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NS5A Resistance: Clinical Implications and Treatment Possibilities

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Abstract

Treatments with interferon-free direct-acting antiviral agents have high efficacy, with sustained virological response rates of more than 90%. Nevertheless, they fail to eliminate the infection in 1-7% of patients. The majority of virological failures are due to relapse following treatment discontinuation, while virological rebound during therapy is rare. Although not the only factor, the presence of resistance-associated variants is one of the major causes for said failure. Resistance-associated variants affect the sequence involved in protein synthesis on which different direct-acting antiviral agents act (NS3/4A, NS5A, NS5B). Of all these variants, the ones with the greatest impact are resistance-associated variants that affect the NS5A region due to their long-term persistence. In this article we will describe the most significant NS5A resistance-associated variants, the clinical relevance of their detection both before and after treatment, their persistence over time, and lastly, we will devote particular attention to discussing what approach to adopt when dealing with treatment failure to an antiviral regimen that includes NS5A inhibitors. **(AIDS Rev. 2016;18:15-22)**

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Key words

Hepatitis C. NS5A inhibitors. Drug resistance. Ledipasvir. Daclatasvir. Ombitasvir. Simeprevir.

Introduction

Hepatitis C virus was discovered in 1989[1]. In just over 20 years since its discovery, in record time, detailed understanding of the virus' life cycle has led to the development of four classes of direct-acting antiviral agents (DAA), which has allowed a cure for the infection to be provided for the majority of patients[2]. Four combinations are currently available: sofosbuvir/simeprevir, sofosbuvir/daclatasvir, sofosbuvir/ledipasvir, and paritaprevir/ritonavir plus dasabuvir/ombitasvir. They can all be used with or without ribavirin for between 8-24 weeks. These interferon (IFN)-free treatment regimens have a high rate of efficacy in the majority of patients, who in many cases only require treatment for a short duration. However, between 1-7% of patients do not manage to eliminate the infection[3].

The majority of virological failures are due to relapse following treatment discontinuation, while virological rebound during therapy is rare. On occasion, failure may be due to poor treatment adherence or early discontinuation due to some side effect.

Virological failure, i.e., the inability of DAAs to eliminate the virus, occurs more frequently in genotype 3-infected patients with cirrhosis and in those who have received short courses of treatment (< 12 weeks)

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Table 1. Factors involved in the failure of treatment with direct antiviral agents

Host
Cirrhosis
IL28B, non-CC
Male
Non-responder to PEG + RBV
Virus
Genotype 1a
Genotyping errors
Genetic recombination processes
Baseline or acquired resistance to NS5A inhibitors
Q80K
Genotype 3
Treatment regimen
Absence of ribavirin
Short duration of treatment
Poor adherence
Combination of two or more factors

PEG: pegylated interferon; RBV: ribavirin.

(Table 1). Other less frequent reasons for virological failure include an inadequate treatment regimen due to an error in the HCV genotyping, genetic recombination, or resistance-associated variants (pre-existing or acquired following initial exposure to DAAs). In this brief review, we will focus on an analysis of resistance-associated variants (RAV) in the NS5A region, their potential clinical impact, and what to do in the event of occurrence.

Concept and methodology for resistance-associated variant detection

HCV has a high turnover rate, estimated to be 2-5 hours, which means an extraordinary production of virions per day (10^{10} -10^{12}). This high replication activity and the absence of mechanisms to repair errors introduced by the RNA-dependent RNA polymerase during the replication process explain the extraordinary variability of HCV, which has a mutation rate that is estimated to be around 10^{-4} to $\times$ 10^{-5} per nucleotide and per replication cycle. Although many mutations are eliminated by the immune system or are not viable, others persist over time, accounting for all of the HCV genotypes, subtypes, and quasispecies. As a matter of fact, there are seven HCV genotypes whose nucleotide sequences differ by 30-35%, and 67 subtypes whose genotypic differences vary between 20-25%. Sequence variation in a single patient may be as much as 10%, defined as quasispecies. And among the thousands of mutations that occur, some may play a key role in resistance to antiviral therapy[4,5].

Resistance-associated variants are variants of the nucleotide sequence that are associated with resistance to different drugs, resulting from the genetic variability we have mentioned above. These RAVs may be present at baseline in treatment-naive patients or, conversely, they may occur in patients in whom prior antiviral treatment has failed. RAVs affect the sequence involved in protein synthesis on which different DAAs act (NS3/4A, NS5A, NS5B).

The genetic diversity of the virus populations and RAVs can be analyzed using three different methods:

- Direct population sequencing of the amplified PCR products, which is only able to detect sequences that represent between 15-25% of the total molecules in circulation.
- Clonal sequencing, i.e., cloning of the PCR product in plasmid vectors for subsequent direct sequencing, which allows a significant increase in the detection of minor variants (up to almost 1-5%).
- Next-generation sequencing that uses deep sequencing to generate thousands of sequences of the HCV circulating in the serum. The identification of thousands of viral sequences in the same serum panel allows resistances and compensatory mutations present in the same genome to be studied, identification of recombinant viruses, subtyping, and detection of mixed infections[6,7], which occasionally has implications for the efficacy of the treatment and therapeutic decision making. In addition, it allows the replicative capacity of the virus (viral fitness) to be studied.

The sequencing method is very important for the analysis of the clinical significance of the RAVs. In fact, the clinical consequences of the variants detected by direct sequencing have been clearly determined, while many of minor variants that have been detected through highly sensitive techniques, such as the new deep sequencing platforms, have not been associated with any known clinical phenomenon and no determinants of resistance to treatment have been found.

None of these techniques are available commercially right now. Only a handful of laboratories have the ability to perform sequencing of the different regions of the C virus, but the use of these techniques in clinical practice is hindered by lack of access to them and difficulty in interpreting the results.

There is controversy about the usefulness of testing for the presence of RAVs in treatment-naive patients.

Table 2. Described NS5A resistance variants.

	Genotype 1a									Genotype 1b					
	M28T	Q30R	Q30E	Q30H	L31M	Y93C	Y93H	Y93N	H58D	L28T	L31M	L31 V/F	Q54 H/N	Y93H	Y93N
Ombitasvir	+	+	–	–	+	+	+	+	+	+	–	+	–	+	+
Ledipasvir	+	+	+	+	+	+	+	+	+	–	+	+	–	+	–
Daclatasvir	+	+	+	+	+	+	+	+	–	–	–	+	+	+	+
Elbasvir	+	+	–	+	+	–	+	+	+	–	–	+	–	+	–

This table allows cross-resistances in nearly every variant described to be clearly seen.

- The guidelines agree on screening for the presence of NS3 Q80K polymorphism before administering treatment with pegylated IFN plus ribavirin plus simeprevir in patients with genotype 1a, even though this polymorphism has no effect on patients without cirrhosis who receive IFN-free treatment and screening is only recommended for cirrhotic patients with genotype 1a before receiving simeprevir plus sofosbuvir.
- The lack of evidence for the clinical significance of the presence of NS5A resistant variants at baseline for the rates of sustained virological response (SVR) in treatment-naive patients means that the majority of guidelines do not recommend screening for them before initiating antiviral therapy, and yet in patients with several factors associated with poor response to treatment (cirrhosis, genotype 1a), the presence of any of these resistant variants may significantly reduce SVR[8]. Likewise, a slight decrease in the rate of SVR has been demonstrated in treatment-naive patients infected with genotype 1a in whom NS5A resistant variants were detected and who were treated with a combination of grazoprevir/elbasvir[9].
- The majority of experts would consider resistance testing for all patients who fail to respond to DAAs to be desirable in order to guide rescue therapy. Despite the existence of different methods for determining resistance, they have different degrees of sensitivity and clinical interpretation is not easy as there is neither a certified method for doing so nor agreement on how to interpret the findings. This makes the standardization and subsequent marketing of a resistance test to guide treatment for these patients urgent. In the meantime, common sense would indicate that a change in the class of drugs used would be advisable in order for rescue therapy to be more effective.

Description of NS5A resistance-associated variants

The NS5A protein is key to the processes of replication, assembly, and cell egress of viral particles and also plays a role in some interactions with the host, although the exact mechanism by which it regulates viral replication is unknown[10]. NS5A protein inhibitors are pan-genotypic in spectrum, though the specific function of each one is variable and they have a relatively low genetic barrier.

The rate of detection of NS5A RAVs in various direct population-sequencing studies is around 0.3-3.5%. Of these, there are two RAVs in genotype 1b isolates that stand out: RAV L31M, which confers low-to-medium level resistance to ledipasvir and daclatasvir in 2.1-6.3% of patients, and RAV Y93H, which is the one most frequently detected in 3.8-14.1% of patients. This variant confers medium-to-high level resistance to all drugs that inhibit NS5A. This variant is more frequent in European patients (15%) in comparison to the USA (9.3%)[11]. When more sensitive detection methods are used, NS5A variants have been shown to be more frequent. Thus, a deep sequencing analysis of 2,000 patients revealed resistance variants to ledipasvir in 15.7 and 16.4% of HCV genotype 1a and 1b infected patients, respectively[11]. In a more detailed analysis of the RAVs that affect each one of the drugs that act on NS5A[12], these can be classified as follows:

- Daclatasvir: The most frequently reported RAVs in patients with genotype 1a who do not achieve SVR are M28T, Q30E/H/R, L31M, H58D, and Y93H/N. L31M/V and Y93H are most common for genotype 1b and Q30H/S for genotype 4.
- Ledipasvir: The variants reported for patients with genotype 1a who do not achieve SVR are Q30E/R, L31M, and Y93C/H/N and Y93H for genotype 1b.
- Ombitasvir: The most frequent for patients with genotype 1a who do not achieve SVR are M28T

and Q30R, while for genotype 1b it is Y93H and for genotype 4 L28V has been reported.

– Elbasvir: Antiviral activity in genotype 1a is reduced by single NS5A substitutions M28A/G/T, Q30D/E/H/K/R, L31M/V, H58D, and Y93C/H/N and in genotype 1b by variants L28M, L31F, and Y93H. In genotype 4, L30S, M31V, and Y93H have been reported (Ref. FDA Label).

As shown in table 2, there is cross-resistance to the majority of the NS5A RAVs that have been reported, i.e., resistance to one NS5A inhibitor usually confers resistance to other drugs in the same class, which suggests that this is a feature of the NS5A inhibitors drug class.

Pre-existing or baseline resistance-associated variants

As mentioned previously, over the natural course of hepatitis C there are many variants that can be associated with resistance to DAAs, either directly (mutations that occur at the position where the DAA and the virus bind) or indirectly, by affecting the functions of the virus protein. Detection of potential RAVs prior to treatment is controversial. The prevalence of these baseline polymorphisms is connected with the HCV genotype and subtype, and although the effects upon the efficacy of the DAAs are variable, generally speaking the clinical impact of these minority variants is very low[13]. Baseline NS3A RAVs have a low replication capacity and in every case they vanish within a relatively short period of time and can be seen with a relatively low frequency prior to treatment (< 3% in treatment-naive patients and < 7% in previously treated patients[14]. Cirrhotic patients with genotype 1a should be systematically screened for Q80K polymorphism at baseline as it is associated with a reduction in sensitivity to NS3A protease inhibitors (simeprevir, asunaprevir, paritaprevir). This recommendation is of particular value when regimens that combine simeprevir, ribavirin, and IFN are going to be used. There is significant geographical variability in the prevalence of this polymorphism, ranging from 48% in North America to between 9-19% in countries in South America and Europe, with significant variability from one country to another[15]. Q80K prevalence in patients with genotype 1a in Spain is around 7%. The impact of NS5A RAVs on SVR rates in treatment-naive patients does not appear to justify the need for conducting a study of baseline resistance in these patients. It may, however, be useful in patients previously treated with DAAs who have failed to treatment, especially if a patient has advanced liver disease. In any event, in the absence of a certified testing method, an assessment of the case, and clinical reasoning based on treatment history should guide the choice of a retreatment strategy. Pretreatment NS5A RAVs are more frequent in genotype 1b and there is high geographic variability. The presence of NS5A resistance variants at baseline differs according to genotype and it is more common in genotype 1a than in genotype 1b. Furthermore, it varies according to the geographical region under study, ranging from 6% in Spain up to 17% in Italy for genotype 1a, and from 8% in Germany up to 17% in New Zealand for genotype 1b[16].

In 0.2-3.1% of genotype 1a cases, pre-existing RAVs associated with resistance to NS5B, such as dasabuvir, and to other nonnucleoside inhibitors, such as tegobuvir, have been described. These RAVs occur much more frequently than the ones that affect nucleotide inhibitors and are associated with resistance and viral breakthrough. In general, they are more frequent in genotype 1a than in genotype 1b[17]. The exceptions are C316N, which was identified in genotype 1b in 10.9-35.6% of cases[18] and S556G, also in genotype 1b, both of which are associated with the conferral of low-level resistance to dasabuvir. RAV C316N/H/F has been detected at baseline in six patients with genotype 1b HCV who failed to treatment with sofosbuvir, and in one patient with genotype 1a who presented with a relapse after the end of sofosbuvir treatment. Nonetheless, further studies are required to accurately determine the role played by RAVs in the resistance to treatment with sofosbuvir[19]. The S282T substitution in the NS5B region described in the ELECTRON study has been associated with reduced susceptibility to nucleotide NS5B inhibitors, of which sofosbuvir is the molecule of choice, it is rarely seen and is not related to any other RAVs in NS5B[16]; it was detected in one patient with genotype 2 infection who had a relapse at four weeks after treatment[20].

An interesting aspect to consider is whether the treatment regimen should be guided by previous presence of RAVs. A comprehensive review of mutations that have been described in HCV was recently published[12]. A European study with 312 participants was conducted of RAVs at baseline in the NS3, NS5A, and NS5B regions, with potential relevance for treatment with telaprevir, simeprevir, asunaprevir, daclatasvir, ledipasvir, ombitasvir, and dasabuvir. No RAVs resistant to sofosbuvir were seen at baseline. RAVs were detected within NS3 in 20.5% of cases, within NS5A in 11.5% of patients (more frequently in genotype 1b than in genotype 1a), and within NS5B in 21.5% (also more frequently in genotype 1b)[21]. The authors suggested that an analysis of baseline RAVs could be used to guide selection of the

most appropriate antiviral treatment option. However, it is not currently recommended by any clinical guidelines.

Persistence and durability of resistance-associated variants

Protease inhibitor RAVs disappear, both when they emerge in treatment with simeprevir[22] as when they do in regimens that include paritaprevir[23] or grazoprevir[9]. However, the RAVs that concern us in this review, which are related to treatment with NS5A inhibitors, persist long term in patients treated with ledipasvir[24] as well as with ombitasvir[23] or elbasvir[9]. Likewise, RAVs associated with nonnucleotide NS5B inhibitors such as dasabuvir also persist over time[25]. This persistence may be prolonged as other studies have shown, which have reported the presence of RAVs in around 85% of NS5A subjects up to 96 weeks after the end of treatment, which is suggestive of the fitness of these variants[24,26,27]. As an example, the study by Dvory-Sobol, et al.[24] should be noted, in which RAVs to ledipasvir persisted in 50 of 58 patients who had them for 96 weeks after discontinuation of treatment.

Clinical significance of resistance-associated variants

There is greater information available about RAVs that can confer resistance to DAAs; however, many aspects are not yet very well known. Although not the only one, RAVs are an obvious predisposing factor for the future emergence of resistance to treatment. This resistance will be dependent on at least the following factors: (i) its quantitative importance (i.e., whether or not it is preponderant in the infecting virus population), (ii) the potency of the antiviral regimen (a more determining factor than the number of DAAs used), (iii) the genetic barrier of the DAA (number of mutations required for HCV to become resistant to the DAA) and, (iv) viral fitness (the efficient replication of the viral variant). And, though there is not yet any scientific support for this claim, it is probable that the development of RAVs increases as a result of suboptimal adherence to treatment.

The main clinical implication of RAVs is the impact they may have on achieving SVR. As a general rule, baseline RAVs do not have a decisive impact on the probability of achieving SVR, especially NS3 RAVs in minority populations (< 1%). The impact of RAVs to NS5A is variable, with absence of SVR being more frequent when associated with other negative predictive factors such as the presence of cirrhosis[28]. Some authors show that the impact of RAVs on the activity of NS5A inhibitors is greater in genotype 1a than in genotype 1b[29]. Their potential impact on retreatment has also been observed, as shown in two studies. In the first, all treatment failures occurred in the group of patients with RAVs[24] and in the second, nearly all of the 22 patients (of 471 patients treated with grazoprevir and elbasvir) who did not achieve SVR had RAVs, and furthermore, in many cases these were already present before initiation of the treatment[9]. However, not all studies are in agreement, as demonstrated by the fact that in 94 patients who had baseline RAVs (out of a series of 511, of whom 18% had cirrhosis) and who received treatment with sofosbuvir and ledipasvir, the rates of SVR were similar to those of patients without RAVs at baseline (91 vs. 98%)[8]. On the other hand, the ION-3 study showed that in patients who had received prior treatment for eight weeks and did not achieve SVR, retreatment for 24 weeks was associated with a very low rate of SVR (< 9%) if they had presented with one or more baseline NS5A RAVs[30].

In conclusion, the majority of studies published suggest that the presence of resistant variants at baseline reduces SVR rates when other negative predictive factors, such as presence of cirrhosis, genotype 1a or 3, or a short duration of treatment (under 12 weeks) are present.

Virologic failure in patients with antiviral regimens that include NS5A inhibitors: Treatment studies that have been conducted

Failure to sofosbuvir plus ledipasvir

From a theoretical point of view, in cases of failure to treatment with sofosbuvir plus ledipasvir, failure is almost certainly due to the emergence of NS5A resistance variants with low sensitivity to sofosbuvir, since the S282T mutation has not been reported in any registration trials. As a matter of fact, NS5A RAVs are found in 76% of failures to treatment with sofosbuvir/ledipasvir[11]. Experience with retreatment after failure to sofosbuvir/ledipasvir is low and different strategies have been used, including retreatment for 24 weeks using the same antiviral regimen. This strategy was of little use in patients who had been previously treated for 12 weeks (46% SVR) and only relatively efficacious (80%) in patients treated for eight weeks[30]. RAVs were detected in 19 cases, with a high impact on chances of SVR: 60% of SVR in patients with RAVs vs. 100% in patients without RAVs, with the efficacy of retreatment being lowest in patients with more than one RAV. Moreover, the presence of the Y93H/N variant was correlated with high resistance to treatment (only two of the six carriers of this RAV achieved SVR). This study did not include use of ribavirin. Lastly, we may be using functional

monotherapy with sofosbuvir as a matter of principle, with the subsequent risk of emergence of RAVs to sofosbuvir that are especially resistant to antiviral treatment. Retreatment with sofosbuvir/ledipasvir ± GS-9669 ± GS-9451 has been tried recently in patients who failed to sofosbuvir/ledipasvir and has shown high antiviral efficacy, although in one case it resulted in highly complex resistance (L31M, Y93H, S282T, V321) for which there are no treatment options at present[31].

Regimens that include daclatasvir

With regard to failure to sofosbuvir/ledipasvir, the studies have shown results that are similar to those described above, even though there is less experience with this combination. The sofosbuvir/daclatasvir combination was evaluated in 152 genotype 3 patients who were either treatment-naive or treatment-experienced and resulted in a relapse rate of 9% in the treatment-naive patients and 14% in the treatment-experienced patients, especially those who had cirrhosis. All of the patients who relapsed had NS5A resistance variants, six of whom presented with them prior to beginning treatment and 10 in whom it emerged after treatment[32].

An article published in Hepatology evaluated the results of a pilot study that showed the efficacy of sofosbuvir/simeprevir without ribavirin for 12 weeks in 16 patients who did not respond to daclatasvir/pegylated ribavirin (n = 12) or daclatasvir/asunaprevir/pegylated ribavirin (n = 3). A response was obtained in 14 cases. The two patients who relapsed had genotype 1a, cirrhosis and presented with baseline RAVs in both NS3 and NS5A (R155K, Q80K and V170I) and NS5A (M28T, L31M)[33].

AbbVie's 3D regimen

As with the sofosbuvir/ledipasvir combination, the high efficacy of the 3D regimen causes the number of patients who experience virologic failure to be very low so, therefore, experience with retreatment following virologic failure to this regimen is very small. Sequencing analyses of virologic failure were conducted in a study of more than 1,000 treated patients. Resistant-conferring variants were detected in NS3-NS4 in 78% of patients with genotype 1a and in 57% of those with genotype 1b, with prevalence being greatest at D168. NS5A resistance variants were found in 72% of patients with genotype 1a, but only 29% of patients with genotype 1b. This study did not report the number of patients with resistance variants with respect to the three targets (NS3, NS5A, NS5B), but on the basis of the data it is estimated to be more than 70%[23].

The following options have been explored: the addition of interferon and ribavirin to the 3D regimen and adding sofosbuvir to the 3D regimen, although the results of these two strategies are not yet fully known. The QUARTZ-1 study investigated retreatment after failure to the 3D regimen (and to other combination treatments) in 22 patients who received a combination of paritaprevir, ombitasvir, dasabuvir, and sofosbuvir with or without ribavirin for 12 or 24 weeks, with SVR achieved in 20 cases. The two patients who had relapses had genotype 1a with cirrhosis and were treated for 12 weeks[34]. In any event, these strategies should be approached with caution and the impact or need for associating more than three drugs should be analyzed, both from the perspective of efficacy (the "logic" of not using the same NS5A inhibitor for retreatment) as from the perspective of tolerability (association of two polymerase inhibitors).

What to do in the event of failure to a treatment regimen containing an NS5A inhibitor

Now that it is possible to clear HCV infections in most cases, it is time to pay special attention to those patients who fail therapy. The failure to DAAs typically occurs after the end of treatment, and virologic breakthrough is very rare during therapy. Given the difficulties for understanding the underlying mechanisms that have caused the therapy to fail, and in the absence of clear guidelines with regard to the approach that should be taken in such cases, it may be appropriate to make a few recommendations before new antiviral treatment is initiated.

In this respect, although this review aims to analyze the clinical significance of RAVs, it is worth remembering that resistance is not the be all and end all in treatment failure[35]:

- Without doubt, the first thing that we need to consider is our patient, i.e., we should properly classify the disease: whether or not the patient has cirrhosis, portal hypertension, risk for hepatic decompensation, possible need to be placed on the waiting list for liver transplantation, or if there is reinfection of liver graft. That is, we must assess the urgency of retreatment.
- In the second place, careful assessment must be made of the antiviral therapy prescribed. In this regard, the use of DAAs against NS5A resistance is of particular interest as we have already seen; NS5A RAVs are persistent over time so retreatment that does not include this class must be considered. In addition, it is necessary to rule out potential

interactions, the use and actual time of administration of the proton pump inhibitor, time at which treatment failure occurred, whether ribavirin was used or not, and adherence to the treatment.

- And undoubtedly, we need to assess the virologic characteristics of HCV: viral load, genotype, and subtype. In this regard, and although no guidelines explicitly recommend doing so, we believe that it is advisable for all patients who fail to a latest-generation antiviral treatment regimen to undergo testing to confirm or rule out the existence of RAVs and/or other factors which may explain the virological failure. Since not every hospital is able to avail itself of sequencing technology, it is indispensable that referral hospitals be equipped for this purpose. In any event, one thing that is within reach for everyone is preservation of a patient sample at –70ºC before beginning retreatment, in order for as detailed an in-depth virological study as possible to be undertaken.

Both the EASL and the AASLD acknowledge that there is not enough scientific evidence to recommend any given treatment regimen after failure of an IFN-free antiviral therapy that includes two or more DAAs. However, since in some cases treatment should be urgently initiated, it does seems reasonable to try and take a practical approach to the problem. Keeping the general principles that have been set out in mind, a few general recommendations can be made that presumably will change in a very short time and which, as indicated in the guidelines of both the EASL and the AASLD, are largely based on indirect evidence.

- As sofosbuvir has a high genetic barrier to resistance and RAVs are uncommon, most patients who fail to antiviral therapy with DAAs should be treated with a regimen that includes sofosbuvir. This regimen should be IFN-free and, if possible, a DAA of a class that has not been used previously should be included.
- Treatment should probably include ribavirin, although it is not known whether adding it or extending treatment duration are equally effective. It is not known whether ribavirin is able to reduce resistance to treatment. An analysis of 513 patients with genotype 1 and cirrhosis, both treatment-naive and previously treated, who received sofosbuvir/ledipasvir ± ribavirin for 12 or 24 weeks, allows us to conclude that ribavirin is especially useful in patients with RAVs, as the chances of SVR increase by nearly 10%[36].
- It is very important to remember that failure to a regimen containing an NS5A inhibitor is likely to contain RAVs with cross-resistance to any NS5A inhibitor and that these variants tend to not disappear[11,25,37].
- The majority of the patients who will fail to treatment in our milieu in the near future will have advanced fibrosis and will therefore require retreatment very rapidly. Patients who do not have an urgent need for treatment and with only early-stage fibrosis should be able to wait until more information and/or alternative treatment options that have been investigated in clinical trials intended to solve this problem become available.
- It may also be appropriate to ask the sponsors of the trials for a roadmap, a predefined strategy with regard to the approach that should be taken in the event of failure of the antiviral therapy, and adapting this approach to the reason for the failure.

The EASL[38] recommends including sofosbuvir because of its high genetic barrier and:

- In the event of failure to NS5A complex inhibitors, add simeprevir plus sofosbuvir for a duration of 12 or 24 weeks, depending on the stage of fibrosis (sofosbuvir plus simeprevir plus ribavirin for 12 weeks for patients with fibrosis stage ≤ F2 and sofosbuvir plus simeprevir plus ribavirin for 12 weeks for patients with fibrosis stage > F2).
- For failure to a 3D regimen, add simeprevir or ledipasvir for 12 or 24 weeks depending on the stage of fibrosis (sofosbuvir/ledipasvir/ribavirin or sofosbuvir/simeprevir/ribavirin for patients with fibrosis stage ≤ 2 and sofosbuvir/ledipasvir/ribavirin or sofosbuvir/simeprevir/ribavirin for 24 weeks for patients with fibrosis stage > 2).

For its part, the AASLD[39] recommends that patients with genotype 1a or 1b in whom an NS5A inhibitor-containing regimen has failed be tested for RAVs and receive treatment as follows below in accordance with the results:

- If there is evidence of NS5A RAVs (or testing has not been conducted) a combination of simeprevir/sofosbuvir/ribavirin seems most reasonable.
- If there are no NS5A RAVs, the most appropriate regimen would be a combination of sofosbuvir/ledipasvir/ribavirin, and in our view, simeprevir and sofosbuvir also constitute a good option for retreatment. In the event of RAVs in the NS5A and NS3 regions, the most reasonable approach would be to try and include the patient in a clinical trial of new DAAs.
- In both cases, the duration of treatment should be 24 weeks and, although the role of ribavirin remains to be elucidated, the most reasonable option is to add it to the treatment regimen because of its ability to reduce relapses.

Conclusion

In conclusion, resistance to antivirals is an issue that will become relevant in the coming months because of the large absolute number of patients who are receiving antiviral treatment. There is not enough evidence to enable making a solid recommendation regarding treatment of choice for patients who have failed to a regimen with two or more direct antivirals. As long as this information is nonexistent, the most sensible recommendation is for caution, especially for patients with mild or moderate fibrosis. Patients with cirrhosis or in urgent need of treatment should be treated with drugs without cross-resistance to the drugs used in the previous treatment. As long as there is no other information, the duration of treatment should be 24 weeks and ribavirin should be used.

Numerous studies to assess the use of triple therapies that are aimed at the three targets are being conducted. From a theoretical perspective, it could be an excellent option for the retreatment of patients with virological failure to two or more antivirals. We await new data.

Declaration of interest

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