UNIVERSIDAD DISTRITAL
FRANCISCO JOSÉ DE CALDAS

Revista Vínculos
http://revistas.udistrital.edu.co/ojs/index.php/vinculos

Revista VÍNCULOS
Ciencia, Tecnología y Sociedad: Un Enlace hacia el Futuro

I+D Investigación y Desarrollo

Computational analysis of resistance mutations in the hepatitis C virus

Análisis computacional de resistencia a mutaciones en el virus de la hepatitis C

Karina Salvatierra[1] Hector Florez[2]

Para citar este artículo: K. Salvatierra y H. Florez, "Computational analysis of resistance mutations in the hepatitis C virus". *Revista Vínculos*, vol 15, no 1, enero-junio 2018, 58-64. DOI: https://doi.org/10.14483/2322939X.12973.

Recibido: 13-01-2018 / Aprobado: 21-02-2018

Abstract

Hepatitis C virus (HCV) continues to be a public health problem worldwide. The development of drugs that target virus proteins such as NS5B, aims to provide more effective treatment in the future. These direct-acting antivirals (DAAs) have demonstrated potent in vitro and in vivo effect; however, mutations have been described in HCV NS5B associated with resistance to DAA. Detecting mutations that are resistant to DAAs is important to prevent potential treatment failures. In this work, we developed a software to analyze mutations that are resistant to DAAs in NS5B gene of HCV. For this, we used the NS5B gene sequence isolate Con1 genotype 1b HCV, based on the main positions mutations associated with resistance to DAAs in vitro and in vivo described in the literature. The software algorithm allows computer analysis of possible changes in nucleotide and amino acid positions associated with resistance mutations to DAAs in NS5B of HCV.

Keywords: antivirals, computational analysis, hepatitis C virus, NS5B.

Resumen

El virus de la hepatitis C (VHC) continúa siendo un problema de salud pública en todo el mundo. El desarrollo de medicamentos que se dirigen a las proteínas del virus como NS5B, tiene como objetivo proporcionar un tratamiento más eficaz en el futuro. Estos antivirales de acción directa (DAA) han demostrado un potente efecto in vitro e in vivo; sin embargo, se han descrito mutaciones en la NS5B del VHC asociada a la resistencia a DAA. La detección de mutaciones que son resistentes a DAA es importante para evitar posibles fallas de tratamiento. En este trabajo, desarrollamos un software para analizar mutaciones que son resistentes a los DAA en el gen NS5B del VHC. Para esto, utilizamos la secuencia del gen NS5B aislada Con1 genotipo 1b HCV, con base en las principales posiciones asociadas con la resistencia a DAA in vitro e in vivo descritas en la literatura. El algoritmo del software permite el análisis computacional de posibles cambios en las posiciones de nucleótidos y aminoácidos asociadas con mutaciones de resistencia a DAA en NS5B de HCV.

Palabras clave: antivirales, análisis computacional, virus de la hepatitis C, NS5B.

1. Doctor en Biotecnología. Universidad Nacional de Misiones, Argentina. Correo electrónico: karinasalvatierra@fceqyn.unam.edu.ar
2. Doctor en Ingeniería. Afiliación institucional: Universidad Distrital Francisco José de Caldas, Colombia. Correo electrónico: haflorezf@udistrital.edu.co

ISSN 1794-211X • e-ISSN 2322-939X • Vol 15, No 1 (enero-junio 2018). pp. 58-64. Universidad Distrital Francisco José de Caldas-Facultad Tecnológica.

1. Introduction

The hepatitis C virus (HCV) was molecularly characterized in 1989 [1], following numerous investigations to identify the genome of the non-A and non-B (NANB) hepatitis viruses, recognized as the major cause of hepatitis C and a major cause of chronic hepatitis. It is estimated that 200 million patients worldwide are infected with HCV and approximately 130-150 million (3% of the world's population) live with chronic hepatitis C. About 3-4 million people are infected every year, and more than 350,000 people die every year from hepatitis C-related diseases [2].
Until very recently, the treatment used for HCV was a combination of two non-specific drugs immune modulators pegylated interferon-α (peg-IFN-α) and ribavirin (RBV) [3], as the effectiveness is unsatisfactory in approximately 50-60% of infected patients, and relapse occurs in 5-20% of patients treated. The long duration of treatment makes it difficult for patients to tolerate the associated side effects such as asthenia, myalgia, fever, headache, anorexia, anemia, skin rashes, fatigue, etc. [4], and most patients have to cease treatment due to these side effects. These limitations brought about the development of new and more effective drugs with fewer side effects that target virus proteins, called "Direct-acting antivirals" (DAAs)[5].
Each step in the HCV life cycle is a potential target for inhibiting molecules. Among different viral enzymes, the most research has been done into protease NS3, NS5A and NS5B RNA-dependent RNA polymerase [6]. NS3/4A protease is necessary for cutting polyprotein, and is required for the formation of the replicase complex. Protease inhibitors prevent this process and block the formation of the replicase complex. Furthermore, the virus needs NS5B polymerase to replicate as it joins the replicase complex to the host cell and amplifies its genetic material. Therefore the inhibition of NS5B polymerase stops the replication process [7]. The first inhibitory agents of these two virus enzymes quickly demonstrated high antiviral efficacy in vitro and in vivo. Currently, there are more than 30 DAAs, some of which are in the advanced stages of clinical development and others, which have already been approved for use. However, mutations resistant to new DAAs, both in vitro and in vivo have been observed [8, 9]. Current data indicate that viral strains with mutations resistant to NS3 protease inhibitors, NS5A inhibitors, and NS5B polymerase inhibitors are circulating in 7.7%, 16.2% and 22.5%, of infected patients [10]. It is likely that these viral strains contribute to the development of a resistance to DAAs during the initial weeks of monotherapy treatment [11]. Resistance can also develop quickly when the combination of two DAAs is used in treatment. Similar to the treatment of HIV, a combination of agents may be required to optimize viral suppression and prevent the development of resistance [12].
The detection of viral strains resistant to new AADs is a factor that should be considered at the beginning of and during the treatment of infected patients to prevent future errors [13].
Advances in technology, particularly advances in bioinformatics, can certainly contribute to the development of invaluable computational methods for managing treatment of HCV-infected patients. The aim of this paper was to develop an online information system which included the profiles of viral strains resistant to DAAs for the HCV NS5B gene.

2. Materials and methods

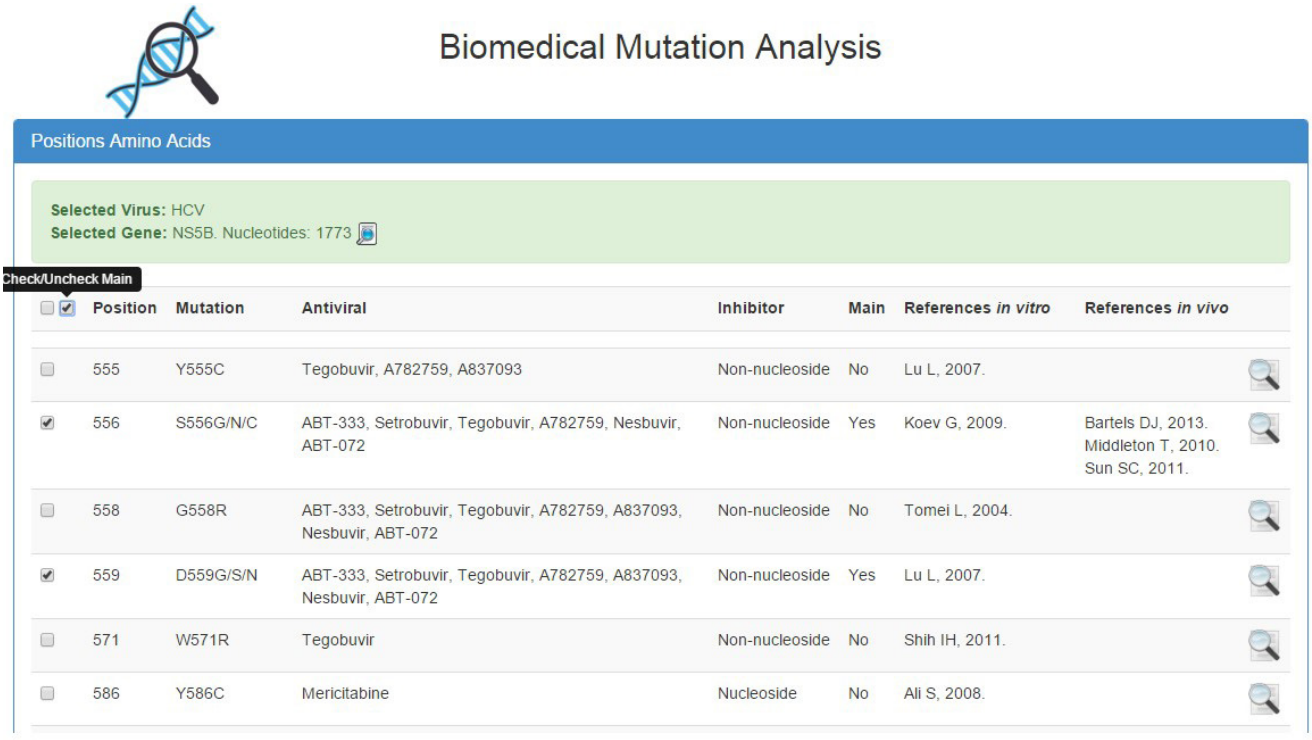
HCV isolate Con1 was used as a reference sequence to develop the online information system. This information was extracted from the GenBank database (code AJ238799) (www.ncbi.nlm.nih.gov/genbank/). This isolate was chosen as a reference sequence because it is the prototype used to build the replicon used in the development of most new DAAs. The nucleotide sequence of the NS5B gene was selected in plain text format (1,773 bp) for the design of the computational analysis of mutations. The positions of mutations associated with resistance DAAs in vitro and in vivo considered for algorithm development, are described in the literature for the HCV NS5B gene genotype1 subtipo1b[14-16].

ISSN 1794-211X • e-ISSN 2322-939X • Vol 15, No 1 (enero-junio 2018). pp. 58-64. Universidad Distrital Francisco José de Caldas-Facultad Tecnológica.

The algorithm was designed so that the nucleotide sequences of the NS5B gene being analyzed are compared with the reference sequence to show nucleotide and amino acid changes at positions related to resistance mutations known to occur in the HCV NS5B gene. The resultant algorithm is part of an information system, which was developed using free software tools to provide an easy to use application for users to calculate results immediately without the need to download and install components or additional applications. This software is available on the web site: http://bma.itiud.org

3. Results

The information system provides an explanation of the mutation positions, antivirals, and a bibliography of resistance mutations AADs (Figure 1).
The algorithm was developed for the computational analysis of the positions associated with mutations described in the literature as causing resistance to DAAs which occur in the NS5B gene. The algorithm can incorporate (n) the numbers of nucleotide sequences which it then compares with the reference sequence of isolate Con1 genotype 1b, of HCV. It then calculates the number and the types of nucleotide and amino acid changes and highlights the amino acid changes that have occurred due to nucleotide changes.
In a simulation test to analyze mutations at positions associated with mutations described in the literature as causing resistance: 159, 282, 316, 320, 414, 419, 423, 426, 448, 482, 494, 554, 556, 559, the online information system analyzed the nucleotide sequence problem of the NS5B gene of HCV from a patient quickly and easily. When executing the algorithm with the nucleotide sequence, the frequency and the number of nucleotide changes in positions of interest are detailed for better interpretation: TGC → AAC = 1, CTT → CTC = 1, ATG → TTA = 1 y AGC →



Position	Mutation	Antiviral	Inhibitor	Main	References *in vitro*	References *in vivo*
555	Y555C	Tegobuvir, A782759, A837093	Non-nucleoside	No	Lu L, 2007.	
556	S556G/N/C	ABT-333, Setrobuvir, Tegobuvir, A782759, Nesbuvir, ABT-072	Non-nucleoside	Yes	Koev G, 2009.	Bartels DJ, 2013. Middleton T, 2010. Sun SC, 2011.
558	G558R	ABT-333, Setrobuvir, Tegobuvir, A782759, A837093, Nesbuvir, ABT-072	Non-nucleoside	No	Tomei L, 2004.	
559	D559G/S/N	ABT-333, Setrobuvir, Tegobuvir, A782759, A837093, Nesbuvir, ABT-072	Non-nucleoside	Yes	Lu L, 2007.	
571	W571R	Tegobuvir	Non-nucleoside	No	Shih IH, 2011.	
586	Y586C	Mericitabine	Nucleoside	No	Ali S, 2008.	

Figure 1. Mutations positions, antivirals and references of the HCV NS5B gene.
Source: Own.

ISSN 1794-211X • e-ISSN 2322-939X • Vol 15, No 1 (enero-junio 2018). pp. 58-64. Universidad Distrital Francisco José de Caldas-Facultad Tecnológica.

GGC = 1. The online information system highlights in red the amino acid changes found in positions analyzed 316: TGC (C) → AAC (N), 426: ATG (M) → TTA (L) y 556: AGC (S) → GGC (G) (Figure 2).
A "force-directed" graph that identifies mutations of each patient sequence through node grouping, which corresponds to each analyzed sequence (Figure 3).

4. Discussion

HCV infection is a major public health problem. It is estimated that 200 million people worldwide are currently infected. The conventional treatment used for HCV based on the combination of two nonspecific drugs peg-IFN-α/RBV, is a long-term treatment, associated with numerous side effects and effective

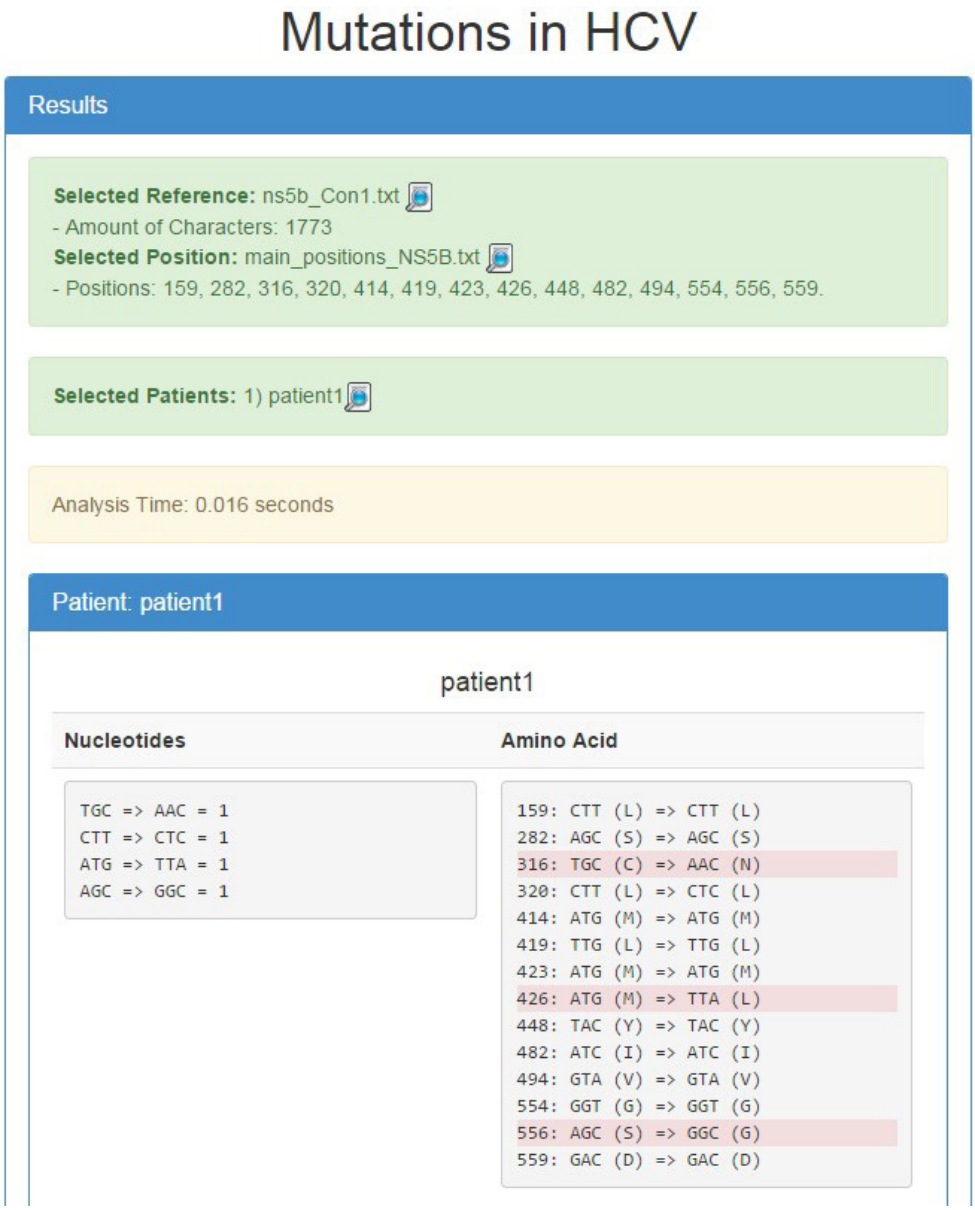


Figure 2. Result of the simulation performed in the mutation analysis for HCV NS5B gene.
Source: Own.

ISSN 1794-211X • e-ISSN 2322-939X • Vol 15, No 1 (enero-junio 2018). pp. 58-64. Universidad Distrital Francisco José de Caldas-Facultad Tecnológica.

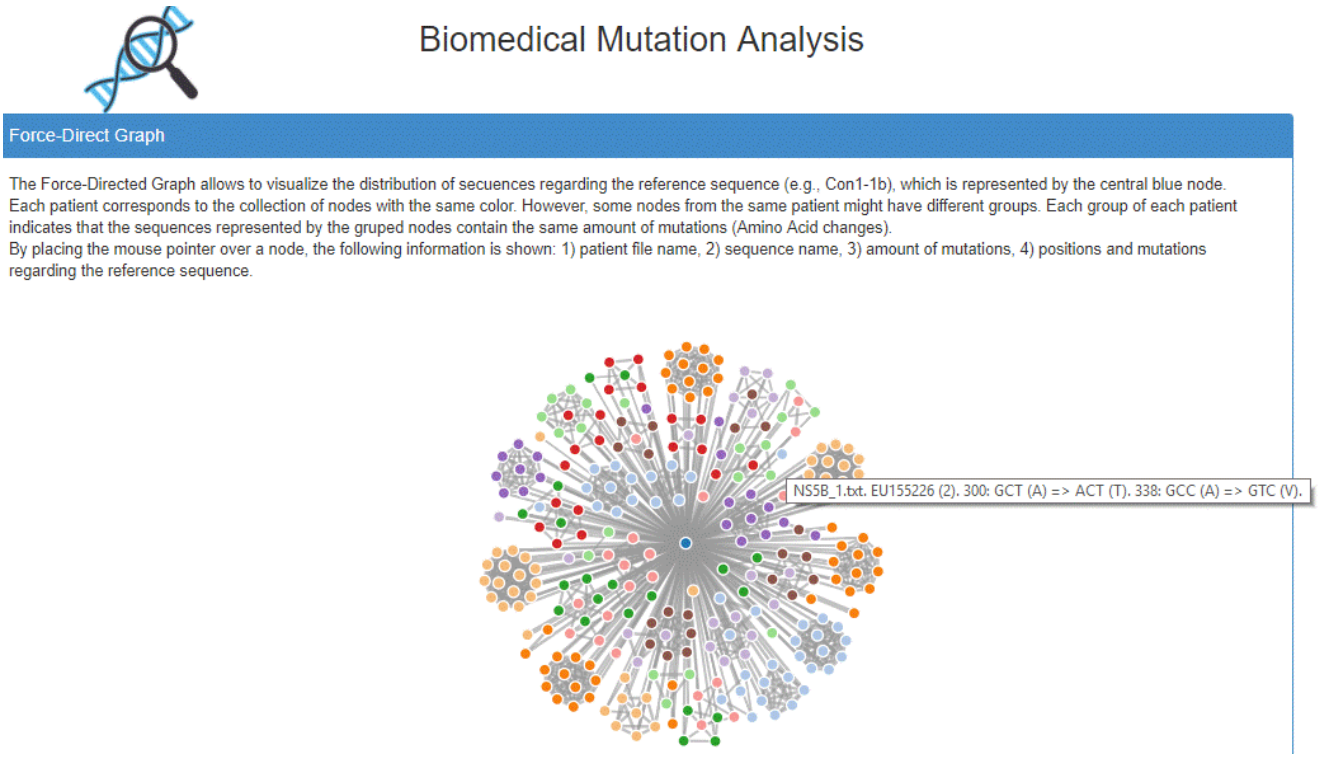


Figure 3. Force Directed Graph visualization. Result of the simulation performed in the mutation analysis for HCV NS5B gene.
Source: Own.

in approximately 50% of patients, with little success in patients infected with genotype 1. For this reason, new DAAs have been developed, some of which are specifically designed to inhibit NS5B polymerase which is essential for virus replication [17-19].

The use of antivirals that specifically target HCV proteins means it is possible to select preexisting resistant strains by selective pressure. Antiviral resistance is as a result of amino acid changes that produce conformational changes that interfere with the interaction between the drug and the target. The existence of resistant virus among in the infected population can be positively selected during therapy and result in a treatment failure [19]. Theoretically, before undergoing treatment, all patients infected with HCV have a heterogeneous population of genomes which may include DAA-resistant strains. Hence, the importance of identifying and analyzing the existence of such substitutions in the infected people to avoid treatment failure.

Most published studies have evaluated the mutations that confer resistance to new DAAs in HCV genotype 1, because most of antivirals have been developed using enzyme assays based on polymerases of this genotype, especially German isolated Con1. Therefore the nucleotide sequence of the NS5B gene isolate Con1 genotype 1b HCV, has served as a reference to design the online algorithm information system for computational analysis of the positions associated with mutations of clinical significance described in the literature as causing resistance to DAAs active in the NS5B gene.

Computational detection of mutations associated with resistance to new DAAs would be an important software tool for sequence analysis, because it is freely available software and it is not the same as other free software such as Bioedit, or MEGA. Although these other systems allow the researcher to

ISSN 1794-211X • e-ISSN 2322-939X • Vol 15, No 1 (enero-junio 2018). pp. 58-64. Universidad Distrital Francisco José de Caldas-Facultad Tecnológica.

perform analyses for the identification of mutations, our software design provides detailed information on positions of the mutations, amino acid changes that are of clinical interest, antivirals and literature on mutations resistant to DAAs.

Using the information system for the analysis of mutations resistant to DAAs designed and developed in this paper, a clinician would have additional information that would allow him/her to choose the correct DAAs for HCV treatment. However, it is important to mention that the NS5B nucleotide sequence of the gene obtained by the Sanger sequencing method is required to use the analysis algorithm information system.

It would be important to carry out the analysis of mutations resistant to DAAs before the treatment, during the first days of treatment and at the end of treatment in order to control the possible emergence of viral strains resistant to DAAs in the short and long term. Thus, by using antiviral tracking, it is possible to prevent treatment failures involving new DAAs.

5. Conclusions

The online information system allows quick, easy and effective computational analysis of mutations, providing a detailed view of changes in nucleotides and amino acids and supplying the clinician with additional information necessary to adjust the treatment of the HCV infection with DAAs.

Acknowledgements

The authors would like to thank the ITI Research Group of the Universidad Distrital Francisco José de Caldas (Colombia), for its collaboration in the project, making this research possible, and M.Sc. James Morgan for interpreting and translation.

Conflict of interest

The authors declare that there is no conflict of interests surrounding the manuscript.

References

[1] Q. Choo,G. Ku, A. Weiner, L. Overby, D. Bradley, M. Houghton, "Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome". *Science, New Series*, vol. 244, n°. 4902, pp. 359-362, 1989. https://doi.org/10.1126/science.2523562

[2] World Health Organization (OMS) "Guidelines for the screening, care and treatment of persons with hepatitis C infection", julio de 2017, [Online] Available: http://apps.who.int/iris/bitstream/handle/10665/111747/9789241548755_eng.EE4A59CFD8179A760BD71236?sequence=1

[3] C. Sarrazin, T. Berg, R.S. Ross, P. Schirmacher, H. Wedemeyer, U. Neumann, *et al.,* "Prophylaxis, diagnosis and therapy of hepatitis C virus (HCV) infection: the German guidelines on the management of HCV infection". *Z Gastroenterol,* vol. 48, n°. 2, pp. 289-351, 2010. https://doi.org/10.1055/s-0028-1110008

[4] M.P. Manns, H. Wedemeyer, M. Cornberg, "Treating viral hepatitis C: efficacy, side effects, and complications". *Gut.*, vol. 55, n°. 9, pp. 1350-9, 2006. https://doi.org/10.1136/gut.2005.076646

[5] V. Soriano, E. Vispo, E. Poveda, P. Labarga, L. Martin-Carbonero, J. V. Fernandez-Montero, *et al.*, "Directly acting antivirals against hepatitis C virus". *J AntimicrobChemother*, vol. 66, n°. 8, pp. 1673-86, 2011. https://doi.org/10.1093/jac/dkr215

[6] A. J. Thompson, S. Locarnini, "Direct-acting antiviral agents for the treatment of HCV". *AntivirTher.*, vol. 17, n°. 6 Pt B, pp. 1105-7, 2012.

[7] A. D. Kwong, L. McNair, I. Jacobson, S. George, "Recent progress in the development of selected hepatitis C virus NS3/4A protease and NS5B polymerase inhibitors". *CurrOpinPharmacol.,* vol. 8, n°. 5, pp. 522-31, 2008. https://doi.org/10.1016/j.coph.2008.09.007

[8] C. Welsch, F. S. Domingues, S. Susser, I. Antes, C. Hartmann, G. Mayr, *et al.*, "Molecular basis of telaprevir resistance due to V36 and T54 mutations in the NS3/4A protease of the hepatitis

ISSN 1794-211X • e-ISSN 2322-939X • Vol 15, No 1 (enero-junio 2018). pp. 58-64. Universidad Distrital Francisco José de Caldas-Facultad Tecnológica.

C virus". *Genome Biol.,* vol.9, n°. 1, pp. R16, 2008. https://doi.org/10.1186/gb-2008-9-1-r16

[9] D. L. Wyles, "Antiviral resistance and the future landscape of hepatitis C virus infection therapy". *J Infect Dis.,* vol. 207, Suppl 1, pp. S33-9, 2013. https://doi.org/10.1093/infdis/jis761

[10] D. L. Wyles, J. A. Gutierrez, "Importance of HCV genotype 1 subtypes for drug resistance and response to therapy". *J Viral Hepat.,* vol. 21, n°. 4, pp. 229-40, 2014. https://doi.org/10.1111/jvh.12230

[11] I. Najera, "Resistance to HCV nucleoside analogue inhibitors of hepatitis C virus RNA-dependent RNA polymerase". *CurrOpinVirol.,* vol. 3, n°. 5, pp. 508-13, 2013. https://doi.org/10.1016/j.coviro.2013.08.011

[12] K. J. Cortez, F. Maldarelli, "Clinical management of HIV drug resistance". *Viruses,* vol. 3, n°. 4, pp. 347-78, 2011. https://doi.org/10.3390/v3040347

[13] P. Halfon, C. Sarrazin, "Future treatment of chronic hepatitis C with direct acting antivirals: is resistance important?". *Liver Int.,* vol. 32, Suppl 1, pp. 79-87, 2012. https://doi.org/10.1111/j.1478-3231.2011.02716.x

[14] K. Salvatierra, S. Fareleski, A. Forcada, F. X. López-Labrador, "Hepatitis C virus resistance to new specifically-targeted antiviral therapy: A public health perspective". *World J Virol.*, vol. 2, n°. 1, pp. 6-15, 2013. https://doi.org/10.5501/wjv.v2.i1.6

[15] X. Tong, S. Le Pogam, L. Li , K. Haines, K. Piso, V. Baronas, *et al.*, "*In vivo* emergence of a novel mutant L159F/L320F in the NS5B polymerase confers low-level resistance to the HCV polymerase inhibitors mericitabine and sofosbuvir". *J Infect Dis.,* vol. 209, n°. 5, pp. 668-75, 2014. https://doi.org/10.1093/infdis/jit562

[16] X. Tong, L. Li, K. Haines, I. Najera, "The NS5B S282T Resistant Variant and Two Novel Amino Acid Substitutions That Affect Replication Capacity Were Identified in Hepatitis C Virus Infected Patients Treated with Mericitabine and Danoprevir". *Antimicrob Agents Chemother,* vol. 58, n°. 6, pp. 3105-3114, 2014. https://doi.org/10.1128/AAC.02672-13

[17] J. M. Pawlotsky, "New antiviral agents for hepatitis C". *F1000 Biol Rep.,* vol. 4, n°. 5, pp. 1-7, 2012. https://doi.org/10.3410/B4-5

[18] M. J. Sofia, W. Chang, P. A. Furman, R. T. Mosley, B. S. Ross, "Nucleoside, nucleotide, and non-nucleoside inhibitors of hepatitis C virus NS5B RNA-dependent RNA-polymerase". *J Med Chem.*, vol. 55, n°. 6, pp. 2481-531, 2012. https://doi.org/10.1021/jm201384j

[19] A. J. Thompson, J. G.McHutchison, "Antiviral resistance and specifically targeted therapy for HCV (STAT-C)". *J ViralHepat.*, vol. 16, n°. 6, pp. 377-87, 2009. https://doi.org/10.1111/j.1365-2893.2009.01124.x

ISSN 1794-211X • e-ISSN 2322-939X • Vol 15, No 1 (enero-junio 2018). pp. 58-64. Universidad Distrital Francisco José de Caldas-Facultad Tecnológica.