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# Detection of Hepatitis C Virus Resistant Mutants in the era of Direct Acting Antivirals

**Livia Maria Gonçalves Rossi and Paula Rahal**

*Department of Biology, Institute of Bioscience, Language and Exact Science, São Paulo State University, São José do Rio Preto, SP, Brazil*

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Corresponding Author:
Livia Maria Gonçalves Rossi,
Department of Biology,
Institute of Bioscience,
Language and Exact Science,
São Paulo State, University, São José do Rio Preto, SP, Brazil
Email: liv.rossi@yahoo.com

**Abstract:** Hepatitis C Virus (HCV) infects millions of people and represents an important public health problem in different regions of the world. Drug resistance is a major challenge for HCV-infection related control. Importantly, the arrival of a plethora of novel, more powerful drugs has revolutionized the field of HCV treatment. Here, we discuss the relevance of identification of HCV resistant mutants from a clinical standpoint. The advantage and limitations of molecular testing in clinical setting is presented.

**Keywords:** Hepatitis C Virus, Resistant Mutants, Therapy, Direct Acting-Antiviral

## Introduction

Hepatitis C Virus (HCV) infects millions of people worldwide (Lavanchy, 2009; Mohd Hanafiah *et al.*, 2013) and despite being subject of extensive research for more them 25 year (Choo *et al.*, 1989), effective antiviral therapies have just recently been developed (Afdhal *et al.*, 2014a; 2014b; Jacobson *et al.*, 2013; Kowdley *et al.*, 2013; Lawitz *et al.*, 2013a; 2013c; Sulkowski *et al.*, 2014b). HCV is a single-stranded, positive polarity, enveloped RNA flavivirus. The viral genome is ~9.6 kb in length and contains a single open reading frame encoding for a large polyprotein which is processed by viral and host proteases into three structural proteins and seven nonstructural proteins (Chevaliez and Pawlotsky, 2006; Stanley *et al.*, 2007). HCV is characterized by a high degree of genetic variability. The virus high replication rate and lack of proofreading activity of the viral polymerase (NS5B) are the main contributors to genetic variability (Cruz-Rivera *et al.*, 2013a; 2013b; Preciado *et al.*, 2014). The molecular plasticity of HCV allows for a rapid rearrangement of the intrahost viral population under different selection pressures (Ralston *et al.*, 2011; Von Hahn, 2007). Consequently, HCV genetic variation plays an important role in antiviral drug escape, especially in this era of Direct Acting Antivirals (DAA) (Marascio *et al.*, 2014).

Originally, anti-HCV therapy was based on Interferon (IFN), initially with IFN alpha (IFNα) in the early 90s and then in combination with Ribavirin (RBV) in 1998. Introduction of pegylated IFN in 2001 increased the likelihood of a Sustained Virological Response (SVR) in combined therapy regimens. The first generation of approved DAA came as a direct result of advancement in the understanding of the HCV life cycle aided by the subgenomic replicons strategies (Lohmann *et al.*, 1999) and the identification of a cell culture infectious clone (Wakita *et al.*, 2005). DAA directly inhibit specific steps in the HCV viral life cycle, particularly targeting the NS3/4A protease, NS5B polymerase and NS5A phosphoprotein, which are indispensable for viral replication. The first-generation of protease inhibitors telaprevir (Vertex, Janssen, Mitsubishi) and boceprevir (Merck) were licensed for use in combination with peg-IFN and RBV in patients infected with HCV genotype 1 in 2011 (Dore *et al.*, 2011). However, these drugs present low genetic barriers to emergence of resistant mutation (Sarrazin *et al.*, 2007; Susser *et al.*, 2009). A second-wave of NS3-4A protease inhibitors have reached phase II or III clinical trials, including Simeprevir (Janssen), already approved in the United States and Europe (Sheridan, 2014). However, these drugs also exhibit a low genetic barrier to resistance mutations and share extensive cross-resistance with telaprevir and boceprevir (Pawlotsky, 2014). Nucleoside/nucleotide analogues, another class of anti-HCV drugs, act as substrates for the HCV-RNA-dependent RNA polymerase (RdRp). This family of drugs tends to have higher genetic barriers to resistant mutants because selected mutations decreased viral fitness significantly. Sofosbuvir (Gilead) was the first polymerase inhibitor approved by FDA and several other candidates are in phase II clinical trials. Additionally, HCV NS5A protein inhibitors have been also developed (Sulkowski *et al.*, 2014b). The first NS5A inhibitors, Daclatasvir, developed by Bristol-Myers-Squibb

(Gao *et al.*, 2010), was followed by a number of alternative compounds, such as Ledipasvir (Gilead), Ombitasvir (Abbvie), among others (Table 1). The second-generation of NS5A inhibitors is already under development and the arrival of a plethora of improved drugs is expected in the near future. NS5A inhibitors have a dual mechanism of action: (i) Blocking the replication complex and (ii) inhibiting assembly and release of viral particles (Guedj *et al.*, 2013; Pawlotsky, 2013). Resistance mutations conferring resistance to these type of drugs are commonly found in the domain I of the NS5A protein. Not surprisingly, some of these mutations are shared by both first and second generation molecules, e.g., Q30, L31 and Y93 (Fridell *et al.*, 2010; Pawlotsky, 2014). Nonetheless, Daclatasvir exhibits a high genetic barrier to resistance *in vivo*, where multiple mutations are required to developed a resistant phenotype (Ross-Thriepland and Harris, 2014). Thus, with the arrival of DAA, a new era in the treatment of chronic hepatitis C has begun and viral eradication with interferon-free, DAA-based therapies is now feasible. The current HCV-treatment options are the following: (i) Simeprevir/faldaprevir/peg/RBV for genotype 1 infected patients, (ii) sofosbuvir/peg/RBV for genotype 1-6 infected patients, (iii) sofosbuvir/RBV (interferon-free) for genotype 1-4 infected patients, (iv) sofosbuvir/simeprevir for genotype 1 patients and (v) sofosbuvir/daclatasvir for genotype 1-3 patients.

So, the relevant question is, does resistance mutation screening in the era of DAA still make sense from a clinical standpoint? Natural occurring HCV resistant mutants commonly take place in the HCV life cycle. The incidence and frequency of resistant variants is variable and depends on a number of factors (Schneider and Sarrazin, 2014). Several studies have shown that resistant mutants at very low frequencies have no impact on treatment response. For instance, treatment-naïve patients with pre-existing resistant mutants prior to treatment achieved similar SVR rates compared to patients with undetected resistant HCV variants (Bartels *et al.*, 2013; Sulkowski *et al.*, 2013). Moreover, treatment response with the first generation of protease inhibitors is independent of pre-existing HCV resistant mutants if a good response to the peg/RBV-lead backbone is attained. However, among poor peg/RBV responders, those bearing resistant mutants showed lower SVR rates compared to those with sensitive viral populations (Howe *et al.*, 2014).

*In vitro* studies suggest that sofosbuvir has a pan-genotypic activity (Lam *et al.*, 2012). However, SVR rates differ between genotypes showing consistently lower SVR rates for genotype 3 (Lawitz *et al.*, 2013b), Moreover, differences between genotypes and subtypes have also been observed (Lawitz *et al*., 2013b). The reasons for these differences are not well understood.

Table 1. Current list of anti-HCV drugs (approved and clinical trials)

| Compound class | Generation/type | Agent name | Manufacturer | Status | Barrier | GT |
|---|---|---|---|---|---|---|
| NS3/4A protease inhibitors | First-generation, first-wave | Telaprevir | Janssen | Approved | Low | 1 |
| | | Boceprevir | Merk | Approved | Low | 1 |
| | First-generation, second-wave | Simeprevir | Tibotec | Approved | Moderate | 1,2,5,6 |
| | | Faldaprevir | Boehringer Ingelheim | Phase III | Moderate | 1 |
| | | Asunaprevir | Bristol-Myers Squibb | Pending approval | Moderate | 1,4 |
| | | ABT-450/r | Abbvie | Phase III | Moderate | 1 |
| | | Danoprevir | Roche | Phase II | High | 1,2,4 |
| | | Sovaprevir | Achillion | Phase II | Moderate | 1 |
| | | Vedroprevir | Gilead | Phase II | Moderate | 1 |
| | | IDX320 | Idenix | Phase II | Moderate | 1,2,3,4 |
| | | Vaniprevir | Merck | Phase III | Moderate | 1 |
| | Second-generation | MK-5172 | Merck | Phase III | Moderate | 1,2,4,5,6 |
| | | ACH-2684 | Achillion | Phase II | High | 1,2,4,5,6 |
| NS5B polymerase inhibitors | Nucleoside inhibitors | Sofosbuvir | Gilead sciences | Approved | High | 1-6 |
| | | Mericitabine | Roche | Phase III | High | 1,4 |
| | Non-nucleoside inhibitors | Deleobuvir | Boehringer Ingelheim | Phase II | | 1 |
| | | GS-9669 | Gilead Sciences | Phase I | | |
| | | Filibuvir | Pfizer | Phase II | | 1 |
| | | VX-222 | Vertex | Phase II | | 1 |
| | | BMS-791325 | Bristol-Myers Squibb | Phase III | | |
| | | Dasabuvir | Abbott | Pending approval | | 1 |
| | | ABT-072 | Abbott | Phase II | | 1 |
| | | Setrobuvir | Roche | Phase II | | 1 |
| | | Tegobuvir | Gilead sciences | Phase II | | 1 |
| NS5A inhibitors | First-generation | Daclatasvir | Bristol-Myers Squibb | Phase III | | |
| | | Ledipasvir | Gilead sciences | Phase III | | |
| | | Ombitasvir | AbbVie | Phase III | | |
| | | PPI-668 | Presidio pharmaceuticals | Phase II | | |
| | | PPI-461 | Presidio pharmaceuticals | Phase II | | |
| | | ACH-2928 | Achillion | Phase II | | |
| | | GSK-2336805 | GlaxoSmithKline | Phase II | | |
| | | BMS-824393 | Bristol–Myers Squibb | Phase II | | |
| | | Samatasvir | Idenix pharmaceuticals | Phase II | | |
| | Second-generation | MK-8742 | Merck | Phase II | | |
| | | ACH-3102 | Achillion | Phase II | | |
| | | GS-5816 | Gilead sciences | Phase II | | |

HCV mutants conferring resistance to sofosbuvir have been identified *in vitro*, mainly associated with S282T mutation (Lam *et al*., 2012). Additionally, in HCV 1a infected patients, viral variants bearing the I434M mutation were shown to be selected by sofosbuvir, leading to relatively low susceptibility.

Clinical trials showed that few patients who relapse after sofosbuvir monotherapy developed the S282T mutation. Importantly, no mutations associated with resistance to sofosbuvir have been detected in patients who showed a relapse after sofosbuvir combination therapy with either RBV or a second DAA. Moreover, no resistance associated with viral breakthrough has been observed in patients treated with any sofosbuvir regimens and robust and rapid SVR is commonly observed. Interestingly, patients who relapse after treatment with sofosbuvir and simeprevir develop Q80K, D168E and I170T mutations but emergence of sofosbuvir resistant mutants has not been reported.

The high genetic barrier for resistance mutants exhibited by sofosbuvir is likely multi-factorial. Fitness of S282T is severely impaired owed to the malfunction of the HCV RdRp activity, resulting in a decreased replication rate of ~90% in HCV 1a and 1b, resulting in the absence of viral breakthrough.

Relapse after completion of sofosbuvir-based therapy has exclusively been observed in patients treated with sofosbuvir alone or in combination with RBV. However, relapsing has not been reported in patients treated with combinations including a second DAA or Peg-IFNα. Relapse rates are particularly high in HCV 1 and 3-infected patients with previous null response to Peg-IFNα and RBV therapy and those with cirrhosis.

Deep-sequencing analysis allows detection of low frequency viral variants at a remarkable sensitivity (Cruz-Rivera *et al*., 2013b). While these powerful sequencing methods allow for the detection of HCV resistant mutants (Fonseca-Coronado *et al*., 2012), clinical usefulness of such expensive methods in clinical settings remains intriguing. While molecular characterization of HCV variants is a valuable tool for molecular epidemiology studies, outbreak investigation and genetic relatedness studies (Goncalves Rossi and Rahal, 2014), its utility for the monitoring of HCV resistant mutants in clinical settings remains controversial.

## Conclusion

Treatment of HCV has evolved rapidly resulting in improved SVR rates. Telaprevir and boceprevir have now been replaced by sofosbuvir and simeprevir, with improved efficacy and safety; and therefore, identification of protease inhibitor resistance mutants is no longer needed. While detection of Q80K mutation prior to treatment with simeprevir-based regimens might provide some guidance, decision making process should not rely exclusively on the pre-existence of such mutant. As a result, testing for Q80K mutation is not strongly recommended by the American Association for the Study of Liver Diseases/Infectious Diseases Society of America. Particularly now, that the IFN-free therapy is expected to become the standard of care for HCV in the near future.

In conclusion, more studies are required to evaluate the influence of pre-existing or persistent resistant mutations prior treatment on the efficacy of DAA-containing therapies. Currently, there is no evidence supporting for a general resistance testing before start of HCV-treatment (Schneider and Sarrazin, 2014). In some instances, resistance testing can assist in the decision making process and patient management. e.g., success of treatment among treatment-naïve patients and in patients after treatment failure with peg/RBV depends on the frequency of pre-existing resistant mutants and with sufficient treatment adherence. For simeprevir, the relative high frequency of pre-existing Q80K variants in genotype 1a in European and North American populations is associated with lower SVR and rapid virologic response rates. Therefore, for HCV 1a infected patients undergoing treatment with simeprevir is important to consider resistance testing for the Q80K mutant. For sofosbuvir, however, no pre-existing mutants are known; and therefore, resistance testing is not indicated.

In general, patients with poor interferon-responses are more likely to develop resistant variants. Importantly, no cross-resistance exists between NS3 and NS5A or NS5B inhibitors. Therefore, resistance testing prior to sofosbuvir-based, interferon-free therapy in conjunction with an NS5a inhibitor is not required.

For HCV 2 and 3 the sofosbuvir-based, interferon-free therapy and RBV, testing for pre-existing resistant variants is not necessary. In experienced patients treated with sofosbuvir and a protease inhibitor have shown to bear the Q80K variants at baseline. Consequently, prior to PI treatment failure, resistance testing may be useful to detect either Q80K and/or other NS3-variants in patients undergoing sofosbuvir/protease inhibitor re-treatment.

In a recent study the use of sofosbuvir and declastavir with/without RBV in HCV 1-3 infected patients showed baseline mutations associated with DCV resistance. Nevertheless, virologic relapse was detected in only one patient (Sulkowski *et al.*, 2014a). Thus, general resistance testing is not recommended in this case.

Resistant testing for HCV might add “too little and too late” information to include it as part of the decision making process and routine clinical testing required in the management of HCV cases.

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## Conflict of Interest

The authors of this manuscript declare no conflict of interest.

## Author's Contributions

All authors equally contributed in this work.

## Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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