known to those of skill in the art of high throughput diagnostic analysis.

          Any addressable array technology known in the art can be employed. One particular  
15       embodiment of polynucleotide arrays is known as Genechips™, and has been generally described in US Patent 5,143,854; PCT publications WO 90/15070 and 92/10092. These arrays are generally produced using mechanical synthesis methods or light directed synthesis methods, which incorporate a combination of photolithographic methods and solid phase oligonucleotide synthesis. (Fodor et al., *Science*, 251:767-777, (1991)). The immobilization of arrays of oligonucleotides on  
20       solid supports has been rendered possible by the development of a technology generally identified as "Very Large Scale Immobilized Polymer Synthesis" (VLSPIS™) in which, typically, probes are immobilized in a high density array on a solid surface of a chip. Examples of VLSPIS™ technologies are provided in US Patents 5,143,854 and 5,412,087 and in PCT Publications WO 90/15070, WO 92/10092 and WO 95/11995, which describe methods for forming oligonucleotide  
25       arrays through techniques such as light-directed synthesis techniques. In designing strategies aimed at providing arrays of nucleotides immobilized on solid supports, further presentation strategies were developed to order and display the oligonucleotide arrays on the chips in an attempt to maximize hybridization patterns and diagnostic information. Examples of such presentation strategies are disclosed in PCT Publications WO 94/12305, WO 94/11530, WO 97/29212, and WO  
30       97/31256.

          A wide variety of labels and conjugation techniques are known by those skilled in the art and can be used in various nucleic acid assays. There are several ways to produce labeled nucleic acids for hybridization or PCR including, but not limited to, oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, a nucleic acid encoding  
35       an HCV peptide can be cloned into a vector for the production of an mRNA probe. Such vectors



are known in the art, are commercially available, and can be used to synthesize RNA probes *in vitro* by addition of an appropriate RNA polymerase such as T7, T3 or SP6 and labeled nucleotides. A number of companies such as Pharmacia Biotech (Piscataway N.J.), Promega (Madison Wis.), and U.S. Biochemical Corp (Cleveland Ohio) supply commercial kits and protocols for these procedures. Suitable reporter molecules or labels include those radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as, substrates, cofactors, inhibitors, magnetic particles and the like.

The presence of an HCV peptide in a protein sample obtained from a patient can also be detected by using conventional assays and the embodiments described herein. For example, antibodies that are immunoreactive with the disclosed HCV peptides can be used to screen biological samples for the presence of HCV infection. In preferred embodiments, antibodies that are reactive to the embodied HCV peptides are used to immunoprecipitate the disclosed HCV peptides from biological samples or are used to react with proteins obtained from a biological sample on Western or Immunoblots. Favored diagnostic embodiments also include enzyme-linked immunosorbant assays (ELISA), radioimmunoassays (RIA), immunoradiometric assays (IRMA) and immunoenzymatic assays (IEMA), including sandwich assays using monoclonal and/or polyclonal antibodies specific for the disclosed HCV peptides. Exemplary sandwich assays are described by David et al., in U.S. Patent Nos. 4,376,110 and 4,486,530. Other embodiments employ aspects of the immune-strip technology disclosed in U.S. Patent Nos. 5,290,678; 5,604,105; 5,710,008; 5,744,358; and 5,747,274.

In another preferred protein-based diagnostic, the antibodies described herein are attached to a support in an ordered array, wherein a plurality of antibodies are attached to distinct regions of the support that do not overlap with each other. As with the nucleic acid-based arrays, the protein-based arrays are ordered arrays that are designed to be "addressable" such that the distinct locations are recorded and can be accessed as part of an assay procedure. These probes are joined to a support in different known locations. The knowledge of the precise location of each probe makes these "addressable" arrays particularly useful in binding assays. For example, an addressable array can comprise a support having several regions to which are joined a plurality of antibody probes that specifically recognize HCV peptides present in a biological sample and differentiate the isotype of HCV identified herein.

By one approach, proteins are obtained from biological samples and are then labeled by conventional approaches (e.g., radioactivity, colorimetrically, or fluorescently). The labeled samples are then applied to the array under conditions that permit binding. If a protein in the sample binds to an antibody probe on the array, then a signal will be detected at a position on the support that corresponds to the location of the antibody-protein complex. Since the identity of

each labeled sample is known and the region of the support on which the labeled sample was applied is known, an identification of the presence, concentration, and/or expression level can be rapidly determined. That is, by employing labeled standards of a known concentration of HCV peptide, an investigator can accurately determine the protein concentration of the particular peptide in a tested sample and can also assess the expression level of the HCV peptide. Conventional methods in densitometry can also be used to more accurately determine the concentration or expression level of the HCV peptide. These approaches are easily automated using technology known to those of skill in the art of high throughput diagnostic analysis.

In another embodiment, an opposite approach to that presented above can be employed. Proteins present in biological samples can be disposed on a support so as to create an addressable array. Preferably, the protein samples are disposed on the support at known positions that do not overlap. The presence of an HCV peptide in each sample is then determined by applying labeled antibody probes that recognize epitopes specific for the HCV peptide. Because the identity of the biological sample and its position on the array is known, an identification of the presence, concentration, and/or expression level of an HCV peptide can be rapidly determined.

That is, by employing labeled standards of a known concentration of HCV peptide, an investigator can accurately determine the concentration of peptide in a sample and from this information can assess the expression level of the peptide. Conventional methods in densitometry can also be used to more accurately determine the concentration or expression level of the HCV peptide. These approaches are also easily automated using technology known to those of skill in the art of high throughput diagnostic analysis. As detailed above, any addressable array technology known in the art can be employed. The section below describes some of the compositions that can have one or more of the embodied HCV nucleic acids or HCV peptides.

*Compositions comprising the embodied HCV nucleic acids or peptides*

Some embodiments contain at least one of the HCV nucleic acids or peptides joined to a support. Preferably, these supports are manufactured so as to create a multimeric agent. These multimeric agents provide the HCV peptide or nucleic acid in such a form or in such a way that a sufficient affinity to the molecule is achieved. A multimeric agent having an HCV nucleic acid or peptide can be obtained by joining the desired molecule to a macromolecular support. A "support" can be termed a carrier, a protein, a resin, a cell membrane, or any macromolecular structure used to join or immobilize such molecules. Solid supports include, but are not limited to, the walls of wells of a reaction tray, test tubes, polystyrene beads, magnetic beads, nitrocellulose strips, membranes, microparticles such as latex particles, animal cells, Duracyte®, artificial cells, and others. An HCV nucleic acid or peptide can also be joined to inorganic carriers, such as silicon

oxide material (e.g., silica gel, zeolite, diatomaceous earth or aminated glass) by, for example, a covalent linkage through a hydroxy, carboxy or amino group and a reactive group on the carrier.

In several multimeric agents, the macromolecular support has a hydrophobic surface that interacts with a portion of the HCV nucleic acid or peptide by a hydrophobic non-covalent interaction. In some cases, the hydrophobic surface of the support is a polymer such as plastic or  
5 any other polymer in which hydrophobic groups have been linked such as polystyrene, polyethylene or polyvinyl. Additionally, HCV nucleic acid or peptide can be covalently bound to carriers including proteins and oligo/polysaccharides (e.g. cellulose, starch, glycogen, chitosane or aminated sepharose). In these later multimeric agents, a reactive group on the molecule, such as a  
10 hydroxy or an amino group, is used to join to a reactive group on the carrier so as to create the covalent bond. Additional multimeric agents comprise a support that has other reactive groups that are chemically activated so as to attach the HCV nucleic acid or peptide. For example, cyanogen bromide activated matrices, epoxy activated matrices, thio and thiopropyl gels, nitrophenyl chloroformate and N-hydroxy succinimide chloroformate linkages, or oxirane acrylic supports are  
15 used. (Sigma).

Carriers for use in the body, (i.e. for prophylactic or therapeutic applications) are desirably physiological, non-toxic and preferably, non-immunoresponsive. Suitable carriers for use in the body include poly-L-lysine, poly-D, L-alanine, liposomes, and Chromosorb® (Johns-Manville Products, Denver Co.). Ligand conjugated Chromosorb® (Synsorb-Pk) has been tested in humans  
20 for the prevention of hemolytic-uremic syndrome and was reported as not presenting adverse reactions. (*Armstrong et al. J. Infectious Diseases 171:1042-1045 (1995)*). For some embodiments, a "naked" carrier (i.e., lacking an attached HCV nucleic acid or peptide) that has the capacity to attach an HCV nucleic acid or peptide in the body of a organism is administered. By this approach, a "prodrug-type" therapy is envisioned in which the naked carrier is administered  
25 separately from the HCV nucleic acid or peptide and, once both are in the body of the organism, the carrier and the HCV nucleic acid or peptide are assembled into a multimeric complex.

The insertion of linkers, such as linkers (e.g., " $\lambda$  linkers" engineered to resemble the flexible regions of  $\lambda$  phage) of an appropriate length between the HCV nucleic acid or peptide and the support are also contemplated so as to encourage greater flexibility of the HCV peptide, hybrid,  
30 or binding partner and thereby overcome any steric hindrance that can be presented by the support. The determination of an appropriate length of linker that allows for an optimal cellular response or lack thereof, can be determined by screening the HCV nucleic acid or peptide with varying linkers in the assays detailed in the present disclosure.

A composite support comprising more than one type of HCV nucleic acid or peptide is also  
35 envisioned. A "composite support" can be a carrier, a resin, or any macromolecular structure used

to attach or immobilize two or more different HCV nucleic acids or peptides. As above, the insertion of linkers, such as  $\lambda$  linkers, of an appropriate length between the HCV nucleic acid or peptide and the support is also contemplated so as to encourage greater flexibility in the molecule and thereby overcome any steric hindrance that can occur. The determination of an appropriate length of linker that allows for an optimal cellular response or lack thereof, can be determined by screening the HCV nucleic acid or peptide with varying linkers in the assays detailed in the present disclosure.

In other embodiments, the multimeric and composite supports discussed above can have attached multimerized HCV nucleic acids or peptides so as to create a "multimerized-multimeric support" and a "multimerized-composite support", respectively. A multimerized ligand can, for example, be obtained by coupling two or more HCV nucleic acids or peptides in tandem using conventional techniques in molecular biology. The multimerized form of the HCV nucleic acid or peptide can be advantageous for many applications because of the ability to obtain an agent with a higher affinity, for example. The incorporation of linkers or spacers, such as flexible  $\lambda$  linkers, between the individual domains that make-up the multimerized agent can also be advantageous for some embodiments. The insertion of  $\lambda$  linkers of an appropriate length between protein binding domains, for example, can encourage greater flexibility in the molecule and can overcome steric hindrance. Similarly, the insertion of linkers between the multimerized HCV nucleic acid or peptide and the support can encourage greater flexibility and limit steric hindrance presented by the support. The determination of an appropriate length of linker can be determined by screening the HCV nucleic acids or peptides in the assays detailed in this disclosure.

Embodiments of the invention also include genetic vaccines, as described above. Preferably these compositions contain ribavirin and a nucleic acid encoding NS3/4A (SEQ. ID. NO.: 17), NS3 (SEQ. ID. NO.: 29), or a mutant (e.g., SEQ. ID. NOs.: 30 - 32 and 43-49) or a fragment thereof (e.g., SEQ. ID. NOs.: 25-27, and 33-42). The following example describes the preparation of a genetic vaccine suitable for use in humans.

#### EXAMPLE 14

An HCV expression plasmid is designed to express the NS3/4A peptide. The NS3/4A coding sequence of NS3/4A-pVAX is removed by digestion with *EcoRI* and *XbaI*, and the isolated fragment is inserted into plasmid A so that it is under the transcriptional control of the CMV promoter and the RSV enhancer element. (See U.S. Pat. No. 6,235,888 to Pachuk, et al.). Plasmid backbone A is 3969 base pairs in length; it contains a PBR origin of replication for replicating in *E. coli* and a kanamycin resistance gene. Inserts such as the NS3/4A, are cloned into a polylinker region, which places the insert between and operably linked to the promoter and polyadenylation signal. Transcription of the cloned inserts is under the control of the CMV promoter and the RSV

enhancer elements. A polyadenylation signal is provided by the presence of an SV40 poly A signal situated just 3' of the cloning site. An NS3/4A containing vaccine composition is then made by mixing 500µg of the rNS3/4A construct with 1mg of ribavirin.

Said vaccine composition can be used to raise antibodies in a mammal (e.g., mice or rabbits) or can be injected intramuscularly into a human so as to raise antibodies, preferably a human that is chronically infected with the HCV virus. The recipient preferably receives three immunization boosts of the mixture at 4-week intervals, as well. By the third boost, the titer of antibody specific for HCV will be significantly increased. Additionally, at this time, said subject will experience an enhanced antibody and T-cell mediated immune response against NS3, as evidenced by an increased fraction of NS3 specific antibodies as detected by EIA, and a reduction in viral load as detected by RT-PCR.

Embodiments also include NS3/4A fusion proteins or nucleic acids encoding these molecules. For instance, production and purification of recombinant protein may be facilitated by the addition of auxiliary amino acids to form a "tag". Such tags include, but are not limited to, His-6, Flag, Myc and GST. The tags may be added to the C-terminus, N-terminus, or within the NS3/4A amino acid sequence. Further embodiments include NS3/4A fusion proteins with amino or carboxy terminal truncations, or internal deletions, or with additional polypeptide sequences added to the amino or carboxy terminal ends, or added internally. Other embodiments include NS3/4A fusion proteins, or truncated or mutated versions thereof, where the residues of the NS3/4A proteolytic cleavage site have been substituted. Such substitutions include, but are not limited to, sequences where the P1' site is a Ser, Gly, or Pro, or the P1 position is an Arg, or where the P8 to P4' sequence is Ser-Ala-Asp-Leu-Glu-Val-Val-Thr-Ser-Thr-Trp-Val (SEQ. ID. NO.: 28).

Other embodiments concern an immunogen comprising the NS3/4A fusion protein, or a truncated or modified version thereof, capable of eliciting an enhanced immune response against NS3. The immunogen can be provided in a substantially purified form, which means that the immunogen has been rendered substantially free of other proteins, lipids, carbohydrates or other compounds with which it naturally associates. Embodiments also include vaccine compositions comprising the NS3/4A fusion protein (SEQ. ID. NO.: 17), or a truncated or mutated version thereof (e.g., SEQ. ID. NOS.: 29- 32 and 43-49) or a fragment thereof (e.g., SEQ. ID. NOS.: 25- 27, and 33-42), and an adjuvant, such as ribavirin. The following example describes one approach to prepare a vaccine composition comprising the NS3/4A fusion protein and an adjuvant.

## Example 15

To generate a tagged NS3/4A construct, the NS3/4A coding sequence of NS3/4A-pVAX is removed by digestion with *EcoRI* and *XbaI*, and the isolated fragment is inserted into an Xpress vector (Invitrogen). The Xpress vector allows for the production of a recombinant fusion protein having a short N-terminal leader peptide that has a high affinity for divalent cations. Using a nickel-chelating resin (Invitrogen), the recombinant protein can be purified in one step and the leader can be subsequently removed by cleavage with enterokinase. A preferred vector is the pBlueBacHis2 Xpress. The pBlueBacHis2 Xpress vector is a Baculovirus expression vector containing a multiple cloning site, an ampicillin resistance gene, and a *lac z* gene. Accordingly, the digested amplification fragment is cloned into the pBlueBacHis2 Xpress vector and SF9 cells are infected. The expression protein is then isolated or purified according to the manufacturer's instructions. An NS3/4A containing vaccine composition is then made by mixing 100µg of the rNS3/4A with 1mg of ribavirin.

Said vaccine composition can be used to raise antibodies in a mammal (e.g., mice or rabbits) or can be injected intramuscularly into a human so as to raise antibodies, preferably a human that is chronically infected with the HCV virus. The recipient preferably receives three immunization boosts of the mixture at 4-week intervals. By the third boost, the titer of antibody specific for HCV will be significantly increased. Additionally, at this time, said subject will experience an enhanced antibody and T-cell mediated immune response against NS3, as evidenced by an increased fraction of NS3 specific antibodies as detected by EIA, and a reduction in viral load as detected by RT-PCR. The section below provides more explanation concerning the methods of using the compositions described herein.

*Methods of using compositions comprising ribavirin and an antigen*

Routes of administration of the vaccines described herein include, but are not limited to, transdermal, parenteral, gastrointestinal, transbronchial, and transalveolar. Transdermal administration can be accomplished by application of a cream, rinse, gel, or other compounds capable of allowing ribavirin and antigen to penetrate the skin. Parenteral routes of administration include, but are not limited to, electrical or direct injection such as direct injection into a central venous line, intravenous, intramuscular, intraperitoneal, intradermal, or subcutaneous injection. Gastrointestinal routes of administration include, but are not limited to, ingestion and rectal. Transbronchial and transalveolar routes of administration include, but are not limited to, inhalation, either via the mouth or intranasally.

Compositions having ribavirin and an antigen that are suitable for transdermal administration include, but are not limited to, pharmaceutically acceptable suspensions, oils, creams, and ointments applied directly to the skin or incorporated into a protective carrier such as a

transdermal device ("transdermal patch"). Examples of suitable creams, ointments, etc. can be found, for instance, in the Physician's Desk Reference. Examples of suitable transdermal devices are described, for instance, in U.S. Patent No. 4,818,540 issued April 4, 1989 to Chinen, et al.

5 Compositions having ribavirin and an antigen that are suitable for parenteral administration include, but are not limited to, pharmaceutically acceptable sterile isotonic solutions. Such solutions include, but are not limited to, saline, phosphate buffered saline and oil preparations for injection into a central venous line, intravenous, intramuscular, intraperitoneal, intradermal, or subcutaneous injection.

10 Compositions having ribavirin and an antigen that are suitable for transbronchial and transalveolar administration include, but not limited to, various types of aerosols for inhalation. Devices suitable for transbronchial and transalveolar administration of these are also embodiments. Such devices include, but are not limited to, atomizers and vaporizers. Many forms of currently available atomizers and vaporizers can be readily adapted to deliver vaccines having ribavirin and an antigen.

15 Compositions having ribavirin and an antigen that are suitable for gastrointestinal administration include, but not limited to, pharmaceutically acceptable powders, pills or liquids for ingestion and suppositories for rectal administration.

The gene constructs described herein, in particular, may be administered by means including, but not limited to, traditional syringes, needleless injection devices, or "microprojectile bombardment gene guns". Alternatively, the genetic vaccine may be introduced by various means  
20 into cells that are removed from the individual. Such means include, for example, *ex vivo* transfection, electroporation, microinjection and microprojectile bombardment. After the gene construct is taken up by the cells, they are reimplanted into the individual. It is contemplated that otherwise non-immunogenic cells that have gene constructs incorporated therein can be implanted  
25 into the individual even if the vaccinated cells were originally taken from another individual.

According to some embodiments, the gene construct is administered to an individual using a needleless injection device. According to some embodiments, the gene construct is simultaneously administered to an individual intradermally, subcutaneously and intramuscularly using a needleless injection device. Needleless injection devices are well known and widely  
30 available. One having ordinary skill in the art can, following the teachings herein, use needleless injection devices to deliver genetic material to cells of an individual. Needleless injection devices are well suited to deliver genetic material to all tissue. They are particularly useful to deliver genetic material to skin and muscle cells. In some embodiments, a needleless injection device may be used to propel a liquid that contains DNA molecules toward the surface of the individual's skin.  
35 The liquid is propelled at a sufficient velocity such that upon impact with the skin the liquid

penetrates the surface of the skin, permeates the skin and muscle tissue therebeneath. Thus, the genetic material is simultaneously administered intradermally, subcutaneously and intramuscularly. In some embodiments, a needleless injection device may be used to deliver genetic material to tissue of other organs in order to introduce a nucleic acid molecule to cells of  
5 that organ.

The vaccines containing ribavirin and an antigen can be used to treat and prevent a vast spectrum of diseases and can enhance the immune response of an animal to an antigen. As one of skill in the art will appreciate, conventional vaccines have been administered to subjects in need of treatment or prevention of bacterial diseases, viral diseases, fungal diseases, and cancer. Because  
10 the vaccines described herein include conventional vaccines, which have been modified by the addition of ribavirin, the methods described herein include the treatment and prevention of a disease using a vaccine that comprises an antigen and ribavirin.

Preferred embodiments concern methods of treating or preventing hepatitis infection. In these embodiments, an animal in need is provided a hepatitis antigen (e.g., a peptide antigen or  
15 nucleic acid-based antigen) and an amount of ribavirin sufficient to exhibit an adjuvant activity in said animal. Accordingly, an animal can be identified as one in need by using currently available diagnostic testing or clinical evaluation. The range of hepatitis viral antigens that can be used with these embodiments is diverse. Preferred hepatitis viral antigens include an HBV antigen, an HAV antigen, an HCV antigen, nucleic acids encoding these antigens, or any combination thereof.  
20 Highly preferred embodiments include an HBV antigen selected from the group consisting of hepatitis B surface antigen (HBsAg), hepatitis core antigen (HBcAg), and hepatitis E antigen (HBeAg), in particular, the peptide and nucleic acid-based antigens described *supra*. The ribavirin and antigen can be provided separately or in combination, and other adjuvants (e.g., oil, alum, or other agents that enhance an immune response) can also be provided to the animal in need. Thus,  
25 preferred embodiments include methods of treating or preventing hepatitis in an animal (e.g., HBV) by identifying an infected animal or an animal at risk of infection and providing said animal a hepatitis antigen (e.g., HBsAg, HBcAg, and HBeAg) and an amount of ribavirin sufficient to exhibit adjuvant activity.

Other embodiments include methods of enhancing an immune response to an antigen by  
30 providing an animal in need with an amount of ribavirin that is effective to enhance said immune response. In these embodiments, an animal in need of an enhanced immune response to an antigen is identified by using currently available diagnostic testing or clinical evaluation. Oftentimes these individuals will be suffering from a disease (e.g., bacterial, fungal, mold, viral, or cancer) or are at risk from contracting the disease. However, an animal in need of an enhanced immune response  
35 can be an animal that has been poisoned (e.g., bit by a poisonous insect or animal) or that has been



exposed to a toxin or other toxic compound. Once identified, these animals are provided an appropriate antigen and an amount of ribavirin effective to enhance an immune response in the animal.

As above, the hepatitis viral antigens that can be used with these embodiments include, but are not limited to, an HBV antigen, an HAV antigen, an HCV antigen, a nucleic acid encoding these molecules, or any combination thereof. Highly preferred embodiments include an HBV antigen selected from the group consisting of hepatitis B surface antigen (HBsAg), hepatitis core antigen (HBcAg), and hepatitis E antigen (HBeAg), in particular, the peptide and nucleic acid-based antigens described *supra*. The ribavirin and antigen can be provided separately or in combination, and other adjuvants (e.g., oil, alum, or other agents that enhance an immune response) can also be provided to the animal in need. Thus, preferred embodiments include methods of enhancing an immune response to a hepatitis antigen (e.g., HBV) by identifying an animal in need and providing the animal a hepatitis antigen (e.g., HBsAg, HBcAg, and HBeAg) and an amount of ribavirin that is effective to enhance an immune response in the animal.

By one approach, for example, an uninfected individual is provided with the above mentioned vaccine compositions in an amount sufficient to elicit a cellular and humoral immune response to NS3 so as to protect said individual from becoming infected with HCV. In another embodiment, an HCV-infected individual is identified and provided with a vaccine composition comprising ribavirin and NS3 in an amount sufficient to enhance the cellular and humoral immune response against NS3 so as to reduce or eliminate the HCV infection. Such individual may be in the chronic or acute phase of the infection. In yet another embodiment, an HCV-infected individual suffering from HCC is provided with a composition comprising ribavirin and the NS3/4A fusion gene in an amount sufficient to elicit a cellular and humoral immune response against NS3-expressing tumor cells.

Although the invention has been described with reference to embodiments and examples, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

WHAT IS CLAIMED IS:

1. A composition comprising ribavirin and the nucleic acid of **SEQ. ID. NO.: 16**.
2. A composition comprising Ribavrin and the peptide of **SEQ. ID. NO.: 17**.
3. A composition comprising Ribavrin and the nucleic acid of **SEQ. ID. NO.: 13** or a  
5 fragment thereof at least 18 consecutive nucleotides in length.
4. A composition comprising ribavirin and the peptide of **SEQ. ID. NO.: 1** or a  
fragment thereof at least 6 consecutive amino acids in length.
5. A composition comprising ribavirin and an antigen.
6. The composition of Claim 5, wherein said antigen is a nucleic acid.
- 10 7. The composition of Claim 5, wherein said antigen is a peptide.
8. The composition of Claim 6, wherein said nucleic acid is derived from a virus  
selected from the group consisting of hepatitis A virus (HAV), hepatitis B virus (HBV), and  
hepatitis C virus (HCV).
9. The composition of Claim 7, wherein said peptide is derived from a virus selected  
15 from the group consisting of hepatitis A virus (HAV), hepatitis B virus (HBV), and hepatitis C  
virus (HCV).
10. The composition of Claim 5, wherein said antigen is a nucleic acid or a peptide  
corresponding to an antigen selected from the group consisting of hepatitis B surface antigen  
(HBsAg), hepatitis core antigen (HBcAg), and hepatitis E antigen (HBeAg).
- 20 11. The composition of Claim 7, wherein said peptide comprises at least three  
consecutive amino acids of a sequence selected from the group consisting of **SEQ. ID. NOs.: 1-12**.
12. The composition of Claim 6, wherein said nucleic acid comprises at least 9  
consecutive nucleotides of a sequence selected from the group consisting of **SEQ. ID. NOs.: 13-  
15**.
- 25 13. A method of enhancing an immune response to a hepatitis C antigen comprising:  
identifying an animal in need of an enhanced immune response to a hepatitis C  
antigen; and  
providing to said animal a composition comprising ribavirin and the nucleic acid  
of **SEQ. ID. NO.: 16**.
- 30 14. A method of enhancing an immune response to a hepatitis C antigen comprising:  
identifying an animal in need of an enhanced immune response to a hepatitis C  
antigen; and  
providing to said animal a composition comprising ribavirin and the peptide of  
**SEQ. ID. NO.: 17**.

15. A method of enhancing an immune response to a hepatitis C antigen comprising:  
identifying an animal in need of an enhanced immune response to a hepatitis C  
antigen; and  
providing to said animal a composition comprising ribavirin and the nucleic acid  
of SEQ. ID. NO.: 13 or a fragment thereof at least 18 consecutive nucleotides in length.
16. A method of enhancing an immune response to a hepatitis C antigen comprising:  
identifying an animal in need of an enhanced immune response to a hepatitis C  
antigen; and  
providing to said animal a composition comprising ribavirin and the peptide of  
SEQ. ID. NO.: 1 or a fragment thereof at least 6 consecutive amino acids in length.
17. A method of making a vaccine comprising:  
providing ribavirin;  
providing the nucleic acid of SEQ. ID. NO.: 16; and  
mixing said ribavirin and said nucleic acid so as to formulate said vaccine.
18. A method of making a vaccine comprising:  
providing ribavirin;  
providing the peptide of SEQ. ID. NO.: 17; and  
mixing said ribavirin and said peptide so as to formulate said vaccine.
19. A method of making a vaccine comprising:  
providing ribavirin;  
providing the nucleic acid of SEQ. ID. NO.: 13 or a fragment thereof at least at  
least 18 consecutive nucleotides in length; and  
mixing said ribavirin and said nucleic acid so as to formulate said vaccine.
20. A method of making a vaccine comprising:  
providing ribavirin;  
providing the nucleic acid of SEQ. ID. NO.: 1 or a fragment thereof at least 6  
consecutive amino acids in length; and  
mixing said ribavirin and said nucleic acid so as to formulate said vaccine.

1/5

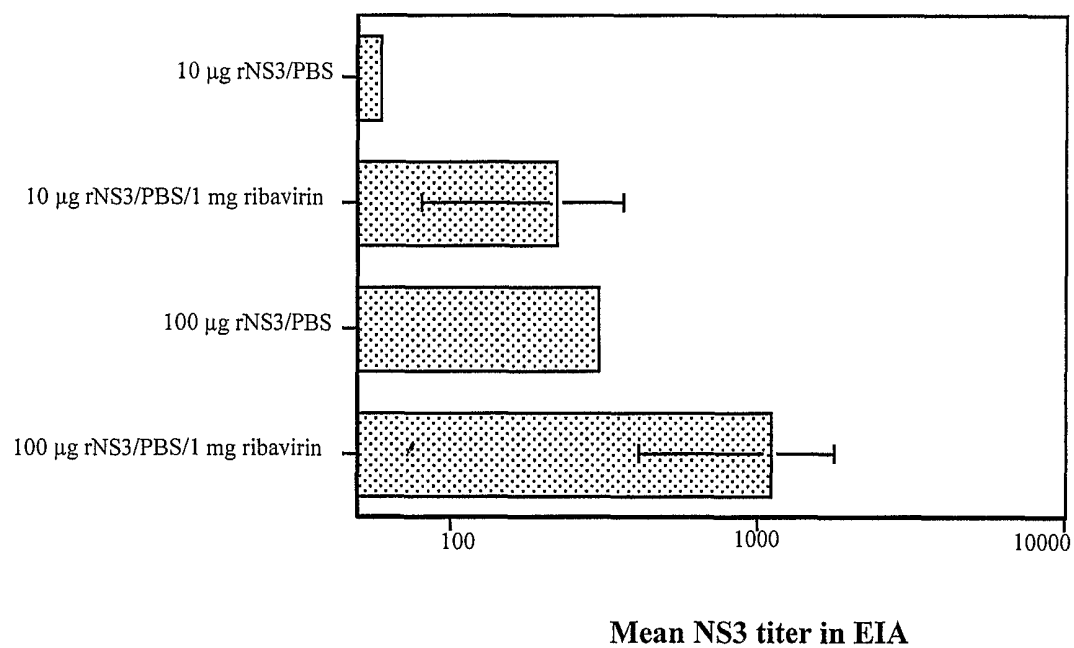
**FIGURE 1**

FIGURE 2

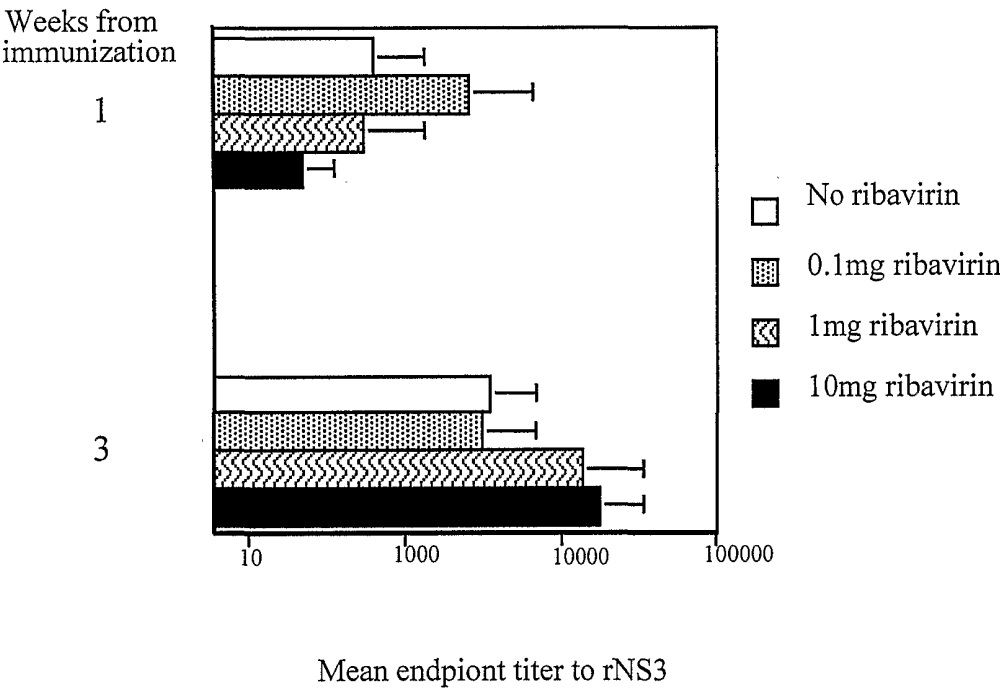


FIGURE 3

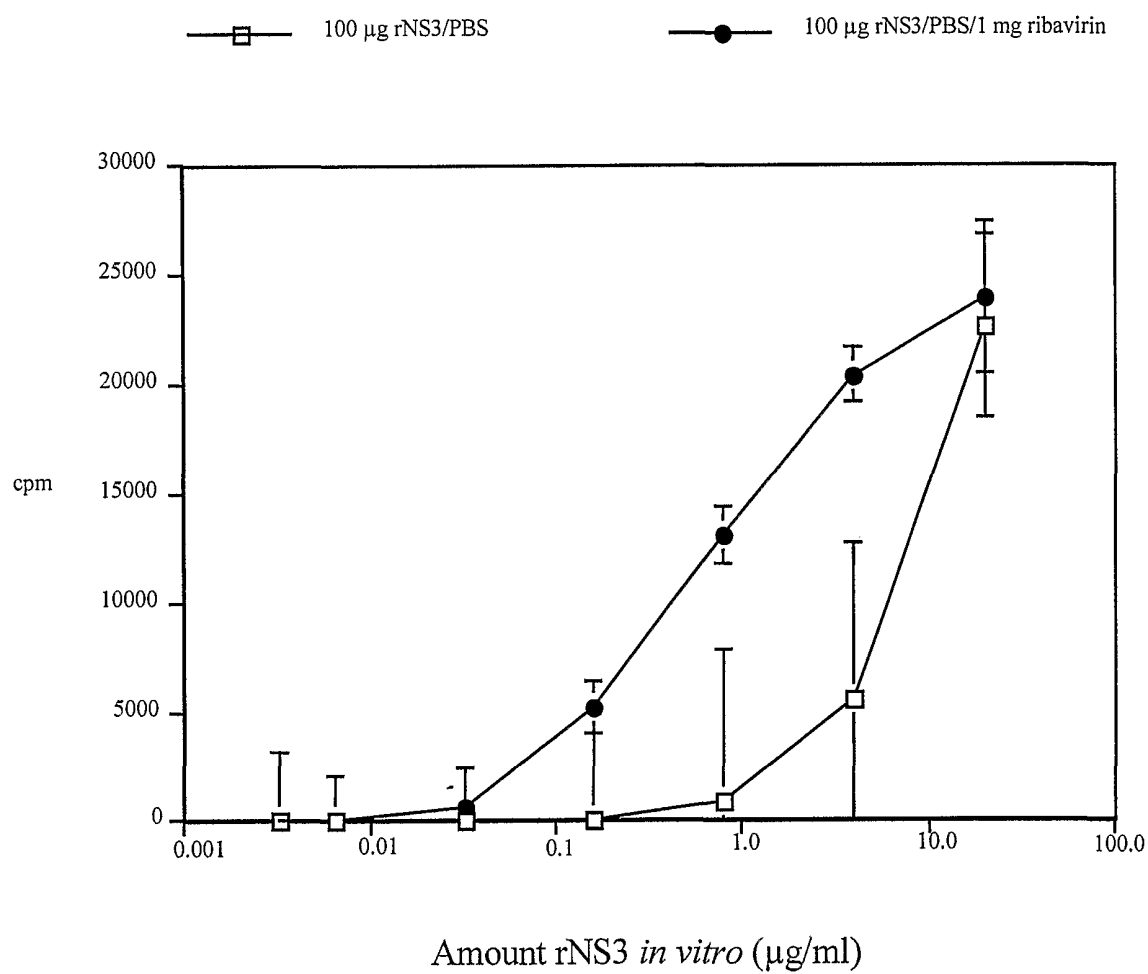


FIGURE 4

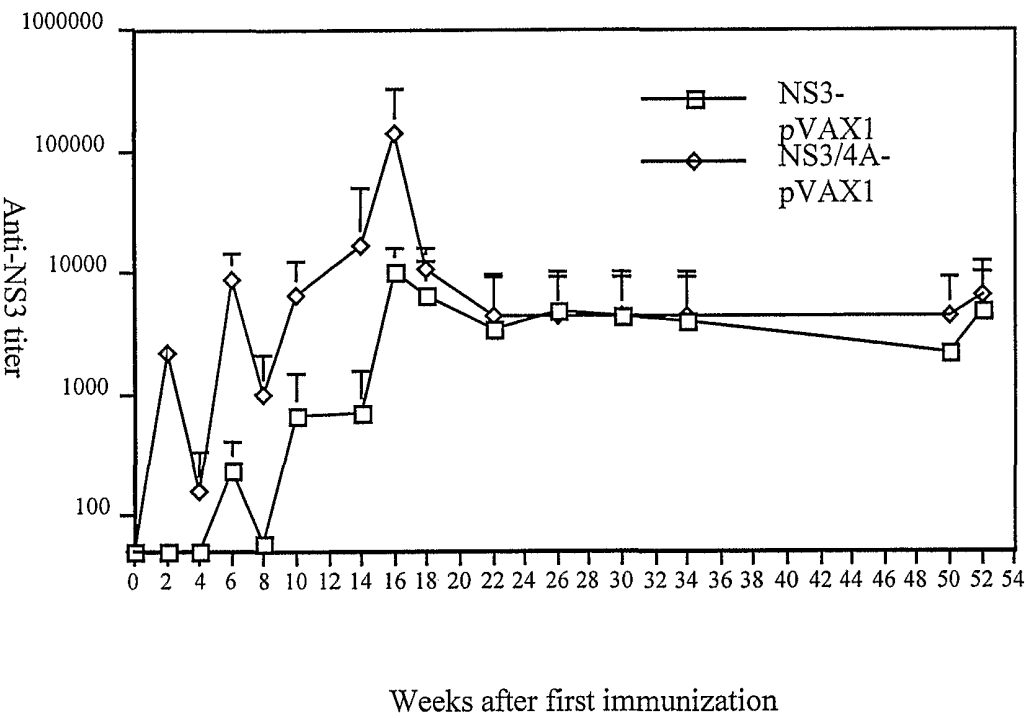


FIG. 5A

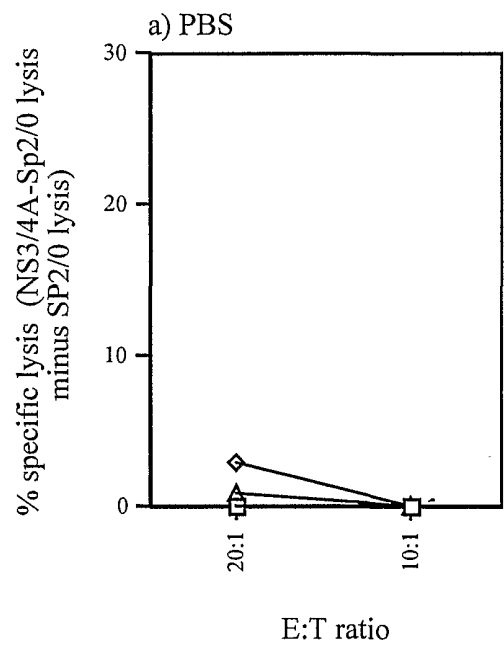
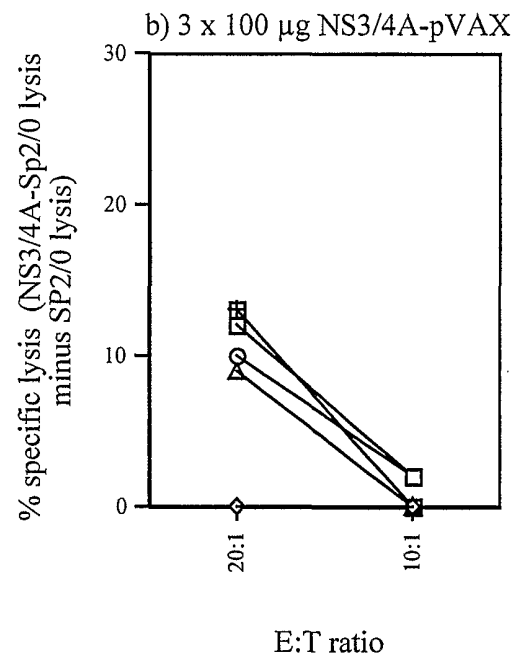


FIG. 5B





## SEQUENCE LISTING

<110> TRIPEP AB  
 Matti SALLBERG  
 Catharina HULTGREN

<120> VACCINES CONTAINING RIBAVIRIN AND  
 METHODS OF USE THEREOF

<130> TRIPEP.023VPC

<150> US 09/705,547  
 <151> 2000-11-03

<150> US 60/229,175  
 <151> 2000-08-29

<150> US 60/225,767  
 <151> 2000-08-17

<160> 49

<170> FastSEQ for Windows Version 4.0

<210> 1  
 <211> 3011  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Hepatitis C virus sequence

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 1 5 10 15  
 Arg Arg Pro Gln Asp Val Lys Phe Pro Gly Gly Gly Gln Ile Val Gly  
 20 25 30  
 Gly Val Tyr Leu Leu Pro Arg Arg Gly Pro Arg Leu Gly Val Arg Ala  
 35 40 45  
 Thr Arg Lys Thr Ser Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro  
 50 55 60  
 Ile Pro Lys Ala Arg Arg Pro Glu Gly Arg Thr Trp Ala Gln Pro Gly  
 65 70 75 80  
 Tyr Pro Trp Pro Leu Tyr Gly Asn Glu Gly Cys Gly Trp Ala Gly Trp  
 85 90 95  
 Leu Leu Ser Pro Arg Gly Ser Arg Pro Ser Trp Gly Pro Thr Asp Pro  
 100 105 110  
 Arg Arg Arg Ser Arg Asn Leu Gly Lys Val Ile Asp Thr Leu Thr Cys  
 115 120 125  
 Gly Phe Ala Asp Leu Met Gly Tyr Ile Pro Leu Val Gly Ala Pro Leu  
 130 135 140  
 Gly Gly Ala Ala Arg Ala Leu Ala His Gly Val Arg Val Leu Glu Asp  
 145 150 155 160

Gly	Val	Asn	Tyr	Ala	Thr	Gly	Asn	Leu	Pro	Gly	Cys	Ser	Phe	Ser	Ile	165	170	175
Phe	Leu	Leu	Ala	Leu	Leu	Ser	Cys	Leu	Thr	Val	Pro	Ala	Ser	Ala	Tyr	180	185	190
Gln	Val	Arg	Asn	Ser	Ser	Gly	Leu	Tyr	His	Val	Thr	Asn	Asp	Cys	Pro	195	200	205
Asn	Ser	Ser	Val	Val	Tyr	Glu	Ala	Ala	Asp	Ala	Ile	Leu	His	Thr	Pro	210	215	220
Gly	Cys	Val	Pro	Cys	Val	Arg	Glu	Gly	Asn	Ala	Ser	Arg	Cys	Trp	Val	225	230	235
Ala	Val	Thr	Pro	Thr	Val	Ala	Thr	Arg	Asp	Gly	Lys	Leu	Pro	Thr	Thr	245	250	255
Gln	Leu	Arg	Arg	His	Ile	Asp	Leu	Leu	Val	Gly	Ser	Ala	Thr	Leu	Cys	260	265	270
Ser	Ala	Leu	Tyr	Val	Gly	Asp	Leu	Cys	Gly	Ser	Val	Phe	Leu	Val	Gly	275	280	285
Gln	Leu	Phe	Thr	Phe	Ser	Pro	Arg	His	His	Trp	Thr	Thr	Gln	Asp	Cys	290	295	300
Asn	Cys	Ser	Ile	Tyr	Pro	Gly	His	Ile	Thr	Gly	His	Arg	Met	Ala	Trp	305	310	315
Asn	Met	Met	Met	Asn	Trp	Ser	Pro	Thr	Ala	Ala	Leu	Val	Val	Ala	Gln	325	330	335
Leu	Leu	Arg	Ile	Pro	Gln	Ala	Ile	Met	Asp	Met	Ile	Ala	Gly	Ala	His	340	345	350
Trp	Gly	Val	Leu	Ala	Gly	Ile	Lys	Tyr	Phe	Ser	Met	Val	Gly	Asn	Trp	355	360	365
Ala	Lys	Val	Leu	Val	Val	Leu	Leu	Phe	Ala	Gly	Val	Asp	Ala	Glu		370	375	380
Thr	His	Val	Thr	Gly	Gly	Asn	Ala	Gly	Arg	Thr	Thr	Ala	Gly	Leu	Val	385	390	395
Gly	Leu	Leu	Thr	Pro	Gly	Ala	Lys	Gln	Asn	Ile	Gln	Leu	Ile	Asn	Thr	405	410	415
Asn	Gly	Ser	Trp	His	Ile	Asn	Ser	Thr	Ala	Leu	Asn	Cys	Asn	Glu	Ser	420	425	430
Leu	Asn	Thr	Gly	Trp	Leu	Ala	Gly	Leu	Phe	Tyr	Gln	His	Lys	Phe	Asn	435	440	445
Ser	Ser	Gly	Cys	Pro	Glu	Arg	Leu	Ala	Ser	Cys	Arg	Arg	Leu	Thr	Asp	450	455	460
Phe	Ala	Gln	Gly	Trp	Gly	Pro	Ile	Ser	Tyr	Ala	Asn	Gly	Ser	Gly	Leu	465	470	475
Asp	Glu	Arg	Pro	Tyr	Cys	Trp	His	Tyr	Pro	Pro	Arg	Pro	Cys	Gly	Ile	485	490	495
Val	Pro	Ala	Lys	Ser	Val	Cys	Gly	Pro	Val	Tyr	Cys	Phe	Thr	Pro	Ser	500	505	510
Pro	Val	Val	Val	Gly	Thr	Thr	Asp	Arg	Ser	Gly	Ala	Pro	Thr	Tyr	Ser	515	520	525
Trp	Gly	Ala	Asn	Asp	Thr	Asp	Val	Phe	Val	Leu	Asn	Asn	Thr	Arg	Pro	530	535	540
Pro	Leu	Gly	Asn	Trp	Phe	Gly	Cys	Thr	Trp	Met	Asn	Ser	Thr	Gly	Phe	545	550	555
Thr	Lys	Val	Cys	Gly	Ala	Pro	Pro	Cys	Val	Ile	Gly	Gly	Val	Gly	Asn	565	570	575
Asn	Thr	Leu	Leu	Cys	Pro	Thr	Asp	Cys	Phe	Arg	Lys	Tyr	Pro	Glu	Ala	580	585	590
Thr	Tyr	Ser	Arg	Cys	Gly	Ser	Gly	Pro	Arg	Ile	Thr	Pro	Arg	Cys	Met			

595	600	605
Val Asp Tyr Pro Tyr Arg Leu Trp His Tyr Pro Cys Thr Ile Asn Tyr		
610	615	620
Thr Ile Phe Lys Val Arg Met Tyr Val Gly Gly Val Glu His Arg Leu		
625	630	635
Glu Ala Ala Cys Asn Trp Thr Arg Gly Glu Arg Cys Asp Leu Glu Asp		640
645	650	655
Arg Asp Arg Ser Glu Leu Ser Pro Leu Leu Leu Ser Thr Thr Gln Trp		
660	665	670
Gln Val Leu Pro Cys Ser Phe Thr Thr Leu Pro Ala Leu Ser Thr Gly		
675	680	685
Leu Ile His Leu His Gln Asn Ile Val Asp Val Gln Tyr Leu Tyr Gly		
690	695	700
Val Gly Ser Ser Ile Ala Ser Trp Ala Ile Lys Trp Glu Tyr Val Val		
705	710	715
Leu Leu Phe Leu Leu Leu Ala Asp Ala Arg Val Cys Ser Cys Leu Trp		720
725	730	735
Met Met Leu Leu Ile Ser Gln Ala Glu Ala Ala Leu Glu Asn Leu Val		
740	745	750
Ile Leu Asn Ala Ala Ser Leu Ala Gly Thr His Gly Leu Val Ser Phe		
755	760	765
Leu Val Phe Phe Cys Phe Ala Trp Tyr Leu Lys Gly Arg Trp Val Pro		
770	775	780
Gly Ala Val Tyr Ala Leu Tyr Gly Met Trp Pro Leu Leu Leu Leu Leu		
785	790	795
Leu Ala Leu Pro Gln Arg Ala Tyr Ala Leu Asp Thr Glu Val Ala Ala		800
805	810	815
Ser Cys Gly Gly Val Val Leu Val Gly Leu Met Ala Leu Thr Leu Ser		
820	825	830
Pro Tyr Tyr Lys Arg Tyr Ile Ser Trp Cys Met Trp Trp Leu Gln Tyr		
835	840	845
Phe Leu Thr Arg Val Glu Ala Gln Leu His Val Trp Val Pro Pro Leu		
850	855	860
Asn Val Arg Gly Gly Arg Asp Ala Val Ile Leu Leu Thr Cys Val Val		
865	870	875
His Pro Ala Leu Val Phe Asp Ile Thr Lys Leu Leu Leu Ala Ile Phe		
885	890	895
Gly Pro Leu Trp Ile Leu Gln Ala Ser Leu Leu Lys Val Pro Tyr Phe		
900	905	910
Val Arg Val Gln Gly Leu Leu Arg Ile Cys Ala Leu Ala Arg Lys Ile		
915	920	925
Ala Gly Gly His Tyr Val Gln Met Ala Ile Ile Lys Leu Gly Ala Leu		
930	935	940
Thr Gly Thr Cys Val Tyr Asn His Leu Ala Pro Leu Arg Asp Trp Ala		
945	950	955
His Asn Gly Leu Arg Asp Leu Ala Val Ala Val Glu Pro Val Val Phe		
965	970	975
Ser Arg Met Glu Thr Lys Leu Ile Thr Trp Gly Ala Asp Thr Ala Ala		
980	985	990
Cys Gly Asp Ile Ile Asn Gly Leu Pro Val Ser Ala Arg Arg Gly Gln		
995	1000	1005
Glu Ile Leu Leu Gly Pro Ala Asp Gly Met Val Ser Lys Gly Trp Arg		
1010	1015	1020
Leu Leu Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr Arg Gly Leu Leu		
1025	1030	1035
		1040

Gly Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu  
 1045 1050 1055  
 Gly Glu Val Gln Ile Val Ser Thr Ala Thr Gln Thr Phe Leu Ala Thr  
 1060 1065 1070  
 Cys Ile Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Thr Arg  
 1075 1080 1085  
 Thr Ile Ala Ser Pro Lys Gly Pro Val Ile Gln Thr Tyr Thr Asn Val  
 1090 1095 1100  
 Asp Gln Asp Leu Val Gly Trp Pro Ala Pro Gln Gly Ser Arg Ser Leu  
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 Thr Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr Arg His  
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 Ala Asp Val Ile Pro Val Arg Arg Arg Gly Asp Ser Arg Gly Ser Leu  
 1140 1145 1150  
 Leu Ser Pro Arg Pro Ile Ser Tyr Leu Lys Gly Ser Ser Gly Gly Pro  
 1155 1160 1165  
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 1170 1175 1180  
 Cys Thr Arg Gly Val Ala Lys Ala Val Asp Phe Ile Pro Val Glu Asn  
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 Leu Glu Thr Thr Met Arg Ser Pro Val Phe Thr Asp Asn Ser Ser Pro  
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 Gly Ala Tyr Met Ser Lys Ala His Gly Val Asp Pro Asn Ile Arg Thr  
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 Gly Val Arg Thr Ile Thr Thr Gly Ser Pro Ile Thr Tyr Ser Thr Tyr  
 1285 1290 1295  
 Gly Lys Phe Leu Ala Asp Ala Gly Cys Ser Gly Gly Ala Tyr Asp Ile  
 1300 1305 1310  
 Ile Ile Cys Asp Glu Cys His Ser Thr Asp Ala Thr Ser Ile Ser Gly  
 1315 1320 1325  
 Ile Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Val  
 1330 1335 1340  
 Val Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr Val Ser His Pro  
 1345 1350 1355 1360  
 Asn Ile Glu Glu Val Ala Leu Ser Thr Thr Gly Glu Ile Pro Phe Tyr  
 1365 1370 1375  
 Gly Lys Ala Ile Pro Leu Glu Val Ile Lys Gly Gly Arg His Leu Ile  
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 Phe Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Val  
 1395 1400 1405  
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 Val Ile Pro Thr Ser Gly Asp Val Val Val Val Ser Thr Asp Ala Leu  
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 Met Thr Gly Phe Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr  
 1445 1450 1455  
 Cys Val Thr Gln Thr Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile  
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 Glu Thr Thr Thr Leu Pro Gln Asp Ala Val Ser Arg Thr Gln Arg Arg

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Gly Arg Thr Gly Arg Gly Lys Pro Gly Ile Tyr Arg Phe Val Ala Pro		
1490	1495	1500
Gly Glu Arg Pro Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys		
1505	1510	1515
Tyr Asp Ala Gly Cys Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Thr		
1525	1530	1535
Val Arg Leu Arg Ala Tyr Met Asn Thr Pro Gly Leu Pro Val Cys Gln		
1540	1545	1550
Asp His Leu Gly Phe Trp Glu Gly Val Phe Thr Gly Leu Thr His Ile		
1555	1560	1565
Asp Ala His Phe Leu Ser Gln Thr Lys Gln Ser Gly Glu Asn Phe Pro		
1570	1575	1580
Tyr Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro		
1585	1590	1595
Pro Pro Ser Trp Asp Gln Met Arg Lys Cys Leu Ile Arg Leu Lys Pro		
1605	1610	1615
Thr Leu His Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln		
1620	1625	1630
Asn Glu Val Thr Leu Thr His Pro Ile Thr Lys Tyr Ile Met Thr Cys		
1635	1640	1645
Met Ser Ala Asp Leu Glu Val Val Thr Ser Thr Trp Val Leu Val Gly		
1650	1655	1660
Gly Val Leu Ala Ala Leu Ala Ala Tyr Cys Leu Ser Thr Gly Cys Val		
1665	1670	1675
Val Ile Val Gly Arg Ile Val Leu Ser Gly Lys Pro Ala Ile Ile Pro		
1685	1690	1695
Asp Arg Glu Val Leu Tyr Gln Glu Phe Asp Glu Met Glu Glu Cys Ser		
1700	1705	1710
Gln His Leu Pro Tyr Ile Glu Gln Gly Met Met Leu Ala Glu Gln Phe		
1715	1720	1725
Lys Gln Lys Ala Leu Gly Leu Leu Gln Thr Ala Ser Arg His Ala Glu		
1730	1735	1740
Val Ile Thr Pro Ala Val Gln Thr Asn Trp Gln Lys Leu Glu Val Phe		
1745	1750	1755
Trp Ala Lys His Met Trp Asn Phe Ile Ser Gly Ile Gln Tyr Leu Ala		
1765	1770	1775
Gly Leu Ser Thr Leu Pro Gly Asn Pro Ala Ile Ala Ser Leu Met Ala		
1780	1785	1790
Phe Thr Ala Ala Val Thr Ser Pro Leu Thr Thr Gly Gln Thr Leu Leu		
1795	1800	1805
Phe Asn Ile Leu Gly Gly Trp Val Ala Ala Gln Leu Ala Ala Pro Gly		
1810	1815	1820
Ala Ala Thr Ala Phe Val Gly Ala Gly Leu Ala Gly Ala Ala Leu Asp		
1825	1830	1835
Ser Val Gly Leu Gly Lys Val Leu Val Asp Ile Leu Ala Gly Tyr Gly		
1845	1850	1855
Ala Gly Val Ala Gly Ala Leu Val Ala Phe Lys Ile Met Ser Gly Glu		
1860	1865	1870
Val Pro Ser Thr Glu Asp Leu Val Asn Leu Leu Pro Ala Ile Leu Ser		
1875	1880	1885
Pro Gly Ala Leu Ala Val Gly Val Val Phe Ala Ser Ile Leu Arg Arg		
1890	1895	1900
Arg Val Gly Pro Gly Glu Gly Ala Val Gln Trp Met Asn Arg Leu Ile		
1905	1910	1915
		1920

Ala Phe Ala Ser Arg Gly Asn His Val Ser Pro Thr His Tyr Val Pro  
 1925 1930 1935  
 Glu Ser Asp Ala Ala Ala Arg Val Thr Ala Ile Leu Ser Ser Leu Thr  
 1940 1945 1950  
 Val Thr Gln Leu Leu Arg Arg Leu His Gln Trp Ile Ser Ser Glu Cys  
 1955 1960 1965  
 Thr Thr Pro Cys Ser Gly Ser Trp Leu Arg Asp Ile Trp Asp Trp Ile  
 1970 1975 1980  
 Cys Glu Val Leu Ser Asp Phe Lys Thr Trp Leu Lys Ala Lys Leu Met  
 1985 1990 1995 2000  
 Pro Gln Leu Pro Gly Ile Pro Phe Val Ser Cys Gln Arg Gly Tyr Arg  
 2005 2010 2015  
 Gly Val Trp Arg Gly Asp Gly Ile Met His Thr Arg Cys His Cys Gly  
 2020 2025 2030  
 Ala Glu Ile Thr Gly His Val Lys Asn Gly Thr Met Arg Ile Val Gly  
 2035 2040 2045  
 Pro Arg Thr Cys Lys Asn Met Trp Ser Gly Thr Phe Phe Ile Asn Ala  
 2050 2055 2060  
 Tyr Thr Thr Gly Pro Cys Thr Pro Leu Pro Ala Pro Asn Tyr Lys Phe  
 2065 2070 2075 2080  
 Ala Leu Trp Arg Val Ser Ala Glu Glu Tyr Val Glu Ile Arg Arg Val  
 2085 2090 2095  
 Gly Asp Phe His Tyr Val Ser Gly Met Thr Thr Asp Asn Leu Lys Cys  
 2100 2105 2110  
 Pro Cys Gln Ile Pro Ser Pro Glu Phe Phe Thr Glu Leu Asp Gly Val  
 2115 2120 2125  
 Arg Leu His Arg Phe Ala Pro Pro Cys Lys Pro Leu Leu Arg Glu Glu  
 2130 2135 2140  
 Val Ser Phe Arg Val Gly Leu His Glu Tyr Pro Val Gly Ser Gln Leu  
 2145 2150 2155 2160  
 Pro Cys Glu Pro Glu Pro Asp Val Ala Val Leu Thr Ser Met Leu Thr  
 2165 2170 2175  
 Asp Pro Ser His Ile Thr Ala Glu Ala Ala Gly Arg Arg Leu Ala Arg  
 2180 2185 2190  
 Gly Ser Pro Pro Ser Met Ala Ser Ser Ser Ala Ser Gln Leu Ser Ala  
 2195 2200 2205  
 Pro Ser Leu Lys Ala Thr Cys Thr Ala Asn His Asp Ser Pro Asp Ala  
 2210 2215 2220  
 Glu Leu Ile Glu Ala Asn Leu Leu Trp Arg Gln Glu Met Gly Gly Asn  
 2225 2230 2235 2240  
 Ile Thr Arg Val Glu Ser Glu Asn Lys Val Val Ile Leu Asp Ser Phe  
 2245 2250 2255  
 Asp Pro Leu Val Ala Glu Glu Asp Glu Arg Glu Val Ser Val Pro Ala  
 2260 2265 2270  
 Glu Ile Leu Arg Lys Ser Arg Arg Phe Ala Pro Ala Leu Pro Val Trp  
 2275 2280 2285  
 Ala Arg Pro Asp Tyr Asn Pro Leu Leu Val Glu Thr Trp Lys Lys Pro  
 2290 2295 2300  
 Asp Tyr Glu Pro Pro Val Val His Gly Cys Pro Leu Pro Pro Pro Arg  
 2305 2310 2315 2320  
 Ser Pro Pro Val Pro Pro Pro Arg Lys Lys Arg Thr Val Val Leu Thr  
 2325 2330 2335  
 Glu Ser Thr Leu Pro Thr Ala Leu Ala Glu Leu Ala Thr Lys Ser Phe  
 2340 2345 2350  
 Gly Ser Ser Ser Thr Ser Gly Ile Thr Gly Asp Asn Thr Thr Thr Ser

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Ser Glu Pro Ala Pro Ser Gly Cys Pro Pro Asp Ser Asp Val Glu Ser		
2370	2375	2380
Tyr Ser Ser Met Pro Pro Leu Glu Gly Glu Pro Gly Asp Pro Asp Leu		
2385	2390	2395
Ser Asp Gly Ser Trp Ser Thr Val Ser Ser Gly Ala Asp Thr Glu Asp		
2405	2410	2415
Val Val Cys Cys Ser Met Ser Tyr Ser Trp Thr Gly Ala Leu Val Thr		
2420	2425	2430
Pro Cys Ala Ala Glu Glu Gln Lys Leu Pro Ile Asn Ala Leu Ser Asn		
2435	2440	2445
Ser Leu Leu Arg His His Asn Leu Val Tyr Ser Thr Thr Ser Arg Ser		
2450	2455	2460
Ala Cys Gln Arg Lys Lys Lys Val Thr Phe Asp Arg Leu Gln Val Leu		
2465	2470	2475
Asp Ser His Tyr Gln Asp Val Leu Lys Glu Val Lys Ala Ala Ala Ser		
2485	2490	2495
Lys Val Lys Ala Asn Leu Leu Ser Val Glu Glu Ala Cys Ser Leu Ala		
2500	2505	2510
Pro Pro His Ser Ala Lys Ser Lys Phe Gly Tyr Gly Ala Lys Asp Val		
2515	2520	2525
Arg Cys His Ala Arg Lys Ala Val Ala His Ile Asn Ser Val Trp Lys		
2530	2535	2540
Asp Leu Leu Glu Asp Ser Val Thr Pro Ile Asp Thr Thr Ile Met Ala		
2545	2550	2555
Lys Asn Glu Val Phe Cys Val Gln Pro Glu Lys Gly Gly Arg Lys Pro		
2565	2570	2575
Ala Arg Leu Ile Val Phe Pro Asp Leu Gly Val Arg Val Cys Glu Lys		
2580	2585	2590
Met Ala Leu Tyr Asp Val Val Ser Lys Leu Pro Leu Ala Val Met Gly		
2595	2600	2605
Ser Ser Tyr Gly Phe Gln Tyr Ser Pro Gly Gln Arg Val Glu Phe Leu		
2610	2615	2620
Val Gln Ala Trp Lys Ser Lys Lys Thr Pro Met Gly Leu Ser Tyr Asp		
2625	2630	2635
Thr Arg Cys Phe Asp Ser Thr Val Thr Glu Ser Asp Ile Arg Thr Glu		
2645	2650	2655
Glu Ala Ile Tyr Gln Cys Cys Asp Leu Asp Pro Gln Ala Arg Val Ala		
2660	2665	2670
Ile Lys Ser Leu Thr Glu Arg Leu Tyr Val Gly Gly Pro Leu Thr Asn		
2675	2680	2685
Ser Arg Gly Glu Asn Cys Gly Tyr Arg Arg Cys Arg Ala Ser Arg Val		
2690	2695	2700
Leu Thr Thr Ser Cys Gly Asn Thr Leu Thr Arg Tyr Ile Lys Ala Arg		
2705	2710	2715
Ala Ala Cys Arg Ala Ala Gly Leu Gln Asp Cys Thr Met Leu Val Cys		
2725	2730	2735
Gly Asp Asp Leu Val Val Ile Cys Glu Ser Ala Gly Val Gln Glu Asp		
2740	2745	2750
Ala Ala Ser Leu Arg Ala Phe Thr Glu Ala Met Thr Arg Tyr Ser Ala		
2755	2760	2765
Pro Pro Gly Asp Pro Pro Gln Pro Glu Tyr Asp Leu Glu Leu Ile Thr		
2770	2775	2780
Ser Cys Ser Ser Asn Val Ser Val Ala His Asp Gly Ala Gly Lys Arg		
2785	2790	2795
		2800

```

Val Tyr Tyr Leu Thr Arg Asp Pro Thr Thr Pro Leu Ala Arg Ala Ala
      2805                      2810                      2815
Trp Glu Thr Ala Arg His Thr Pro Val Asn Ser Trp Leu Gly Asn Ile
      2820                      2825                      2830
Ile Met Phe Ala Pro Thr Leu Trp Ala Arg Met Ile Leu Met Thr His
      2835                      2840                      2845
Phe Phe Ser Val Leu Ile Ala Arg Asp Gln Leu Glu Gln Ala Leu Asn
      2850                      2855                      2860
Cys Glu Ile Tyr Gly Ala Cys Tyr Ser Ile Glu Pro Leu Asp Leu Pro
2865                      2870                      2875                      2880
Pro Ile Ile Gln Arg Leu His Gly Leu Ser Ala Phe Ser Leu His Ser
      2885                      2890                      2895
Tyr Ser Pro Gly Glu Ile Asn Arg Val Ala Ala Cys Leu Arg Lys Leu
      2900                      2905                      2910
Gly Val Pro Pro Leu Arg Ala Trp Arg His Arg Ala Trp Ser Val Arg
      2915                      2920                      2925
Ala Arg Leu Leu Ala Arg Gly Gly Lys Ala Ala Ile Cys Gly Lys Tyr
      2930                      2935                      2940
Leu Phe Asn Trp Ala Val Arg Thr Lys Leu Lys Leu Thr Pro Ile Thr
2945                      2950                      2955                      2960
Ala Ala Gly Arg Leu Asp Leu Ser Gly Trp Phe Thr Ala Gly Tyr Ser
      2965                      2970                      2975
Gly Gly Asp Ile Tyr His Ser Val Ser His Ala Arg Pro Arg Trp Phe
      2980                      2985                      2990
Trp Phe Cys Leu Leu Leu Leu Ala Ala Gly Val Gly Ile Tyr Leu Leu
      2995                      3000                      3005
Pro Asn Arg
      3010

```

&lt;210&gt; 2

&lt;211&gt; 182

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Hepatitis C virus core protein sequence

&lt;400&gt; 2

```

Met Ser Thr Asn Pro Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn
  1              5              10              15
Arg Arg Pro Gln Asp Val Lys Phe Pro Gly Gly Gly Gln Ile Val Gly
      20              25              30
Gly Val Tyr Leu Leu Pro Arg Arg Gly Pro Arg Leu Gly Val Arg Ala
      35              40              45
Thr Arg Lys Thr Ser Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro
      50              55              60
Ile Pro Lys Ala Arg Arg Pro Glu Gly Arg Thr Trp Ala Gln Pro Gly
65              70              75              80
Tyr Pro Trp Pro Leu Tyr Gly Asn Glu Gly Cys Gly Trp Ala Gly Trp
      85              90              95
Leu Leu Ser Pro Arg Gly Ser Arg Pro Ser Trp Gly Pro Thr Asp Pro
      100             105             110
Arg Arg Arg Ser Arg Asn Leu Gly Lys Val Ile Asp Thr Leu Thr Cys
      115             120             125

```



Gly Phe Ala Asp Leu Met Gly Tyr Ile Pro Leu Val Gly Ala Pro Leu  
 130 135 140  
 Gly Gly Ala Ala Arg Ala Leu Ala His Gly Val Arg Val Leu Glu Asp  
 145 150 155 160  
 Gly Val Asn Tyr Ala Thr Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile  
 165 170 175  
 Phe Leu Leu Ala Leu Leu  
 180

&lt;210&gt; 3

&lt;211&gt; 197

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Hepatitis C virus E1 protein sequence

&lt;400&gt; 3

Ser Cys Leu Thr Val Pro Ala Ser Ala Tyr Gln Val Arg Asn Ser Ser  
 1 5 10 15  
 Gly Leu Tyr His Val Thr Asn Asp Cys Pro Asn Ser Ser Val Val Tyr  
 20 25 30  
 Glu Ala Ala Asp Ala Ile Leu His Thr Pro Gly Cys Val Pro Cys Val  
 35 40 45  
 Arg Glu Gly Asn Ala Ser Arg Cys Trp Val Ala Val Thr Pro Thr Val  
 50 55 60  
 Ala Thr Arg Asp Gly Lys Leu Pro Thr Thr Gln Leu Arg Arg His Ile  
 65 70 75 80  
 Asp Leu Leu Val Gly Ser Ala Thr Leu Cys Ser Ala Leu Tyr Val Gly  
 85 90 95  
 Asp Leu Cys Gly Ser Val Phe Leu Val Gly Gln Leu Phe Thr Phe Ser  
 100 105 110  
 Pro Arg His His Trp Thr Thr Gln Asp Cys Asn Cys Ser Ile Tyr Pro  
 115 120 125  
 Gly His Ile Thr Gly His Arg Met Ala Trp Asn Met Met Met Asn Trp  
 130 135 140  
 Ser Pro Thr Ala Ala Leu Val Val Ala Gln Leu Leu Arg Ile Pro Gln  
 145 150 155 160  
 Ala Ile Met Asp Met Ile Ala Gly Ala His Trp Gly Val Leu Ala Gly  
 165 170 175  
 Ile Lys Tyr Phe Ser Met Val Gly Asn Trp Ala Lys Val Leu Val Val  
 180 185 190  
 Leu Leu Leu Phe Ala  
 195

&lt;210&gt; 4

&lt;211&gt; 350

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Hepatitis C virus E2 protein sequence

&lt;400&gt; 4

Gly Val Asp Ala Glu Thr His Val Thr Gly Gly Asn Ala Gly Arg Thr  
 1 5 10 15  
 Thr Ala Gly Leu Val Gly Leu Leu Thr Pro Gly Ala Lys Gln Asn Ile  
 20 25 30  
 Gln Leu Ile Asn Thr Asn Gly Ser Trp His Ile Asn Ser Thr Ala Leu  
 35 40 45  
 Asn Cys Asn Glu Ser Leu Asn Thr Gly Trp Leu Ala Gly Leu Phe Tyr  
 50 55 60  
 Gln His Lys Phe Asn Ser Ser Gly Cys Pro Glu Arg Leu Ala Ser Cys  
 65 70 75 80  
 Arg Arg Leu Thr Asp Phe Ala Gln Gly Trp Gly Pro Ile Ser Tyr Ala  
 85 90 95  
 Asn Gly Ser Gly Leu Asp Glu Arg Pro Tyr Cys Trp His Tyr Pro Pro  
 100 105 110  
 Arg Pro Cys Gly Ile Val Pro Ala Lys Ser Val Cys Gly Pro Val Tyr  
 115 120 125  
 Cys Phe Thr Pro Ser Pro Val Val Val Gly Thr Thr Asp Arg Ser Gly  
 130 135 140  
 Ala Pro Thr Tyr Ser Trp Gly Ala Asn Asp Thr Asp Val Phe Val Leu  
 145 150 155 160  
 Asn Asn Thr Arg Pro Pro Leu Gly Asn Trp Phe Gly Cys Thr Trp Met  
 165 170 175  
 Asn Ser Thr Gly Phe Thr Lys Val Cys Gly Ala Pro Pro Cys Val Ile  
 180 185 190  
 Gly Gly Val Gly Asn Asn Thr Leu Leu Cys Pro Thr Asp Cys Phe Arg  
 195 200 205  
 Lys Tyr Pro Glu Ala Thr Tyr Ser Arg Cys Gly Ser Gly Pro Arg Ile  
 210 215 220  
 Thr Pro Arg Cys Met Val Asp Tyr Pro Tyr Arg Leu Trp His Tyr Pro  
 225 230 235 240  
 Cys Thr Ile Asn Tyr Thr Ile Phe Lys Val Arg Met Tyr Val Gly Gly  
 245 250 255  
 Val Glu His Arg Leu Glu Ala Ala Cys Asn Trp Thr Arg Gly Glu Arg  
 260 265 270  
 Cys Asp Leu Glu Asp Arg Asp Arg Ser Glu Leu Ser Pro Leu Leu Leu  
 275 280 285  
 Ser Thr Thr Gln Trp Gln Val Leu Pro Cys Ser Phe Thr Thr Leu Pro  
 290 295 300  
 Ala Leu Ser Thr Gly Leu Ile His Leu His Gln Asn Ile Val Asp Val  
 305 310 315 320  
 Gln Tyr Leu Tyr Gly Val Gly Ser Ser Ile Ala Ser Trp Ala Ile Lys  
 325 330 335  
 Trp Glu Tyr Val Val Leu Leu Phe Leu Leu Leu Ala Asp Ala  
 340 345 350

&lt;210&gt; 5

&lt;211&gt; 315

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Hepatitis C virus NS2 protein sequence

&lt;400&gt; 5

```

Arg Val Cys Ser Cys Leu Trp Met Met Leu Leu Ile Ser Gln Ala Glu
 1              5              10              15
Ala Ala Leu Glu Asn Leu Val Ile Leu Asn Ala Ala Ser Leu Ala Gly
      20              25              30
Thr His Gly Leu Val Ser Phe Leu Val Phe Phe Cys Phe Ala Trp Tyr
      35              40              45
Leu Lys Gly Arg Trp Val Pro Gly Ala Val Tyr Ala Leu Tyr Gly Met
      50              55              60
Trp Pro Leu Leu Leu Leu Leu Leu Ala Leu Pro Gln Arg Ala Tyr Ala
      65              70              75              80
Leu Asp Thr Glu Val Ala Ala Ser Cys Gly Gly Val Val Leu Val Gly
      85              90              95
Leu Met Ala Leu Thr Leu Ser Pro Tyr Tyr Lys Arg Tyr Ile Ser Trp
      100             105             110
Cys Met Trp Trp Leu Gln Tyr Phe Leu Thr Arg Val Glu Ala Gln Leu
      115             120             125
His Val Trp Val Pro Pro Leu Asn Val Arg Gly Gly Arg Asp Ala Val
      130             135             140
Ile Leu Leu Thr Cys Val Val His Pro Ala Leu Val Phe Asp Ile Thr
      145             150             155             160
Lys Leu Leu Leu Ala Ile Phe Gly Pro Leu Trp Ile Leu Gln Ala Ser
      165             170             175
Leu Leu Lys Val Pro Tyr Phe Val Arg Val Gln Gly Leu Leu Arg Ile
      180             185             190
Cys Ala Leu Ala Arg Lys Ile Ala Gly Gly His Tyr Val Gln Met Ala
      195             200             205
Ile Ile Lys Leu Gly Ala Leu Thr Gly Thr Cys Val Tyr Asn His Leu
      210             215             220
Ala Pro Leu Arg Asp Trp Ala His Asn Gly Leu Arg Asp Leu Ala Val
      225             230             235             240
Ala Val Glu Pro Val Val Phe Ser Arg Met Glu Thr Lys Leu Ile Thr
      245             250             255
Trp Gly Ala Asp Thr Ala Ala Cys Gly Asp Ile Ile Asn Gly Leu Pro
      260             265             270
Val Ser Ala Arg Arg Gly Gln Glu Ile Leu Leu Gly Pro Ala Asp Gly
      275             280             285
Met Val Ser Lys Gly Trp Arg Leu Leu Ala Pro Ile Thr Ala Tyr Ala
      290             295             300
Gln Gln Thr Arg Gly Leu Leu Gly Cys Ile Ile
      305             310             315

```

&lt;210&gt; 6

&lt;211&gt; 613

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Hepatitis C virus NS3 protein sequence

&lt;400&gt; 6

```

Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu Gly Glu Val Gln
 1              5              10              15
Ile Val Ser Thr Ala Thr Gln Thr Phe Leu Ala Thr Cys Ile Asn Gly

```

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```

Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys Tyr Asp Ala Gly
465                               470                               475                               480
Cys Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Thr Val Arg Leu Arg
                               485                               490                               495
Ala Tyr Met Asn Thr Pro Gly Leu Pro Val Cys Gln Asp His Leu Gly
                               500                               505                               510
Phe Trp Glu Gly Val Phe Thr Gly Leu Thr His Ile Asp Ala His Phe
                               515                               520                               525
Leu Ser Gln Thr Lys Gln Ser Gly Glu Asn Phe Pro Tyr Leu Val Ala
                               530                               535                               540
Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro Pro Pro Ser Trp
545                               550                               555                               560
Asp Gln Met Arg Lys Cys Leu Ile Arg Leu Lys Pro Thr Leu His Gly
                               565                               570                               575
Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln Asn Glu Val Thr
                               580                               585                               590
Leu Thr His Pro Ile Thr Lys Tyr Ile Met Thr Cys Met Ser Ala Asp
                               595                               600                               605
Leu Glu Val Val Thr
610

```

<210> 7  
 <211> 54  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Hepatitis C virus NS4A protein sequence

```

<400> 7
Ser Thr Trp Val Leu Val Gly Gly Val Leu Ala Ala Leu Ala Ala Tyr
1                               5                               10                               15
Cys Leu Ser Thr Gly Cys Val Val Ile Val Gly Arg Ile Val Leu Ser
                               20                               25                               30
Gly Lys Pro Ala Ile Ile Pro Asp Arg Glu Val Leu Tyr Gln Glu Phe
                               35                               40                               45
Asp Glu Met Glu Glu Cys
50

```

<210> 8  
 <211> 260  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Hepatitis C virus NS4B protein sequence

```

<400> 8
Ser Gln His Leu Pro Tyr Ile Glu Gln Gly Met Met Leu Ala Glu Gln
1                               5                               10                               15
Phe Lys Gln Lys Ala Leu Gly Leu Leu Gln Thr Ala Ser Arg His Ala
                               20                               25                               30
Glu Val Ile Thr Pro Ala Val Gln Thr Asn Trp Gln Lys Leu Glu Val

```

```

          35          40          45
Phe Trp Ala Lys His Met Trp Asn Phe Ile Ser Gly Ile Gln Tyr Leu
  50          55          60
Ala Gly Leu Ser Thr Leu Pro Gly Asn Pro Ala Ile Ala Ser Leu Met
  65          70          75          80
Ala Phe Thr Ala Ala Val Thr Ser Pro Leu Thr Thr Gly Gln Thr Leu
          85          90          95
Leu Phe Asn Ile Leu Gly Gly Trp Val Ala Ala Gln Leu Ala Ala Pro
          100          105          110
Gly Ala Ala Thr Ala Phe Val Gly Ala Gly Leu Ala Gly Ala Ala Leu
          115          120          125
Asp Ser Val Gly Leu Gly Lys Val Leu Val Asp Ile Leu Ala Gly Tyr
          130          135          140
Gly Ala Gly Val Ala Gly Ala Leu Val Ala Phe Lys Ile Met Ser Gly
  145          150          155          160
Glu Val Pro Ser Thr Glu Asp Leu Val Asn Leu Leu Pro Ala Ile Leu
          165          170          175
Ser Pro Gly Ala Leu Ala Val Gly Val Val Phe Ala Ser Ile Leu Arg
          180          185          190
Arg Arg Val Gly Pro Gly Glu Gly Ala Val Gln Trp Met Asn Arg Leu
          195          200          205
Ile Ala Phe Ala Ser Arg Gly Asn His Val Ser Pro Thr His Tyr Val
          210          215          220
Pro Glu Ser Asp Ala Ala Ala Arg Val Thr Ala Ile Leu Ser Ser Leu
  225          230          235          240
Thr Val Thr Gln Leu Leu Arg Arg Leu His Gln Trp Ile Ser Ser Glu
          245          250          255
Cys Thr Thr Pro
          260

```

&lt;210&gt; 9

&lt;211&gt; 1040

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Hepatitis C virus NS5A/B protein sequence

&lt;400&gt; 9

```

Cys Ser Gly Ser Trp Leu Arg Asp Ile Trp Asp Trp Ile Cys Glu Val
  1          5          10          15
Leu Ser Asp Phe Lys Thr Trp Leu Lys Ala Lys Leu Met Pro Gln Leu
          20          25          30
Pro Gly Ile Pro Phe Val Ser Cys Gln Arg Gly Tyr Arg Gly Val Trp
          35          40          45
Arg Gly Asp Gly Ile Met His Thr Arg Cys His Cys Gly Ala Glu Ile
          50          55          60
Thr Gly His Val Lys Asn Gly Thr Met Arg Ile Val Gly Pro Arg Thr
  65          70          75          80
Cys Lys Asn Met Trp Ser Gly Thr Phe Phe Ile Asn Ala Tyr Thr Thr
          85          90          95
Gly Pro Cys Thr Pro Leu Pro Ala Pro Asn Tyr Lys Phe Ala Leu Trp
          100          105          110
Arg Val Ser Ala Glu Glu Tyr Val Glu Ile Arg Arg Val Gly Asp Phe

```

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Ala Arg Lys Ala Val Ala His Ile Asn Ser Val Trp Lys Asp Leu Leu  
 565 570 575  
 Glu Asp Ser Val Thr Pro Ile Asp Thr Thr Ile Met Ala Lys Asn Glu  
 580 585 590  
 Val Phe Cys Val Gln Pro Glu Lys Gly Gly Arg Lys Pro Ala Arg Leu  
 595 600 605  
 Ile Val Phe Pro Asp Leu Gly Val Arg Val Cys Glu Lys Met Ala Leu  
 610 615 620  
 Tyr Asp Val Val Ser Lys Leu Pro Leu Ala Val Met Gly Ser Ser Tyr  
 625 630 635 640  
 Gly Phe Gln Tyr Ser Pro Gly Gln Arg Val Glu Phe Leu Val Gln Ala  
 645 650 655  
 Trp Lys Ser Lys Lys Thr Pro Met Gly Leu Ser Tyr Asp Thr Arg Cys  
 660 665 670  
 Phe Asp Ser Thr Val Thr Glu Ser Asp Ile Arg Thr Glu Glu Ala Ile  
 675 680 685  
 Tyr Gln Cys Cys Asp Leu Asp Pro Gln Ala Arg Val Ala Ile Lys Ser  
 690 695 700  
 Leu Thr Glu Arg Leu Tyr Val Gly Gly Pro Leu Thr Asn Ser Arg Gly  
 705 710 715 720  
 Glu Asn Cys Gly Tyr Arg Arg Cys Arg Ala Ser Arg Val Leu Thr Thr  
 725 730 735  
 Ser Cys Gly Asn Thr Leu Thr Arg Tyr Ile Lys Ala Arg Ala Ala Cys  
 740 745 750  
 Arg Ala Ala Gly Leu Gln Asp Cys Thr Met Leu Val Cys Gly Asp Asp  
 755 760 765  
 Leu Val Val Ile Cys Glu Ser Ala Gly Val Gln Glu Asp Ala Ala Ser  
 770 775 780  
 Leu Arg Ala Phe Thr Glu Ala Met Thr Arg Tyr Ser Ala Pro Pro Gly  
 785 790 795 800  
 Asp Pro Pro Gln Pro Glu Tyr Asp Leu Glu Leu Ile Thr Ser Cys Ser  
 805 810 815  
 Ser Asn Val Ser Val Ala His Asp Gly Ala Gly Lys Arg Val Tyr Tyr  
 820 825 830  
 Leu Thr Arg Asp Pro Thr Thr Pro Leu Ala Arg Ala Ala Trp Glu Thr  
 835 840 845  
 Ala Arg His Thr Pro Val Asn Ser Trp Leu Gly Asn Ile Ile Met Phe  
 850 855 860  
 Ala Pro Thr Leu Trp Ala Arg Met Ile Leu Met Thr His Phe Phe Ser  
 865 870 875 880  
 Val Leu Ile Ala Arg Asp Gln Leu Glu Gln Ala Leu Asn Cys Glu Ile  
 885 890 895  
 Tyr Gly Ala Cys Tyr Ser Ile Glu Pro Leu Asp Leu Pro Pro Ile Ile  
 900 905 910  
 Gln Arg Leu His Gly Leu Ser Ala Phe Ser Leu His Ser Tyr Ser Pro  
 915 920 925  
 Gly Glu Ile Asn Arg Val Ala Ala Cys Leu Arg Lys Leu Gly Val Pro  
 930 935 940  
 Pro Leu Arg Ala Trp Arg His Arg Ala Trp Ser Val Arg Ala Arg Leu  
 945 950 955 960  
 Leu Ala Arg Gly Gly Lys Ala Ala Ile Cys Gly Lys Tyr Leu Phe Asn  
 965 970 975  
 Trp Ala Val Arg Thr Lys Leu Lys Leu Thr Pro Ile Thr Ala Ala Gly  
 980 985 990  
 Arg Leu Asp Leu Ser Gly Trp Phe Thr Ala Gly Tyr Ser Gly Gly Asp



	995		1000		1005										
Ile	Tyr	His	Ser	Val	Ser	His	Ala	Arg	Pro	Arg	Trp	Phe	Trp	Phe	Cys
	1010				1015					1020					
Leu	Leu	Leu	Leu	Ala	Ala	Gly	Val	Gly	Ile	Tyr	Leu	Leu	Pro	Asn	Arg
1025				1030					1035					1040	

<210> 10  
 <211> 226  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Hepatitis B virus S antigen (HBsAg) sequence

<400> 10  
 Met Glu Asn Ile Thr Ser Gly Phe Leu Gly Pro Leu Leu Val Leu Gln  
 1 5 10 15  
 Ala Gly Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro Gln Ser Leu  
 20 25 30  
 Asp Ser Trp Trp Thr Ser Leu Asn Phe Leu Gly Gly Thr Thr Val Cys  
 35 40 45  
 Leu Gly Gln Asn Ser Gln Ser Pro Thr Ser Asn His Ser Pro Thr Ser  
 50 55 60  
 Cys Pro Pro Thr Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe  
 65 70 75 80  
 Ile Ile Phe Leu Phe Ile Leu Leu Leu Cys Leu Ile Phe Leu Leu Val  
 85 90 95  
 Leu Leu Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Ile Pro Gly  
 100 105 110  
 Ser Ser Thr Thr Ser Thr Gly Pro Cys Arg Thr Cys Met Thr Thr Ala  
 115 120 125  
 Gln Gly Thr Ser Met Tyr Pro Ser Cys Cys Cys Thr Lys Pro Ser Asp  
 130 135 140  
 Gly Asn Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Gly Lys  
 145 150 155 160  
 Phe Leu Trp Glu Trp Ala Ser Ala Arg Phe Ser Trp Leu Ser Leu Leu  
 165 170 175  
 Val Pro Phe Val Gln Trp Phe Val Gly Leu Ser Pro Thr Val Trp Leu  
 180 185 190  
 Ser Val Ile Trp Met Met Trp Tyr Trp Gly Pro Ser Leu Tyr Ser Ile  
 195 200 205  
 Leu Ser Pro Phe Leu Pro Leu Leu Pro Ile Phe Phe Cys Leu Trp Val  
 210 215 220  
 Tyr Ile  
 225

<210> 11  
 <211> 212  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Hepatitis B virus C antigen and e antigen

## (HBcAg/HBeAg) sequence

&lt;400&gt; 11

```

Met Gln Leu Phe His Leu Cys Leu Ile Ile Ser Cys Ser Cys Pro Thr
 1              5              10              15
Val Gln Ala Ser Lys Leu Cys Leu Gly Trp Leu Trp Gly Met Asp Ile
              20              25              30
Asp Pro Tyr Lys Glu Phe Gly Ala Thr Val Glu Leu Leu Ser Phe Leu
              35              40              45
Pro Ser Asp Phe Phe Pro Ser Val Arg Asp Leu Leu Asp Thr Ala Ser
              50              55              60
Ala Leu Tyr Arg Glu Ala Leu Glu Ser Pro Glu His Cys Ser Pro His
65              70              75              80
His Thr Ala Leu Arg Gln Ala Ile Leu Cys Trp Gly Glu Leu Met Thr
              85              90              95
Leu Ala Thr Trp Val Gly Val Asn Leu Glu Asp Pro Ala Ser Arg Asp
              100             105             110
Leu Val Val Ser Tyr Val Asn Thr Asn Met Gly Leu Lys Phe Arg Gln
              115             120             125
Leu Leu Trp Phe His Ile Ser Cys Leu Thr Phe Gly Arg Glu Thr Val
              130             135             140
Ile Glu Tyr Leu Val Ser Phe Gly Val Trp Ile Arg Thr Pro Pro Ala
145             150             155             160
Tyr Arg Pro Pro Asn Ala Pro Ile Leu Ser Thr Leu Pro Glu Thr Thr
              165             170             175
Val Val Arg Arg Arg Gly Arg Ser Pro Arg Arg Arg Thr Pro Ser Pro
              180             185             190
Arg Arg Arg Arg Ser Gln Ser Pro Arg Arg Arg Arg Ser Gln Ser Arg
              195             200             205
Glu Ser Gln Cys
              210

```

&lt;210&gt; 12

&lt;211&gt; 2227

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Hepatitis A virus sequence

&lt;400&gt; 12

```

Met Asn Met Ser Lys Gln Gly Ile Phe Gln Thr Val Gly Ser Gly Leu
 1              5              10              15
Asp His Ile Leu Ser Leu Ala Asp Ile Glu Glu Glu Gln Met Ile Gln
              20              25              30
Ser Val Asp Arg Thr Ala Val Thr Gly Ala Ser Tyr Phe Thr Ser Val
              35              40              45
Asp Gln Ser Ser Val His Thr Ala Glu Val Gly Ser His Gln Ile Glu
              50              55              60
Pro Leu Lys Thr Ser Val Asp Lys Pro Gly Ser Lys Lys Thr Gln Gly
65              70              75              80
Glu Lys Phe Phe Leu Ile His Ser Ala Asp Trp Leu Thr Thr His Ala
              85              90              95
Leu Phe His Glu Val Ala Lys Leu Asp Val Val Lys Leu Leu Tyr Asn

```

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Gln Gln Leu Asn Asp Pro Val Leu Ala Lys Lys Val Pro Glu Thr Phe  
 545 550 555 560  
 Pro Glu Leu Lys Pro Gly Glu Ser Arg His Thr Ser Asp His Met Ser  
 565 570 575  
 Ile Tyr Lys Phe Met Gly Arg Ser His Phe Leu Cys Thr Phe Thr Phe  
 580 585 590  
 Asn Ser Asn Asn Lys Glu Tyr Thr Phe Pro Ile Thr Leu Ser Ser Thr  
 595 600 605  
 Ser Asn Pro Pro His Gly Leu Pro Ser Thr Leu Arg Trp Phe Phe Asn  
 610 615 620  
 Leu Phe Gln Leu Tyr Arg Gly Pro Leu Asp Leu Thr Ile Ile Ile Thr  
 625 630 635 640  
 Gly Ala Thr Asp Val Asp Gly Met Ala Trp Phe Thr Pro Val Gly Leu  
 645 650 655  
 Ala Val Asp Pro Trp Val Glu Lys Glu Ser Ala Leu Ser Ile Asp Tyr  
 660 665 670  
 Lys Thr Ala Leu Gly Ala Val Arg Phe Asn Thr Arg Arg Thr Gly Asn  
 675 680 685  
 Ile Gln Ile Arg Leu Pro Trp Tyr Ser Tyr Leu Tyr Ala Val Ser Gly  
 690 695 700  
 Ala Leu Asp Gly Leu Gly Asp Lys Thr Asp Ser Thr Phe Gly Leu Phe  
 705 710 715 720  
 Leu Phe Glu Ile Ala Asn Tyr Asn His Ser Asp Glu Tyr Leu Ser Phe  
 725 730 735  
 Ser Cys Tyr Leu Ser Val Thr Glu Gln Ser Glu Phe Tyr Phe Pro Arg  
 740 745 750  
 Ala Pro Leu Asn Ser Asn Ala Met Leu Ser Thr Glu Ser Met Met Ser  
 755 760 765  
 Arg Ile Ala Ala Gly Asp Leu Glu Ser Ser Val Asp Asp Pro Arg Ser  
 770 775 780  
 Glu Glu Asp Arg Arg Phe Glu Ser His Ile Glu Cys Arg Lys Pro Tyr  
 785 790 795 800  
 Lys Glu Leu Arg Leu Glu Val Gly Lys Gln Arg Leu Lys Tyr Ala Gln  
 805 810 815  
 Glu Glu Leu Ser Asn Glu Val Leu Pro Pro Arg Lys Met Lys Gly  
 820 825 830  
 Leu Phe Ser Gln Ala Lys Ile Ser Leu Phe Tyr Thr Glu Glu His Glu  
 835 840 845  
 Ile Met Lys Phe Ser Trp Arg Gly Val Thr Ala Asp Thr Arg Ala Leu  
 850 855 860  
 Arg Arg Phe Gly Phe Ser Leu Ala Ala Gly Arg Ser Val Trp Thr Leu  
 865 870 875 880  
 Glu Met Asp Ala Gly Val Leu Thr Gly Arg Leu Ile Arg Leu Asn Asp  
 885 890 895  
 Glu Lys Trp Thr Glu Met Lys Asp Asp Lys Ile Val Ser Leu Ile Glu  
 900 905 910  
 Lys Phe Thr Ser Asn Lys Tyr Trp Ser Lys Val Asn Phe Pro His Gly  
 915 920 925  
 Met Leu Asp Leu Glu Glu Ile Ala Ala Asn Ser Lys Asp Phe Pro Asn  
 930 935 940  
 Met Ser Glu Thr Asp Leu Cys Phe Leu Leu His Trp Leu Asn Pro Lys  
 945 950 955 960  
 Lys Ile Asn Leu Ala Asp Arg Met Leu Gly Leu Ser Gly Val Gln Glu  
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Asp Ser Ile Ala Gly Thr Leu Lys Ser Met Met Phe Gly Phe His His		
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Ser Val Thr Val Glu Ile Ile Asn Thr Val Leu Cys Phe Val Lys Ser		
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Gly Ile Leu Leu Tyr Val Ile Gln Gln Leu Asn Gln Asp Glu His Ser		
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His Ile Ile Gly Leu Leu Arg Val Met Asn Tyr Ala Asp Ile Gly Cys		1040
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Ser Val Ile Ser Cys Gly Lys Val Phe Ser Lys Met Leu Glu Thr Val		1055
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Phe Asn Trp Gln Met Asp Ser Arg Met Met Glu Leu Arg Thr Gln Ser		1070
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Phe Ser Asn Trp Leu Arg Asp Ile Cys Ser Gly Ile Thr Ile Phe Lys		1085
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Ser Phe Lys Asp Ala Ile Tyr Trp Leu Tyr Thr Lys Leu Lys Asp Phe		1100
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Tyr Glu Val Asn Tyr Gly Lys Lys Lys Asp Ile Leu Asn Ile Leu Lys		1120
	1125	1130
Asp Asn Gln Gln Lys Ile Glu Lys Ala Ile Glu Glu Ala Asp Asn Phe		1135
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Cys Ile Leu Gln Ile Gln Asp Val Glu Lys Phe Asp Gln Tyr Gln Lys		1150
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Gly Val Asp Leu Ile Gln Lys Leu Arg Thr Val His Ser Met Ala Gln		1165
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Ala Arg Val His Gln Lys Leu Lys Asn Leu Gly Ser Ile Asn Gln Ala		1200
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Met Val Thr Arg Cys Glu Pro Val Val Cys Tyr Leu Tyr Gly Lys Arg		1215
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Lys His Tyr Gly Val Glu Pro Glu Lys Asn Ile Tyr Thr Lys Pro Val		1245
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Ala Ser Asp Tyr Trp Asp Gly Tyr Ser Gly Gln Leu Val Cys Ile Ile		1260
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Gln Leu Val Ser Gly Cys Pro Met Arg Leu Asn Met Ala Ser Leu Glu		1295
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Glu Lys Gly Arg His Phe Ser Ser Pro Phe Ile Ile Ala Thr Ser Asn		1310
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Ala Ile Lys Asp Met Ser Cys Val Asp Leu Ile Met Asp Gly His Asn		1375
	1380	1385
Ile Ser Leu Met Asp Leu Leu Ser Ser Leu Val Met Thr Val Glu Ile		1390
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Arg Lys Gln Asn Met Ser Glu Phe Met Glu Leu Trp Ser Gln Gly Ile		1405
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Asp Leu Ser					
2225					

&lt;210&gt; 13

&lt;211&gt; 9416

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Hepatitis C virus sequence

&lt;400&gt; 13

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&lt;210&gt; 14

&lt;211&gt; 3182

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Hepatitis B virus sequence

&lt;400&gt; 14

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&lt;210&gt; 15

&lt;211&gt; 7478

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Hepatitis A virus sequence

&lt;400&gt; 15

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&lt;210&gt; 16

&lt;211&gt; 2061

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Hepatitis C virus NS3/4A, coding region

&lt;400&gt; 16

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aaactgggtcg cgttgggcgt caatgccgtg gcttactacc gcggccttga tgtgtccgtc 1200
atcccgacca gtggtgacgt tgtcgtcgtg gcaactgacg ccctcatgac cggctttacc 1260
ggcgacttcg attcggtgat agactgcaac acgtgtgtca ccagacagat cgacttcagc 1320
cttgacccta ccttcaccat tgagacaatc acgcttcccc aggatgctgt ctcccgtact 1380
caacgtcggg gtaggactgg cagagggaag ccaggcatct acagatttgt ggcaccgggg 1440
gagcgtcctt ctggcatgtt tgactcgtct gtcctctgcg agtgctatga cgcgggttgt 1500
gcttggtatg agcttacgcc cgccgagacc acagttaggc tacgagcata catgaacacc 1560
ccgggacttc ccgtgtgcc aagaccatct gaattttggg agggcgtctt tacgggtctc 1620
acccacatag acgcccactt cctatcccag acaaagcaga gtggggaaaa cctccctat 1680
ctggtagcgt accaagccac cgtgtgcgt agagctcaag cccctcccc gtcgtgggac 1740
cagatgtgga agtgcttgat ccgtctcaag cccaccctcc atgggccaac acctctgcta 1800
tatagactgg gcgctgtcca gaatgaagtc accctgacgc acccagtcac caagtatatc 1860
atgacatgta tgtcggctga cctggaggtc gtcacgagta cctgggtgct cgttggcggc 1920
gttctggctg ctttggccgc gtattgccta tccacaggct gcgtggtcat agtaggtagg 1980
attgtcttgt ccgaaagcc ggcaatcata cccgacaggg aagtcctcta ccgggagttc 2040
gatgaaatgg aagagtgtg a 2061

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&lt;210&gt; 17

&lt;211&gt; 686

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Hepatitis C virus NS3/4A peptide

&lt;400&gt; 17

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Met Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr Arg Gly Leu Leu Gly
 1           5           10           15
Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu Gly
          20           25           30
Glu Val Gln Ile Val Ser Thr Ala Ala Gln Thr Phe Leu Ala Thr Cys
          35           40           45
Ile Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Thr Arg Thr
          50           55           60
Ile Ala Ser Pro Lys Gly Pro Val Ile Gln Met Tyr Thr Asn Val Asp
65           70           75           80
Gln Asp Leu Val Gly Trp Pro Ala Pro Gln Gly Ala Arg Ser Leu Thr
          85           90           95
Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr Arg His Ala
          100          105          110
Asp Val Ile Pro Val Arg Arg Arg Gly Asp Gly Arg Gly Ser Leu Leu
          115          120          125
Ser Pro Arg Pro Ile Ser Tyr Leu Lys Gly Ser Ser Gly Gly Pro Leu
          130          135          140
Leu Cys Pro Ala Gly His Ala Val Gly Ile Phe Arg Ala Ala Val Cys
145          150          155          160
Thr Arg Gly Val Ala Lys Ala Val Asp Phe Ile Pro Val Glu Ser Leu
          165          170          175

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Glu Thr Thr Met Arg Ser Pro Val Phe Ser Asp Asn Ser Ser Pro Pro  
 180 185 190  
 Ala Val Pro Gln Ser Tyr Gln Val Ala His Leu His Ala Pro Thr Gly  
 195 200 205  
 Ser Gly Lys Ser Thr Lys Val Pro Ala Ala Tyr Ala Ala Gln Gly Tyr  
 210 215 220  
 Lys Val Leu Val Leu Asn Pro Ser Val Ala Ala Thr Met Gly Phe Gly  
 225 230 235 240  
 Ala Tyr Met Ser Lys Ala His Gly Ile Asp Pro Asn Ile Arg Thr Gly  
 245 250 255  
 Val Arg Thr Ile Thr Thr Gly Ser Pro Ile Thr Tyr Ser Thr Tyr Gly  
 260 265 270  
 Lys Phe Leu Ala Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile Ile  
 275 280 285  
 Ile Cys Asp Glu Cys His Ser Thr Asp Ala Thr Ser Ile Leu Gly Ile  
 290 295 300  
 Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Thr Val  
 305 310 315 320  
 Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr Val Pro His Pro Asn  
 325 330 335  
 Ile Glu Glu Val Ala Leu Ser Thr Thr Gly Glu Ile Pro Phe Tyr Gly  
 340 345 350  
 Lys Ala Ile Pro Leu Glu Ala Ile Lys Gly Gly Arg His Leu Ile Phe  
 355 360 365  
 Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Val Ala  
 370 375 380  
 Leu Gly Val Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val  
 385 390 395 400  
 Ile Pro Thr Ser Gly Asp Val Val Val Val Ala Thr Asp Ala Leu Met  
 405 410 415  
 Thr Gly Phe Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr Cys  
 420 425 430  
 Val Thr Gln Thr Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile Glu  
 435 440 445  
 Thr Ile Thr Leu Pro Gln Asp Ala Val Ser Arg Thr Gln Arg Arg Gly  
 450 455 460  
 Arg Thr Gly Arg Gly Lys Pro Gly Ile Tyr Arg Phe Val Ala Pro Gly  
 465 470 475 480  
 Glu Arg Pro Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys Tyr  
 485 490 495  
 Asp Ala Gly Cys Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Thr Val  
 500 505 510  
 Arg Leu Arg Ala Tyr Met Asn Thr Pro Gly Leu Pro Val Cys Gln Asp  
 515 520 525  
 His Leu Glu Phe Trp Glu Gly Val Phe Thr Gly Leu Thr His Ile Asp  
 530 535 540  
 Ala His Phe Leu Ser Gln Thr Lys Gln Ser Gly Glu Asn Leu Pro Tyr  
 545 550 555 560  
 Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro Pro  
 565 570 575  
 Pro Ser Trp Asp Gln Met Trp Lys Cys Leu Ile Arg Leu Lys Pro Thr  
 580 585 590  
 Leu His Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln Asn  
 595 600 605  
 Glu Val Thr Leu Thr His Pro Val Thr Lys Tyr Ile Met Thr Cys Met



610	615	620
Ser Ala Asp Leu Glu Val Val Thr Ser Thr Trp Val Leu Val Gly Gly		
625	630	635
Val Leu Ala Ala Leu Ala Ala Tyr Cys Leu Ser Thr Gly Cys Val Val		640
	645	650
Ile Val Gly Arg Ile Val Leu Ser Gly Lys Pro Ala Ile Ile Pro Asp		655
	660	665
Arg Glu Val Leu Tyr Arg Glu Phe Asp Glu Met Glu Glu Cys		670
	675	680
		685

<210> 18  
 <211> 30  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> cloning oligonucleotide

<400> 18  
 ccgtctagat cagcactctt ccatttcac 30

<210> 19  
 <211> 30  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> cloning oligonucleotide

<400> 19  
 cctgaattca tggcgcctat cacggcctat 30

<210> 20  
 <211> 24  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> cloning oligonucleotide

<400> 20  
 ccacgcggcc gcgacgacct acag 24

<210> 21  
 <211> 33  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> cloning oligonucleotide

<400> 21  
 ctggaggtcg tcacgcctac ctgggtgctc gtt 33

<210> 22  
 <211> 33  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> cloning oligonucleotide

<400> 22  
 accgagcacc caggtaggcg tgacgacctc cag 33

<210> 23  
 <211> 33  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> cloning oligonucleotide

<400> 23  
 ctggaggtcg tccgcggtac ctgggtgctc gtt 33

<210> 24  
 <211> 33  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> cloning oligonucleotide

<400> 24  
 accgagcacc caggtaccgc ggacgacctc cag 33

<210> 25  
 <211> 25  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Hepatitis C virus NS3/4A peptide

<400> 25  
 Thr Lys Tyr Met Thr Cys Met Ser Ala Asp Leu Glu Val Val Thr Ser  
 1 5 10 15  
 Thr Trp Val Leu Val Gly Gly Val Leu  
 20 25

<210> 26  
 <211> 25  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Mutant Hepatitis C virus NS3/4A peptide

&lt;400&gt; 26

Thr Lys Tyr Met Thr Cys Met Ser Ala Asp Leu Glu Val Val Thr Gly  
 1 5 10 15  
 Thr Trp Val Leu Val Gly Gly Val Leu  
 20 25

&lt;210&gt; 27

&lt;211&gt; 25

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Mutant Hepatitis C virus NS3/4A peptide

&lt;400&gt; 27

Thr Lys Tyr Met Thr Cys Met Ser Ala Asp Leu Glu Val Val Arg Gly  
 1 5 10 15  
 Thr Trp Val Leu Val Gly Gly Val Leu  
 20 25

&lt;210&gt; 28

&lt;211&gt; 12

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Hepatitis C virus NS3/4A peptide

&lt;400&gt; 28

Ser Ala Asp Leu Glu Val Val Thr Ser Thr Trp Val  
 1 5 10

&lt;210&gt; 29

&lt;211&gt; 632

&lt;212&gt; PRT

&lt;213&gt; Hepatitis C virus NS3 peptideArtificial Sequence

&lt;220&gt;

&lt;223&gt; Hepatitis C virus NS3 peptide

&lt;400&gt; 29

Met Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr Arg Gly Leu Leu Gly  
 1 5 10 15  
 Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu Gly  
 20 25 30  
 Glu Val Gln Ile Val Ser Thr Ala Ala Gln Thr Phe Leu Ala Thr Cys  
 35 40 45  
 Ile Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Thr Arg Thr  
 50 55 60  
 Ile Ala Ser Pro Lys Gly Pro Val Ile Gln Met Tyr Thr Asn Val Asp  
 65 70 75 80

Gln Asp Leu Val Gly Trp Pro Ala Pro Gln Gly Ala Arg Ser Leu Thr  
                                   85                                  90                                  95  
 Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr Arg His Ala  
                                   100                                  105                                  110  
 Asp Val Ile Pro Val Arg Arg Arg Gly Asp Gly Arg Gly Ser Leu Leu  
                                   115                                  120                                  125  
 Ser Pro Arg Pro Ile Ser Tyr Leu Lys Gly Ser Ser Gly Gly Pro Leu  
                                   130                                  135                                  140  
 Leu Cys Pro Ala Gly His Ala Val Gly Ile Phe Arg Ala Ala Val Cys  
 145                                  150                                  155                                  160  
 Thr Arg Gly Val Ala Lys Ala Val Asp Phe Ile Pro Val Glu Ser Leu  
                                   165                                  170                                  175  
 Glu Thr Thr Met Arg Ser Pro Val Phe Ser Asp Asn Ser Ser Pro Pro  
                                   180                                  185                                  190  
 Ala Val Pro Gln Ser Tyr Gln Val Ala His Leu His Ala Pro Thr Gly  
                                   195                                  200                                  205  
 Ser Gly Lys Ser Thr Lys Val Pro Ala Ala Tyr Ala Ala Gln Gly Tyr  
                                   210                                  215                                  220  
 Lys Val Leu Val Leu Asn Pro Ser Val Ala Ala Thr Met Gly Phe Gly  
 225                                  230                                  235                                  240  
 Ala Tyr Met Ser Lys Ala His Gly Ile Asp Pro Asn Ile Arg Thr Gly  
                                   245                                  250                                  255  
 Val Arg Thr Ile Thr Thr Gly Ser Pro Ile Thr Tyr Ser Thr Tyr Gly  
                                   260                                  265                                  270  
 Lys Phe Leu Ala Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile Ile  
                                   275                                  280                                  285  
 Ile Cys Asp Glu Cys His Ser Thr Asp Ala Thr Ser Ile Leu Gly Ile  
                                   290                                  295                                  300  
 Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Thr Val  
 305                                  310                                  315                                  320  
 Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr Val Pro His Pro Asn  
                                   325                                  330                                  335  
 Ile Glu Glu Val Ala Leu Ser Thr Thr Gly Glu Ile Pro Phe Tyr Gly  
                                   340                                  345                                  350  
 Lys Ala Ile Pro Leu Glu Ala Ile Lys Gly Gly Arg His Leu Ile Phe  
                                   355                                  360                                  365  
 Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Val Ala  
                                   370                                  375                                  380  
 Leu Gly Val Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val  
 385                                  390                                  395                                  400  
 Ile Pro Thr Ser Gly Asp Val Val Val Val Ala Thr Asp Ala Leu Met  
                                   405                                  410                                  415  
 Thr Gly Phe Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr Cys  
                                   420                                  425                                  430  
 Val Thr Gln Thr Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile Glu  
                                   435                                  440                                  445  
 Thr Ile Thr Leu Pro Gln Asp Ala Val Ser Arg Thr Gln Arg Arg Gly  
                                   450                                  455                                  460  
 Arg Thr Gly Arg Gly Lys Pro Gly Ile Tyr Arg Phe Val Ala Pro Gly  
 465                                  470                                  475                                  480  
 Glu Arg Pro Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys Tyr  
                                   485                                  490                                  495  
 Asp Ala Gly Cys Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Thr Val  
                                   500                                  505                                  510  
 Arg Leu Arg Ala Tyr Met Asn Thr Pro Gly Leu Pro Val Cys Gln Asp

```

          515                      520                      525
His Leu Glu Phe Trp Glu Gly Val Phe Thr Gly Leu Thr His Ile Asp
          530                      535                      540
Ala His Phe Leu Ser Gln Thr Lys Gln Ser Gly Glu Asn Leu Pro Tyr
545                      550                      555                      560
Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro Pro
          565                      570                      575
Pro Ser Trp Asp Gln Met Trp Lys Cys Leu Ile Arg Leu Lys Pro Thr
          580                      585                      590
Leu His Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln Asn
          595                      600                      605
Glu Val Thr Leu Thr His Pro Val Thr Lys Tyr Ile Met Thr Cys Met
          610                      615                      620
Ser Ala Asp Leu Glu Val Val Thr
625                      630

```

<210> 30  
 <211> 54  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Hepatitis C virus NS4A peptide

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<400> 30
Ser Thr Trp Val Leu Val Gly Gly Val Leu Ala Ala Leu Ala Ala Tyr
 1              5              10              15
Cys Leu Ser Thr Gly Cys Val Val Ile Val Gly Arg Ile Val Leu Ser
          20              25              30
Gly Lys Pro Ala Ile Ile Pro Asp Arg Glu Val Leu Tyr Arg Glu Phe
          35              40              45
Asp Glu Met Glu Glu Cys
          50

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<210> 31  
 <211> 686  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Mutant Hepatitis C virus NS3/4A

```

<400> 31
Met Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr Arg Gly Leu Leu Gly
 1              5              10              15
Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu Gly
          20              25              30
Glu Val Gln Ile Val Ser Thr Ala Ala Gln Thr Phe Leu Ala Thr Cys
          35              40              45
Ile Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Thr Arg Thr
          50              55              60
Ile Ala Ser Pro Lys Gly Pro Val Ile Gln Met Tyr Thr Asn Val Asp
65              70              75              80

```

Gln	Asp	Leu	Val	Gly	Trp	Pro	Ala	Pro	Gln	Gly	Ala	Arg	Ser	Leu	Thr
				85					90					95	
Pro	Cys	Thr	Cys	Gly	Ser	Ser	Asp	Leu	Tyr	Leu	Val	Thr	Arg	His	Ala
			100					105					110		
Asp	Val	Ile	Pro	Val	Arg	Arg	Arg	Gly	Asp	Gly	Arg	Gly	Ser	Leu	Leu
		115					120					125			
Ser	Pro	Arg	Pro	Ile	Ser	Tyr	Leu	Lys	Gly	Ser	Ser	Gly	Gly	Pro	Leu
	130					135					140				
Leu	Cys	Pro	Ala	Gly	His	Ala	Val	Gly	Ile	Phe	Arg	Ala	Ala	Val	Cys
145					150					155					160
Thr	Arg	Gly	Val	Ala	Lys	Ala	Val	Asp	Phe	Ile	Pro	Val	Glu	Ser	Leu
				165					170						175
Glu	Thr	Thr	Met	Arg	Ser	Pro	Val	Phe	Ser	Asp	Asn	Ser	Ser	Pro	Pro
			180					185					190		
Ala	Val	Pro	Gln	Ser	Tyr	Gln	Val	Ala	His	Leu	His	Ala	Pro	Thr	Gly
		195					200					205			
Ser	Gly	Lys	Ser	Thr	Lys	Val	Pro	Ala	Ala	Tyr	Ala	Ala	Gln	Gly	Tyr
	210					215					220				
Lys	Val	Leu	Val	Leu	Asn	Pro	Ser	Val	Ala	Ala	Thr	Met	Gly	Phe	Gly
225					230					235					240
Ala	Tyr	Met	Ser	Lys	Ala	His	Gly	Ile	Asp	Pro	Asn	Ile	Arg	Thr	Gly
				245					250					255	
Val	Arg	Thr	Ile	Thr	Thr	Gly	Ser	Pro	Ile	Thr	Tyr	Ser	Thr	Tyr	Gly
			260				265						270		
Lys	Phe	Leu	Ala	Asp	Gly	Gly	Cys	Ser	Gly	Gly	Ala	Tyr	Asp	Ile	Ile
	275						280					285			
Ile	Cys	Asp	Glu	Cys	His	Ser	Thr	Asp	Ala	Thr	Ser	Ile	Leu	Gly	Ile
	290					295					300				
Gly	Thr	Val	Leu	Asp	Gln	Ala	Glu	Thr	Ala	Gly	Ala	Arg	Leu	Thr	Val
305					310					315					320
Leu	Ala	Thr	Ala	Thr	Pro	Pro	Gly	Ser	Val	Thr	Val	Pro	His	Pro	Asn
				325					330					335	
Ile	Glu	Glu	Val	Ala	Leu	Ser	Thr	Thr	Gly	Glu	Ile	Pro	Phe	Tyr	Gly
			340					345					350		
Lys	Ala	Ile	Pro	Leu	Glu	Ala	Ile	Lys	Gly	Gly	Arg	His	Leu	Ile	Phe
	355						360					365			
Cys	His	Ser	Lys	Lys	Lys	Cys	Asp	Glu	Leu	Ala	Ala	Lys	Leu	Val	Ala
	370					375					380				
Leu	Gly	Val	Asn	Ala	Val	Ala	Tyr	Tyr	Arg	Gly	Leu	Asp	Val	Ser	Val
385					390					395					400
Ile	Pro	Thr	Ser	Gly	Asp	Val	Val	Val	Val	Ala	Thr	Asp	Ala	Leu	Met
				405					410					415	
Thr	Gly	Phe	Thr	Gly	Asp	Phe	Asp	Ser	Val	Ile	Asp	Cys	Asn	Thr	Cys
			420				425						430		
Val	Thr	Gln	Thr	Val	Asp	Phe	Ser	Leu	Asp	Pro	Thr	Phe	Thr	Ile	Glu
	435						440					445			
Thr	Ile	Thr	Leu	Pro	Gln	Asp	Ala	Val	Ser	Arg	Thr	Gln	Arg	Arg	Gly
	450					455					460				
Arg	Thr	Gly	Arg	Gly	Lys	Pro	Gly	Ile	Tyr	Arg	Phe	Val	Ala	Pro	Gly
465					470					475					480
Glu	Arg	Pro	Ser	Gly	Met	Phe	Asp	Ser	Ser	Val	Leu	Cys	Glu	Cys	Tyr
				485					490					495	
Asp	Ala	Gly	Cys	Ala	Trp	Tyr	Glu	Leu	Thr	Pro	Ala	Glu	Thr	Thr	Val
			500					505					510		
Arg	Leu	Arg	Ala	Tyr	Met	Asn	Thr	Pro	Gly	Leu	Pro	Val	Cys	Gln	Asp

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<210> 32
<211> 686
<212> PRT
<213> Artificial Sequence
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<400> 32															
Met	Ala	Pro	Ile	Thr	Ala	Tyr	Ala	Gln	Gln	Thr	Arg	Gly	Leu	Leu	Gly
1				5					10					15	
Cys	Ile	Ile	Thr	Ser	Leu	Thr	Gly	Arg	Asp	Lys	Asn	Gln	Val	Glu	Gly
			20					25					30		
Glu	Val	Gln	Ile	Val	Ser	Thr	Ala	Ala	Gln	Thr	Phe	Leu	Ala	Thr	Cys
		35					40					45			
Ile	Asn	Gly	Val	Cys	Trp	Thr	Val	Tyr	His	Gly	Ala	Gly	Thr	Arg	Thr
	50					55				60					
Ile	Ala	Ser	Pro	Lys	Gly	Pro	Val	Ile	Gln	Met	Tyr	Thr	Asn	Val	Asp
65					70					75					80
Gln	Asp	Leu	Val	Gly	Trp	Pro	Ala	Pro	Gln	Gly	Ala	Arg	Ser	Leu	Thr
				85					90					95	
Pro	Cys	Thr	Cys	Gly	Ser	Ser	Asp	Leu	Tyr	Leu	Val	Thr	Arg	His	Ala
			100					105					110		
Asp	Val	Ile	Pro	Val	Arg	Arg	Arg	Gly	Asp	Gly	Arg	Gly	Ser	Leu	Leu
		115					120					125			
Ser	Pro	Arg	Pro	Ile	Ser	Tyr	Leu	Lys	Gly	Ser	Ser	Gly	Gly	Pro	Leu
	130					135					140				
Leu	Cys	Pro	Ala	Gly	His	Ala	Val	Gly	Ile	Phe	Arg	Ala	Ala	Val	Cys
145					150					155					160
Thr	Arg	Gly	Val	Ala	Lys	Ala	Val	Asp	Phe	Ile	Pro	Val	Glu	Ser	Leu
				165					170					175	
Glu	Thr	Thr	Met	Arg	Ser	Pro	Val	Phe	Ser	Asp	Asn	Ser	Ser	Pro	Pro

- 40 -



Ser Ala Asp Leu Glu Val Val Arg Gly Thr Trp Val Leu Val Gly Gly  
 625 630 635 640  
 Val Leu Ala Ala Leu Ala Ala Tyr Cys Leu Ser Thr Gly Cys Val Val  
 645 650 655  
 Ile Val Gly Arg Ile Val Leu Ser Gly Lys Pro Ala Ile Ile Pro Asp  
 660 665 670  
 Arg Glu Val Leu Tyr Arg Glu Phe Asp Glu Met Glu Glu Cys  
 675 680 685

&lt;210&gt; 33

&lt;211&gt; 25

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Mutant Hepatitis C virus NS3/4A peptide

&lt;400&gt; 33

Thr Lys Tyr Met Thr Cys Met Ser Ala Asp Leu Glu Val Val Thr Pro  
 1 5 10 15  
 Thr Trp Val Leu Val Gly Gly Val Leu  
 20 25

&lt;210&gt; 34

&lt;211&gt; 25

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Mutant Hepatitis C virus NS3/4A peptide

&lt;400&gt; 34

Thr Lys Tyr Met Thr Cys Met Ser Ala Asp Leu Glu Val Val Arg Pro  
 1 5 10 15  
 Thr Trp Val Leu Val Gly Gly Val Leu  
 20 25

&lt;210&gt; 35

&lt;211&gt; 25

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Mutant Hepatitis C virus NS3/4A peptide

&lt;400&gt; 35

Thr Lys Tyr Met Thr Cys Met Ser Ala Asp Leu Glu Val Val Arg Pro  
 1 5 10 15  
 Ala Trp Val Leu Val Gly Gly Val Leu  
 20 25

<210> 36  
 <211> 25  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Mutant Hepatitis C virus, NS3/4A peptide

<400> 36  
 Thr Lys Tyr Met Thr Cys Met Ser Ala Asp Leu Glu Val Val Cys Ser  
 1 5 10 15  
 Thr Trp Val Leu Val Gly Gly Val Leu  
 20 25

<210> 37  
 <211> 25  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Mutant Hepatitis C virus NS3/4A peptide

<400> 37  
 Thr Lys Tyr Met Thr Cys Met Ser Ala Asp Leu Glu Val Cys Cys Ser  
 1 5 10 15  
 Thr Trp Val Leu Val Gly Gly Val Leu  
 20 25

<210> 38  
 <211> 25  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Mutant Hepatitis C virus NS3/4A peptide

<400> 38  
 Thr Lys Tyr Met Thr Cys Met Ser Ala Asp Leu Glu Val Ser Ser Ser  
 1 5 10 15  
 Thr Trp Val Leu Val Gly Gly Val Leu  
 20 25

<210> 39  
 <211> 25  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Mutant Hepatitis C virus NS3/4A peptide

<400> 39  
 Thr Lys Tyr Met Thr Cys Met Ser Ala Asp Ser Ser Ser Ser Cys Ser

1 5 10 15  
 Thr Trp Val Leu Val Gly Gly Val Leu  
 20 25

<210> 40  
 <211> 25  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Mutant Hepatitis C virus NS3/4A peptide

<400> 40  
 Thr Lys Tyr Met Thr Cys Met Ser Ala Asp Val Val Val Val Thr Ser  
 1 5 10 15  
 Thr Trp Val Leu Val Gly Gly Val Leu  
 20 25

<210> 41  
 <211> 16  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Hepatitis C virus NS5 peptide

<400> 41  
 Ala Ser Glu Asp Val Val Cys Cys Ser Met Ser Tyr Thr Trp Thr Gly  
 1 5 10 15

<210> 42  
 <211> 18  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Mutant Hepatitis C virus NS5A/B peptide

<400> 42  
 Ser Ser Glu Asp Val Val Cys Cys Ser Met Trp Val Leu Val Gly Gly  
 1 5 10 15  
 Val Leu

<210> 43  
 <211> 686  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Mutant Hepatitis C virus NS3/4A

&lt;400&gt; 43

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Met Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr Arg Gly Leu Leu Gly
 1           5           10           15
Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu Gly
      20           25           30
Glu Val Gln Ile Val Ser Thr Ala Ala Gln Thr Phe Leu Ala Thr Cys
      35           40           45
Ile Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Thr Arg Thr
      50           55           60
Ile Ala Ser Pro Lys Gly Pro Val Ile Gln Met Tyr Thr Asn Val Asp
65           70           75           80
Gln Asp Leu Val Gly Trp Pro Ala Pro Gln Gly Ala Arg Ser Leu Thr
      85           90           95
Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr Arg His Ala
      100          105          110
Asp Val Ile Pro Val Arg Arg Arg Gly Asp Gly Arg Gly Ser Leu Leu
      115          120          125
Ser Pro Arg Pro Ile Ser Tyr Leu Lys Gly Ser Ser Gly Gly Pro Leu
      130          135          140
Leu Cys Pro Ala Gly His Ala Val Gly Ile Phe Arg Ala Ala Val Cys
145          150          155          160
Thr Arg Gly Val Ala Lys Ala Val Asp Phe Ile Pro Val Glu Ser Leu
      165          170          175
Glu Thr Thr Met Arg Ser Pro Val Phe Ser Asp Asn Ser Ser Pro Pro
      180          185          190
Ala Val Pro Gln Ser Tyr Gln Val Ala His Leu His Ala Pro Thr Gly
      195          200          205
Ser Gly Lys Ser Thr Lys Val Pro Ala Ala Tyr Ala Ala Gln Gly Tyr
      210          215          220
Lys Val Leu Val Leu Asn Pro Ser Val Ala Ala Thr Met Gly Phe Gly
225          230          235          240
Ala Tyr Met Ser Lys Ala His Gly Ile Asp Pro Asn Ile Arg Thr Gly
      245          250          255
Val Arg Thr Ile Thr Thr Gly Ser Pro Ile Thr Tyr Ser Thr Tyr Gly
      260          265          270
Lys Phe Leu Ala Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile Ile
      275          280          285
Ile Cys Asp Glu Cys His Ser Thr Asp Ala Thr Ser Ile Leu Gly Ile
      290          295          300
Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Thr Val
305          310          315          320
Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr Val Pro His Pro Asn
      325          330          335
Ile Glu Glu Val Ala Leu Ser Thr Thr Gly Glu Ile Pro Phe Tyr Gly
      340          345          350
Lys Ala Ile Pro Leu Glu Ala Ile Lys Gly Gly Arg His Leu Ile Phe
      355          360          365
Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Val Ala
      370          375          380
Leu Gly Val Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val
385          390          395          400
Ile Pro Thr Ser Gly Asp Val Val Val Val Ala Thr Asp Ala Leu Met
      405          410          415
Thr Gly Phe Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr Cys

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                420                425                430
Val Thr Gln Thr Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile Glu
                435                440                445
Thr Ile Thr Leu Pro Gln Asp Ala Val Ser Arg Thr Gln Arg Arg Gly
                450                455                460
Arg Thr Gly Arg Gly Lys Pro Gly Ile Tyr Arg Phe Val Ala Pro Gly
465                470                475                480
Glu Arg Pro Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys Tyr
                485                490                495
Asp Ala Gly Cys Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Thr Val
                500                505                510
Arg Leu Arg Ala Tyr Met Asn Thr Pro Gly Leu Pro Val Cys Gln Asp
                515                520                525
His Leu Glu Phe Trp Glu Gly Val Phe Thr Gly Leu Thr His Ile Asp
                530                535                540
Ala His Phe Leu Ser Gln Thr Lys Gln Ser Gly Glu Asn Leu Pro Tyr
545                550                555                560
Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro Pro
                565                570                575
Pro Ser Trp Asp Gln Met Trp Lys Cys Leu Ile Arg Leu Lys Pro Thr
                580                585                590
Leu His Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln Asn
                595                600                605
Glu Val Thr Leu Thr His Pro Val Thr Lys Tyr Ile Met Thr Cys Met
                610                615                620
Ser Ala Asp Leu Glu Val Val Thr Pro Thr Trp Val Leu Val Gly Gly
625                630                635                640
Val Leu Ala Ala Leu Ala Ala Tyr Cys Leu Ser Thr Gly Cys Val Val
                645                650                655
Ile Val Gly Arg Ile Val Leu Ser Gly Lys Pro Ala Ile Ile Pro Asp
                660                665                670
Arg Glu Val Leu Tyr Arg Glu Phe Asp Glu Met Glu Glu Cys
                675                680                685

```

&lt;210&gt; 44

&lt;211&gt; 686

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Mutant Hepatitis C virus NS3/4A

&lt;400&gt; 44

```

Met Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr Arg Gly Leu Leu Gly
 1                5                10                15
Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu Gly
                20                25                30
Glu Val Gln Ile Val Ser Thr Ala Ala Gln Thr Phe Leu Ala Thr Cys
                35                40                45
Ile Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Thr Arg Thr
                50                55                60
Ile Ala Ser Pro Lys Gly Pro Val Ile Gln Met Tyr Thr Asn Val Asp
65                70                75                80
Gln Asp Leu Val Gly Trp Pro Ala Pro Gln Gly Ala Arg Ser Leu Thr

```

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His	Leu	Glu	Phe	Trp	Glu	Gly	Val	Phe	Thr	Gly	Leu	Thr	His	Ile	Asp
530						535					540				
Ala	His	Phe	Leu	Ser	Gln	Thr	Lys	Gln	Ser	Gly	Glu	Asn	Leu	Pro	Tyr
545					550					555					560
Leu	Val	Ala	Tyr	Gln	Ala	Thr	Val	Cys	Ala	Arg	Ala	Gln	Ala	Pro	Pro
				565						570				575	
Pro	Ser	Trp	Asp	Gln	Met	Trp	Lys	Cys	Leu	Ile	Arg	Leu	Lys	Pro	Thr
			580					585					590		
Leu	His	Gly	Pro	Thr	Pro	Leu	Leu	Tyr	Arg	Leu	Gly	Ala	Val	Gln	Asn
		595				600					605				
Glu	Val	Thr	Leu	Thr	His	Pro	Val	Thr	Lys	Tyr	Ile	Met	Thr	Cys	Met
		610				615					620				
Ser	Ala	Asp	Leu	Glu	Val	Val	Arg	Pro	Thr	Trp	Val	Leu	Val	Gly	Gly
625					630					635					640
Val	Leu	Ala	Ala	Leu	Ala	Ala	Tyr	Cys	Leu	Ser	Thr	Gly	Cys	Val	Val
				645					650					655	
Ile	Val	Gly	Arg	Ile	Val	Leu	Ser	Gly	Lys	Pro	Ala	Ile	Ile	Pro	Asp
		660						665					670		
Arg	Glu	Val	Leu	Tyr	Arg	Glu	Phe	Asp	Glu	Met	Glu	Glu	Cys		
		675					680					685			

&lt;210&gt; 45

&lt;211&gt; 686

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Mutant Hepatitis C virus NS3/4A

&lt;400&gt; 45

Met	Ala	Pro	Ile	Thr	Ala	Tyr	Ala	Gln	Gln	Thr	Arg	Gly	Leu	Leu	Gly
1				5				10					15		
Cys	Ile	Ile	Thr	Ser	Leu	Thr	Gly	Arg	Asp	Lys	Asn	Gln	Val	Glu	Gly
			20				25					30			
Glu	Val	Gln	Ile	Val	Ser	Thr	Ala	Ala	Gln	Thr	Phe	Leu	Ala	Thr	Cys
		35				40					45				
Ile	Asn	Gly	Val	Cys	Trp	Thr	Val	Tyr	His	Gly	Ala	Gly	Thr	Arg	Thr
	50				55				60						
Ile	Ala	Ser	Pro	Lys	Gly	Pro	Val	Ile	Gln	Met	Tyr	Thr	Asn	Val	Asp
65				70					75						80
Gln	Asp	Leu	Val	Gly	Trp	Pro	Ala	Pro	Gln	Gly	Ala	Arg	Ser	Leu	Thr
			85					90					95		
Pro	Cys	Thr	Cys	Gly	Ser	Ser	Asp	Leu	Tyr	Leu	Val	Thr	Arg	His	Ala
			100				105						110		
Asp	Val	Ile	Pro	Val	Arg	Arg	Arg	Gly	Asp	Gly	Arg	Gly	Ser	Leu	Leu
		115				120						125			
Ser	Pro	Arg	Pro	Ile	Ser	Tyr	Leu	Lys	Gly	Ser	Ser	Gly	Gly	Pro	Leu
		130			135						140				
Leu	Cys	Pro	Ala	Gly	His	Ala	Val	Gly	Ile	Phe	Arg	Ala	Ala	Val	Cys
145				150					155						160
Thr	Arg	Gly	Val	Ala	Lys	Ala	Val	Asp	Phe	Ile	Pro	Val	Glu	Ser	Leu
			165					170					175		
Glu	Thr	Thr	Met	Arg	Ser	Pro	Val	Phe	Ser	Asp	Asn	Ser	Ser	Pro	Pro
			180					185					190		

Ala Val Pro Gln Ser Tyr Gln Val Ala His Leu His Ala Pro Thr Gly  
 195 200 205  
 Ser Gly Lys Ser Thr Lys Val Pro Ala Ala Tyr Ala Ala Gln Gly Tyr  
 210 215 220  
 Lys Val Leu Val Leu Asn Pro Ser Val Ala Ala Thr Met Gly Phe Gly  
 225 230 235 240  
 Ala Tyr Met Ser Lys Ala His Gly Ile Asp Pro Asn Ile Arg Thr Gly  
 245 250 255  
 Val Arg Thr Ile Thr Thr Gly Ser Pro Ile Thr Tyr Ser Thr Tyr Gly  
 260 265 270  
 Lys Phe Leu Ala Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile Ile  
 275 280 285  
 Ile Cys Asp Glu Cys His Ser Thr Asp Ala Thr Ser Ile Leu Gly Ile  
 290 295 300  
 Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Thr Val  
 305 310 315 320  
 Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr Val Pro His Pro Asn  
 325 330 335  
 Ile Glu Glu Val Ala Leu Ser Thr Thr Gly Glu Ile Pro Phe Tyr Gly  
 340 345 350  
 Lys Ala Ile Pro Leu Glu Ala Ile Lys Gly Gly Arg His Leu Ile Phe  
 355 360 365  
 Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Val Ala  
 370 375 380  
 Leu Gly Val Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val  
 385 390 395 400  
 Ile Pro Thr Ser Gly Asp Val Val Val Val Ala Thr Asp Ala Leu Met  
 405 410 415  
 Thr Gly Phe Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr Cys  
 420 425 430  
 Val Thr Gln Thr Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile Glu  
 435 440 445  
 Thr Ile Thr Leu Pro Gln Asp Ala Val Ser Arg Thr Gln Arg Arg Gly  
 450 455 460  
 Arg Thr Gly Arg Gly Lys Pro Gly Ile Tyr Arg Phe Val Ala Pro Gly  
 465 470 475 480  
 Glu Arg Pro Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys Tyr  
 485 490 495  
 Asp Ala Gly Cys Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Thr Val  
 500 505 510  
 Arg Leu Arg Ala Tyr Met Asn Thr Pro Gly Leu Pro Val Cys Gln Asp  
 515 520 525  
 His Leu Glu Phe Trp Glu Gly Val Phe Thr Gly Leu Thr His Ile Asp  
 530 535 540  
 Ala His Phe Leu Ser Gln Thr Lys Gln Ser Gly Glu Asn Leu Pro Tyr  
 545 550 555 560  
 Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro Pro  
 565 570 575  
 Pro Ser Trp Asp Gln Met Trp Lys Cys Leu Ile Arg Leu Lys Pro Thr  
 580 585 590  
 Leu His Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln Asn  
 595 600 605  
 Glu Val Thr Leu Thr His Pro Val Thr Lys Tyr Ile Met Thr Cys Met  
 610 615 620  
 Ser Ala Asp Leu Glu Val Val Arg Pro Ala Trp Val Leu Val Gly Gly



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625          630          635          640
Val Leu Ala Ala Leu Ala Ala Tyr Cys Leu Ser Thr Gly Cys Val Val
          645          650          655
Ile Val Gly Arg Ile Val Leu Ser Gly Lys Pro Ala Ile Ile Pro Asp
          660          665          670
Arg Glu Val Leu Tyr Arg Glu Phe Asp Glu Met Glu Glu Cys
          675          680          685

```

<210> 46  
 <211> 686  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Mutant Hepatitis C virus NS3/4A

```

<400> 46
Met Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr Arg Gly Leu Leu Gly
  1          5          10          15
Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu Gly
          20          25          30
Glu Val Gln Ile Val Ser Thr Ala Ala Gln Thr Phe Leu Ala Thr Cys
          35          40          45
Ile Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Thr Arg Thr
          50          55          60
Ile Ala Ser Pro Lys Gly Pro Val Ile Gln Met Tyr Thr Asn Val Asp
          65          70          75          80
Gln Asp Leu Val Gly Trp Pro Ala Pro Gln Gly Ala Arg Ser Leu Thr
          85          90          95
Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr Arg His Ala
          100          105          110
Asp Val Ile Pro Val Arg Arg Arg Gly Asp Gly Arg Gly Ser Leu Leu
          115          120          125
Ser Pro Arg Pro Ile Ser Tyr Leu Lys Gly Ser Ser Gly Gly Pro Leu
          130          135          140
Leu Cys Pro Ala Gly His Ala Val Gly Ile Phe Arg Ala Ala Val Cys
          145          150          155          160
Thr Arg Gly Val Ala Lys Ala Val Asp Phe Ile Pro Val Glu Ser Leu
          165          170          175
Glu Thr Thr Met Arg Ser Pro Val Phe Ser Asp Asn Ser Ser Pro Pro
          180          185          190
Ala Val Pro Gln Ser Tyr Gln Val Ala His Leu His Ala Pro Thr Gly
          195          200          205
Ser Gly Lys Ser Thr Lys Val Pro Ala Ala Tyr Ala Ala Gln Gly Tyr
          210          215          220
Lys Val Leu Val Leu Asn Pro Ser Val Ala Ala Thr Met Gly Phe Gly
          225          230          235          240
Ala Tyr Met Ser Lys Ala His Gly Ile Asp Pro Asn Ile Arg Thr Gly
          245          250          255
Val Arg Thr Ile Thr Thr Gly Ser Pro Ile Thr Tyr Ser Thr Tyr Gly
          260          265          270
Lys Phe Leu Ala Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile Ile
          275          280          285
Ile Cys Asp Glu Cys His Ser Thr Asp Ala Thr Ser Ile Leu Gly Ile

```

290	295	300
Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Thr Val		
305	310	315
Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr Val Pro His Pro Asn		
	325	330
Ile Glu Glu Val Ala Leu Ser Thr Thr Gly Glu Ile Pro Phe Tyr Gly		
	340	345
Lys Ala Ile Pro Leu Glu Ala Ile Lys Gly Gly Arg His Leu Ile Phe		
	355	360
Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Val Ala		
	370	375
Leu Gly Val Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val		
385	390	395
Ile Pro Thr Ser Gly Asp Val Val Val Val Ala Thr Asp Ala Leu Met		
	405	410
Thr Gly Phe Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr Cys		
	420	425
Val Thr Gln Thr Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile Glu		
	435	440
Thr Ile Thr Leu Pro Gln Asp Ala Val Ser Arg Thr Gln Arg Arg Gly		
	450	455
Arg Thr Gly Arg Gly Lys Pro Gly Ile Tyr Arg Phe Val Ala Pro Gly		
465	470	475
Glu Arg Pro Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys Tyr		
	485	490
Asp Ala Gly Cys Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Thr Val		
	500	505
Arg Leu Arg Ala Tyr Met Asn Thr Pro Gly Leu Pro Val Cys Gln Asp		
	515	520
His Leu Glu Phe Trp Glu Gly Val Phe Thr Gly Leu Thr His Ile Asp		
	530	535
Ala His Phe Leu Ser Gln Thr Lys Gln Ser Gly Glu Asn Leu Pro Tyr		
545	550	555
Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro Pro		
	565	570
Pro Ser Trp Asp Gln Met Trp Lys Cys Leu Ile Arg Leu Lys Pro Thr		
	580	585
Leu His Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln Asn		
	595	600
Glu Val Thr Leu Thr His Pro Val Thr Lys Tyr Ile Met Thr Cys Met		
	610	615
Ser Ala Asp Leu Glu Val Val Cys Ser Thr Trp Val Leu Val Gly Gly		
625	630	635
Val Leu Ala Ala Leu Ala Ala Tyr Cys Leu Ser Thr Gly Cys Val Val		
	645	650
Ile Val Gly Arg Ile Val Leu Ser Gly Lys Pro Ala Ile Ile Pro Asp		
	660	665
Arg Glu Val Leu Tyr Arg Glu Phe Asp Glu Met Glu Glu Cys		
	675	680
		685

&lt;210&gt; 47

&lt;211&gt; 686

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Mutant Hepatitis C virus NS3/4A

&lt;400&gt; 47

```

Met Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr Arg Gly Leu Leu Gly
 1           5           10           15
Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu Gly
      20           25           30
Glu Val Gln Ile Val Ser Thr Ala Ala Gln Thr Phe Leu Ala Thr Cys
      35           40           45
Ile Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Thr Arg Thr
      50           55           60
Ile Ala Ser Pro Lys Gly Pro Val Ile Gln Met Tyr Thr Asn Val Asp
65           70           75           80
Gln Asp Leu Val Gly Trp Pro Ala Pro Gln Gly Ala Arg Ser Leu Thr
      85           90           95
Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr Arg His Ala
      100          105          110
Asp Val Ile Pro Val Arg Arg Arg Gly Asp Gly Arg Gly Ser Leu Leu
      115          120          125
Ser Pro Arg Pro Ile Ser Tyr Leu Lys Gly Ser Ser Gly Gly Pro Leu
      130          135          140
Leu Cys Pro Ala Gly His Ala Val Gly Ile Phe Arg Ala Ala Val Cys
145          150          155          160
Thr Arg Gly Val Ala Lys Ala Val Asp Phe Ile Pro Val Glu Ser Leu
      165          170          175
Glu Thr Thr Met Arg Ser Pro Val Phe Ser Asp Asn Ser Ser Pro Pro
      180          185          190
Ala Val Pro Gln Ser Tyr Gln Val Ala His Leu His Ala Pro Thr Gly
      195          200          205
Ser Gly Lys Ser Thr Lys Val Pro Ala Ala Tyr Ala Ala Gln Gly Tyr
      210          215          220
Lys Val Leu Val Leu Asn Pro Ser Val Ala Ala Thr Met Gly Phe Gly
225          230          235          240
Ala Tyr Met Ser Lys Ala His Gly Ile Asp Pro Asn Ile Arg Thr Gly
      245          250          255
Val Arg Thr Ile Thr Thr Gly Ser Pro Ile Thr Tyr Ser Thr Tyr Gly
      260          265          270
Lys Phe Leu Ala Asp Gly Gly Cys Ser Gly Gly Ala Tyr Asp Ile Ile
      275          280          285
Ile Cys Asp Glu Cys His Ser Thr Asp Ala Thr Ser Ile Leu Gly Ile
      290          295          300
Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Thr Val
305          310          315          320
Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr Val Pro His Pro Asn
      325          330          335
Ile Glu Glu Val Ala Leu Ser Thr Thr Gly Glu Ile Pro Phe Tyr Gly
      340          345          350
Lys Ala Ile Pro Leu Glu Ala Ile Lys Gly Gly Arg His Leu Ile Phe
      355          360          365
Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Val Ala
      370          375          380
Leu Gly Val Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val
385          390          395          400

```

```

Ile Pro Thr Ser Gly Asp Val Val Val Val Ala Thr Asp Ala Leu Met
              405                      410                      415
Thr Gly Phe Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr Cys
              420                      425                      430
Val Thr Gln Thr Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile Glu
              435                      440                      445
Thr Ile Thr Leu Pro Gln Asp Ala Val Ser Arg Thr Gln Arg Arg Gly
              450                      455                      460
Arg Thr Gly Arg Gly Lys Pro Gly Ile Tyr Arg Phe Val Ala Pro Gly
465              470                      475                      480
Glu Arg Pro Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys Tyr
              485                      490                      495
Asp Ala Gly Cys Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Thr Val
              500                      505                      510
Arg Leu Arg Ala Tyr Met Asn Thr Pro Gly Leu Pro Val Cys Gln Asp
              515                      520                      525
His Leu Glu Phe Trp Glu Gly Val Phe Thr Gly Leu Thr His Ile Asp
              530                      535                      540
Ala His Phe Leu Ser Gln Thr Lys Gln Ser Gly Glu Asn Leu Pro Tyr
545              550                      555                      560
Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro Pro
              565                      570                      575
Pro Ser Trp Asp Gln Met Trp Lys Cys Leu Ile Arg Leu Lys Pro Thr
              580                      585                      590
Leu His Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln Asn
              595                      600                      605
Glu Val Thr Leu Thr His Pro Val Thr Lys Tyr Ile Met Thr Cys Met
              610                      615                      620
Ser Ala Asp Leu Glu Val Cys Cys Ser Thr Trp Val Leu Val Gly Gly
625              630                      635                      640
Val Leu Ala Ala Leu Ala Ala Tyr Cys Leu Ser Thr Gly Cys Val Val
              645                      650                      655
Ile Val Gly Arg Ile Val Leu Ser Gly Lys Pro Ala Ile Ile Pro Asp
              660                      665                      670
Arg Glu Val Leu Tyr Arg Glu Phe Asp Glu Met Glu Glu Cys
              675                      680                      685

```

&lt;210&gt; 48

&lt;211&gt; 686

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Mutant Hepatitis C virus, NS3/4A

&lt;400&gt; 48

```

Met Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr Arg Gly Leu Leu Gly
 1              5              10              15
Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu Gly
              20              25              30
Glu Val Gln Ile Val Ser Thr Ala Ala Gln Thr Phe Leu Ala Thr Cys
              35              40              45
Ile Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Thr Arg Thr
 50              55              60

```

Ile	Ala	Ser	Pro	Lys	Gly	Pro	Val	Ile	Gln	Met	Tyr	Thr	Asn	Val	Asp	
65					70				75						80	
Gln	Asp	Leu	Val	Gly	Trp	Pro	Ala	Pro	Gln	Gly	Ala	Arg	Ser	Leu	Thr	
				85					90					95		
Pro	Cys	Thr	Cys	Gly	Ser	Ser	Asp	Leu	Tyr	Leu	Val	Thr	Arg	His	Ala	
			100					105					110			
Asp	Val	Ile	Pro	Val	Arg	Arg	Arg	Gly	Asp	Gly	Arg	Gly	Ser	Leu	Leu	
	115						120					125				
Ser	Pro	Arg	Pro	Ile	Ser	Tyr	Leu	Lys	Gly	Ser	Ser	Gly	Gly	Pro	Leu	
	130					135					140					
Leu	Cys	Pro	Ala	Gly	His	Ala	Val	Gly	Ile	Phe	Arg	Ala	Ala	Val	Cys	
145					150					155					160	
Thr	Arg	Gly	Val	Ala	Lys	Ala	Val	Asp	Phe	Ile	Pro	Val	Glu	Ser	Leu	
				165					170						175	
Glu	Thr	Thr	Met	Arg	Ser	Pro	Val	Phe	Ser	Asp	Asn	Ser	Ser	Pro	Pro	
			180					185					190			
Ala	Val	Pro	Gln	Ser	Tyr	Gln	Val	Ala	His	Leu	His	Ala	Pro	Thr	Gly	
	195						200					205				
Ser	Gly	Lys	Ser	Thr	Lys	Val	Pro	Ala	Ala	Tyr	Ala	Ala	Gln	Gly	Tyr	
	210					215					220					
Lys	Val	Leu	Val	Leu	Asn	Pro	Ser	Val	Ala	Ala	Thr	Met	Gly	Phe	Gly	
225					230					235					240	
Ala	Tyr	Met	Ser	Lys	Ala	His	Gly	Ile	Asp	Pro	Asn	Ile	Arg	Thr	Gly	
				245					250						255	
Val	Arg	Thr	Ile	Thr	Thr	Gly	Ser	Pro	Ile	Thr	Tyr	Ser	Thr	Tyr	Gly	
			260					265					270			
Lys	Phe	Leu	Ala	Asp	Gly	Gly	Cys	Ser	Gly	Gly	Ala	Tyr	Asp	Ile	Ile	
	275						280					285				
Ile	Cys	Asp	Glu	Cys	His	Ser	Thr	Asp	Ala	Thr	Ser	Ile	Leu	Gly	Ile	
	290					295					300					
Gly	Thr	Val	Leu	Asp	Gln	Ala	Glu	Thr	Ala	Gly	Ala	Arg	Leu	Thr	Val	
305					310					315					320	
Leu	Ala	Thr	Ala	Thr	Pro	Pro	Gly	Ser	Val	Thr	Val	Pro	His	Pro	Asn	
				325					330						335	
Ile	Glu	Glu	Val	Ala	Leu	Ser	Thr	Thr	Gly	Glu	Ile	Pro	Phe	Tyr	Gly	
			340					345					350			
Lys	Ala	Ile	Pro	Leu	Glu	Ala	Ile	Lys	Gly	Gly	Arg	His	Leu	Ile	Phe	
	355						360					365				
Cys	His	Ser	Lys	Lys	Lys	Cys	Asp	Glu	Leu	Ala	Ala	Lys	Leu	Val	Ala	
	370					375					380					
Leu	Gly	Val	Asn	Ala	Val	Ala	Tyr	Tyr	Arg	Gly	Leu	Asp	Val	Ser	Val	
385					390					395					400	
Ile	Pro	Thr	Ser	Gly	Asp	Val	Val	Val	Val	Ala	Thr	Asp	Ala	Leu	Met	
				405					410						415	
Thr	Gly	Phe	Thr	Gly	Asp	Phe	Asp	Ser	Val	Ile	Asp	Cys	Asn	Thr	Cys	
			420					425					430			
Val	Thr	Gln	Thr	Val	Asp	Phe	Ser	Leu	Asp	Pro	Thr	Phe	Thr	Ile	Glu	
	435						440					445				
Thr	Ile	Thr	Leu	Pro	Gln	Asp	Ala	Val	Ser	Arg	Thr	Gln	Arg	Arg	Gly	
	450					455					460					
Arg	Thr	Gly	Arg	Gly	Lys	Pro	Gly	Ile	Tyr	Arg	Phe	Val	Ala	Pro	Gly	
465					470					475					480	
Glu	Arg	Pro	Ser	Gly	Met	Phe	Asp	Ser	Ser	Val	Leu	Cys	Glu	Cys	Tyr	
				485					490					495		
Asp	Ala	Gly	Cys	Ala	Trp	Tyr	Glu	Leu	Thr	Pro	Ala	Glu	Thr	Thr	Val	

```

                    500                      505                      510
Arg Leu Arg Ala Tyr Met Asn Thr Pro Gly Leu Pro Val Cys Gln Asp
                    515                      520                      525
His Leu Glu Phe Trp Glu Gly Val Phe Thr Gly Leu Thr His Ile Asp
                    530                      535                      540
Ala His Phe Leu Ser Gln Thr Lys Gln Ser Gly Glu Asn Leu Pro Tyr
545                      550                      555                      560
Leu Val Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro Pro
                    565                      570                      575
Pro Ser Trp Asp Gln Met Trp Lys Cys Leu Ile Arg Leu Lys Pro Thr
                    580                      585                      590
Leu His Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln Asn
                    595                      600                      605
Glu Val Thr Leu Thr His Pro Val Thr Lys Tyr Ile Met Thr Cys Met
                    610                      615                      620
Ser Ala Asp Leu Glu Val Ser Ser Ser Thr Trp Val Leu Val Gly Gly
625                      630                      635                      640
Val Leu Ala Ala Leu Ala Ala Tyr Cys Leu Ser Thr Gly Cys Val Val
                    645                      650                      655
Ile Val Gly Arg Ile Val Leu Ser Gly Lys Pro Ala Ile Ile Pro Asp
                    660                      665                      670
Arg Glu Val Leu Tyr Arg Glu Phe Asp Glu Met Glu Glu Cys
                    675                      680                      685

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&lt;210&gt; 49

&lt;211&gt; 686

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Mutant Hepatitis C virus NS3/4A

&lt;400&gt; 49

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Met Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr Arg Gly Leu Leu Gly
 1                      5                      10                      15
Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu Gly
                    20                      25                      30
Glu Val Gln Ile Val Ser Thr Ala Ala Gln Thr Phe Leu Ala Thr Cys
                    35                      40                      45
Ile Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Thr Arg Thr
                    50                      55                      60
Ile Ala Ser Pro Lys Gly Pro Val Ile Gln Met Tyr Thr Asn Val Asp
65                      70                      75                      80
Gln Asp Leu Val Gly Trp Pro Ala Pro Gln Gly Ala Arg Ser Leu Thr
                    85                      90                      95
Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr Arg His Ala
                    100                      105                      110
Asp Val Ile Pro Val Arg Arg Arg Gly Asp Gly Arg Gly Ser Leu Leu
                    115                      120                      125
Ser Pro Arg Pro Ile Ser Tyr Leu Lys Gly Ser Ser Gly Gly Pro Leu
                    130                      135                      140
Leu Cys Pro Ala Gly His Ala Val Gly Ile Phe Arg Ala Ala Val Cys
145                      150                      155                      160
Thr Arg Gly Val Ala Lys Ala Val Asp Phe Ile Pro Val Glu Ser Leu

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Glu	Val	Thr	Leu	Thr	His	Pro	Val	Thr	Lys	Tyr	Ile	Met	Thr	Cys	Met
610						615					620				
Ser	Ala	Asp	Ser	Ser	Ser	Ser	Cys	Ser	Thr	Trp	Val	Leu	Val	Gly	Gly
625						630				635					640
Val	Leu	Ala	Ala	Leu	Ala	Ala	Tyr	Cys	Leu	Ser	Thr	Gly	Cys	Val	Val
				645					650					655	
Ile	Val	Gly	Arg	Ile	Val	Leu	Ser	Gly	Lys	Pro	Ala	Ile	Ile	Pro	Asp
			660					665					670		
Arg	Glu	Val	Leu	Tyr	Arg	Glu	Phe	Asp	Glu	Met	Glu	Glu	Cys		
	675						680						685		