

Special Contribution

Understanding Hepatitis C Virus Drug Resistance: Clinical Implications for Current and Future Regimens

David L. Wyles, MD; Anne F. Luetkemeyer, MD

Viral resistance to direct-acting antiviral drugs may impact their effectiveness during treatment of hepatitis C virus (HCV) infection. Most data on HCV drug resistance concern genotypes 1 and 3. The clinical impact of resistance to HCV nonstructural protein 5A (NS5A) inhibitors and a practical approach to indications and methods for resistance testing are discussed.

Keywords: hepatitis C virus, HCV, resistance, resistance-associated substitution, RAS, mutations, HCV treatment, HCV retreatment, direct-acting antiviral, NS5A

Viral resistance to hepatitis C virus (HCV) direct-acting antiviral (DAA) drugs has emerged as an important consideration to optimal DAA use during treatment of HCV infection. The replication dynamics of HCV in chronically infected humans combines a high rate of viral production with an error-prone RNA polymerase, providing a favorable setting for the emergence and enrichment of viral nucleotide substitutions that confer resistance to specific drugs or drug classes, particularly under drug selection pressure. Although, in theory, all resistance-associated substitutions (RASs) in all HCV proteins are generated daily in an infected individual,¹ RASs that have clinical impact are much more limited. These limitations are determined by drug class, viral genotype, replication fitness conferred by the RAS, and patient characteristics such as prior HCV treatment and the presence of cirrhosis.

Most data on the impact and selection of RASs concern HCV genotype 1 infection, and to a lesser extent, genotype 3 infection. Certain polymorphisms that confer resistance to some DAA drug classes are present with other HCV genotypes (eg, genotype 2). However, these polymorphisms have limited clinical impact and there is a lack of commercially available diagnostic testing options. In HCV genotype 1 infection, viral subtype plays an important role in the

prevalence of preexisting (baseline) nonstructural protein 5A (NS5A) RASs and their clinical impact.²

Of the major HCV antiviral drug classes, there is only compelling evidence for the impact of NS5A inhibitor RASs on treatment outcome. The RASs impacting the NS5B nucleotide inhibitor sofosbuvir are not present in individuals who are not exposed to this drug, and these RASs emerge infrequently (in approximately 1%) in those whose therapy with this drug has failed.^{3,4} The signature NS5B mutation, S282T confers a modest level of resistance based on in vitro data ($3 \times -10 \times$ fold-change in median effective concentration [EC₅₀]) and is unfit for viral replication (replication fitness approximately 8% of wild-type).³ However, clinically, S282T has not been shown to impact the efficacy of sofosbuvir. Thus, there is no current role for NS5B resistance testing in treatment-naïve or -experienced individuals.

Clinically significant RASs to NS3 protease inhibitors (PIs) are also rare in the absence of prior drug exposure. Although much attention has been paid to the Q80K polymorphism in HCV genotype 1a, current evidence does not support a substantial effect of this variant on responses to treatment with simeprevir plus sofosbuvir at recommended durations, with the exception of treatment-experienced individuals with cirrhosis, for whom Q80K testing is recommended.⁵ Further, no impact is expected of the Q80K polymorphism on other NS3 inhibitors such as ritonavir-boosted paritaprevir and

Table 1. Resistance-Associated Substitutions in the Hepatitis C Virus (HCV) Nonstructural Protein 5A (NS5A) Gene Associated With Resistance to NS5A Inhibitors, by Genotype^a

	HCV Genotype		
	1a	1b	3
Resistance-associated substitutions	K24E/R/N	M/L28M/G/T	A30K
	M28A/T/G/V	R30H	L31I/F
	Q30R/K/E/H/L/Y/G/T/D/I	L31V/M/F/I	Y93H
	L31M/V/F	P58S	
	H54R	Y93H/N/C/S/R	
	H58D/P/R		
	E62D		
	Y93H/N/C/S/F/L		

^aThe letter preceding the number is the wild-type amino acid; the number is the position on the gene; and the letter or letters after the position are the amino acid substitution(s) at that position that are associated with NS5A inhibitor resistance.

grazoprevir. NS5A RASs emerge in approximately 50% (range, 25%-78%) of cases of virologic failure of a PI-containing regimen,^{6,7} with the most prominent variants at positions 155, 156, and 168. The R155K variant is only observed in genotype 1a HCV and does not impact the activity of grazoprevir.⁸ By contrast, variants of D168 and A156 are the most clinically relevant, as they emerge with relative frequency, impact the activity of all currently available HCV PIs, and are observed in both genotype 1a and 1b infections. Fortunately, most variants at these positions display poor replicative fitness in vitro and are lost rapidly following removal of drug selective pressure.^{6,7} It is not known if previously selected variants can still impact subsequent therapy once they are no longer detectable by sequencing.

RASs in NS5A are the most important clinically. The major RASs are depicted in Table 1. General characteristics of NS5A RASs are outlined in the Box. Substantial cross-resistance among currently available NS5A inhibitors is also notable. RASs at key positions (28, 30, 31, and 93) in HCV genotype 1a result in broad cross-resistance to early generation NS5A inhibitors. Exceptions include the lack of impact of the L31M RAS on ombitasvir and of the M28V RAS on elbasvir or ledipasvir.⁸⁻¹⁰ Although velpatasvir is less impacted by these NS5A RASs, Y93H/N RASs in genotype 1a still confer high levels of resistance to this drug.¹¹ The investigational next-generation NS5A inhibitors pibrentasvir (ABT-530) and ruzasvir (MK-8408), which are anticipated to become available in the next year, retain activity against all of the key single-position NS5A RASs in HCV genotypes 1a and 1b and, therefore, may retain activity despite resistance to current NS5A inhibitors.^{12,13}

Nomenclature and Identification of Resistance

The prevalence and clinical impact of RASs correlate with how RASs are defined and the sequencing methods used to detect them within RNA quasispecies. In order of decreasing prevalence, the RASs studied for NS5A are: 1) NS5A inhibitor resistance-associated polymorphisms, defined as any variation from the consensus sequence at all positions associated with resistance to any NS5A inhibitor (a specific amino acid change does not necessarily impact the in vitro activity of any NS5A inhibitor); 2) NS5A inhibitor class RASs, defined as amino acid variants at all positions associated with resistance to any NS5A inhibitor that confer in vitro resistance to at least 1 NS5A inhibitor; and 3) NS5A drug-specific RASs, defined as amino acid variants that impact the activity in vitro of a specific drug. The threshold for considering the impact of RASs on a specific drug varies based on the in vitro fold-change in EC₅₀ and ranges from 2× to 20× or even 100× fold-change in EC₅₀ (see Table 2).

Assays vary in their threshold for detecting NS5A RASs. Ultradeep sequencing (UDS, or next-generation sequencing) allows the detection of RNA substitutions present in 1% of the viral population (subject to the input RNA and number of reads obtained). This technique is often used in research supporting clinical drug-development programs. This approach

Box. Characteristics of Nonstructural Protein 5A (NS5A) Resistance-Associated Substitutions (RASs)

- Baseline (ie, prior to drug exposure) NS5A RASs are relatively prevalent (13% prevalence in genotype 1a infection,¹⁵ 18% prevalence in genotype 1b infection,¹⁵ and 12%-17% prevalence in genotype 3 infection^{11,22}).
- The clinical impact of baseline NS5A RASs varies by hepatitis C virus (HCV) genotype and subtype, with the largest impact in genotype 1a and 3 infections.
- Key NS5A RASs by genotype are:
 - Genotype 1a. M28A/T/V, Q30E/H/K/R, L31M/V, and Y93C/H/N
 - Genotype 1b. L31I/M/V and Y93H
 - Genotype 3. A30K and Y93H
- Patient characteristics, including the presence of cirrhosis and prior HCV treatment (non-NS5A inhibitor based), increase clinical impact of NS5A RASs.
- Following failed NS5A-based treatment, the majority of individuals have HCV with NS5A RASs (75%-90%).^{4,6,7}
- NS5A RASs persist in most individuals for more than 2 years.^{6,24}
- The impact of NS5A RAS is relative and can often be overcome by increasing the length of therapy and/or by adding ribavirin.¹⁸

provides a detailed assessment of RASs but, at the 1% threshold, is too sensitive for optimal clinical decision making. Population (or Sanger) sequencing generates an average sequence for a viral population. Substitutions must be present in a substantial proportion of viral sequences to be identified, generally, 15% to 25% of the viral population. The risk with this approach is that, in theory, clinically significant minority RASs may be missed.

In studies in which a 1% UDS threshold was compared with either population sequencing or with a 10% to 15% UDS threshold, better discrimination between clinically significant and insignificant baseline NS5A RASs was obtained with population sequencing and a 10% to 15% threshold UDS approach.^{10,14} Data from a comprehensive study of baseline RASs in individuals treated with coformulated (indicated with a /) ledipasvir/sofosbuvir indicated that the difference between 10% and 15% UDS cutoffs was minimal.¹⁵ Commercially available HCV resistance tests in the United States utilize a population sequencing assay (Quest Diagnostics, Madison, New Jersey) or a UDS assay with substitutions called at the 10% level (LabCorp/Monogram Biosciences, South San Francisco, California). Currently, such testing is only available for HCV genotypes 1a, 1b, and 3.

Considerations for Resistance Testing for NS5A Inhibitor Treatment-Naive Individuals With HCV Genotype 1 Infection

For individuals with HCV genotype 1 infection who have not been exposed to an NS5A inhibitor, NS5A RAS testing

Table 2. Fold-Changes in EC₅₀ for Select Resistance-Associated Substitutions for HCV Drugs, by Genotype^a

HCV Drug	Fold-Change								
	RASs in HCV Genotype 1a				RASs in HCV Genotype 1b				
	M28T	Q30R	L31M	L31V	Y93H	Y93N	L31V	Y93H	Y93N
Daclatasvir	>100×	>1000×	>100×	>1000×	>1000×	>10,000×	<10×	20×	50×
Elbasvir	20×	>100×	>10×	>100×	>1000×	>1000×	<10×	>100×	NA
Ledipasvir	20×	>100×	>100×	>100×	>1000×	>10,000×	>20×	>100×	NA
Ombitasvir	>1000×	>100×	<3×	>100×	>10,000×	>10,000×	<10×	20×	50×
Pibrentasvir	<3×	<3×	<3×	<3×	<10×	<10×	<3×	<3×	<3×
Ruzasvir	<10×	<10×	<10×	<10×	<10×	<10×	<10×	<10×	<10×
Velpatasvir	<10×	<3×	20×	50×	>100×	>1000×	<10×	<3×	NA

Abbreviations: EC₅₀, median effective concentration; HCV, hepatitis C virus; NA, data not available; RAS, resistance-associated substitution.

^aRASs highlighted in red are more likely to confer a clinical impact due to the high fold-change. RASs highlighted in orange have an intermediate impact on efficacy. RASs highlighted in shades of green are not likely to have a clinically significant impact.

^bInvestigational drug.

is generally only indicated in those with genotype 1a before treatment with elbasvir/grazoprevir and is a consideration for those with genotype 1a who will receive a ledipasvir/sofosbuvir-based regimen and have cirrhosis or whose prior treatment (non-NS5A inhibitor based) failed (see Table 3).

Baseline NS5A resistance to elbasvir (RASs conferring >5-fold reduced susceptibility) is infrequent, occurring in 5% of individuals tested with population-level sequencing.¹⁴ However, when elbasvir RASs were present, sustained virologic response 12 weeks after cessation of treatment (SVR12) decreased to 58%, compared with 98% in those without elbasvir RASs. Thus, NS5A testing is recommended for individuals infected with HCV genotype 1a considering treatment with elbasvir/grazoprevir. If elbasvir RASs are identified, therapy with elbasvir/grazoprevir should be extended to 16 weeks and weight-based ribavirin should be added, or an alternative regimen should be selected if available.^{16,17} This guidance is extrapolated from data on individuals with HCV genotype 1a infection and prior nonresponse to treatment who were treated with 16 or 18 weeks of elbasvir/grazoprevir, 100% of whom attained an SVR12 regardless of the presence of elbasvir RASs. Elbasvir RASs for genotype 1a are M/L28T/A/G, Q/R30E/H/R/G/K/L/D, L31M/V/F, H58D, and Y93C/H/N/S.

NS5A RAS testing may be considered for individuals with genotype 1a infection who plan to take ledipasvir/sofosbuvir and have cirrhosis or are treatment experienced (see Table 3), as RASs may impact the duration of therapy and the need for addition of ribavirin. For treatment-experienced individuals, the presence of baseline RASs with greater than 100-fold reduced susceptibility to ledipasvir was associated with an SVR12 of 64.7% with 12 weeks of treatment with ledipasvir/sofosbuvir, compared with an SVR12 of 97.4% for those with no NS5A RASs and 100% for those with RASs conferring less than 100-fold reduced susceptibility to ledipasvir.¹⁸ Extending treatment with ledipasvir/sofosbuvir to 24 weeks or adding ribavirin to a 12-week course of ledipasvir/

sofosbuvir mitigated the effect of baseline NS5A RASs. Similarly, individuals with cirrhosis, genotype 1a infection, and ledipasvir RASs with greater than 100-fold reduced susceptibility had a decreased SVR12 of 92% if treatment naive and SVR12 of 67% if treatment experienced.¹⁸ Adding ribavirin or extending treatment with ledipasvir/sofosbuvir to 24 weeks mitigated the impact of NS5A RASs. In contrast, data from registrational trials of sofosbuvir/velpatasvir have not demonstrated an impact of baseline NS5A RASs on HCV cure rate in individuals with HCV genotype 1 infection, including in those who experienced a prior treatment failure (non-NS5A inhibitor based) or those with cirrhosis. Thus, NS5A RAS testing is not recommended before use of sofosbuvir/velpatasvir in these populations.¹⁹

Considerations for Resistance Testing for NS5A Inhibitor Treatment-Naive Individuals With HCV Genotype 3 Infection

Genotype 3 HCV infection is the second most prevalent HCV infection globally, and highly efficacious DAA therapy (>95% SVR) remains elusive in some populations such as those with cirrhosis or prior treatment experience. Recommended regimens for individuals with genotype 3 infection include sofosbuvir plus daclatasvir and sofosbuvir/velpatasvir. Data on the impact of baseline NS5A RASs on the outcome of NS5A inhibitor-containing therapy are limited. Further, clear data on appropriate therapeutic modifications to mitigate the impact of baseline RASs do not exist. The NS5A RAS of most clinical importance in HCV genotype 3 is Y93H, which confers a high level of resistance to daclatasvir and to velpatasvir.^{19,20} The prevalence of the Y93H RAS is 5% to 10% for individuals with HCV genotype 3 infection and no prior exposure to NS5A inhibitors.²¹ In the case of virologic failure following exposure to an NS5A inhibitor, the Y93H RAS emerges in the vast majority of individuals with HCV genotype 3 infection.

Table 3. Recommendations for NS5A RAS Testing by Regimen and Clinical Characteristics (Treatment Experience and Cirrhosis)

HCV Regimen	HCV Genotype 1a				HCV Genotype 1b				HCV Genotype 3			
	TN		TE ^a		TN		TE ^a		TN		TE ^a	
	NC	C	NC	C	NC	C	NC	C	NC	C	NC	C
Elbasvir/grazoprevir ^b	+	+	+	+	-	-	-	-	NA	NA	NA	NA
Ledipasvir/sofosbuvir ^b	-	+/-	+/-	+/-	-	-	-	-	NA	NA	NA	NA
Paritaprevir/ritonavir/ombitasvir ^b plus dasabuvir	-	-	-	-	-	-	-	-	NA	NA	NA	NA
Sofosbuvir plus daclatasvir	-	-	-	-	-	-	-	-	-	+	+	-
Sofosbuvir/velpatasvir ^b	-	-	-	-	-	-	-	-	-	+	+	-

Abbreviations: +, resistance testing recommended; +/-, resistance testing may be considered; -, resistance testing not recommended; C, cirrhosis; HCV, hepatitis C virus; NA, not applicable; NC, no cirrhosis; NS5A, nonstructural protein 5A; RAS, resistance-associated substitution;

^aWith peginterferon alfa-based regimen; excludes prior NS5A-containing regimens.

^bSlash indicates a coformulation.

Daclatasvir Plus Sofosbuvir

The impact of baseline NS5A RASs on treatment with daclatasvir plus sofosbuvir for individuals with HCV genotype 3 infection was suggested by results from the ALLY-3 study.²² The SVR12 among participants treated with daclatasvir plus sofosbuvir (without ribavirin) was 92% in those without baseline NS5A RASs and 54% in those with baseline NS5A RASs. The presence of cirrhosis also impacted the response to this regimen. However, given the small number of participants with both cirrhosis and baseline NS5A RASs (n = 4; SVR12 of 25%), the relative contribution of each is difficult to discern. An SVR12 of 67% was observed in participants without cirrhosis but with the Y93H RAS. The addition of ribavirin improved SVR12 in participants with cirrhosis to 86% (12-16 weeks of therapy).²³

Given these data, the presence of NS5A RASs may impact therapeutic approaches for treatment-naïve persons with cirrhosis or treatment-experienced persons without cirrhosis, and baseline NS5A RAS testing is recommended for these populations. Identification of the Y93H variant should prompt the addition of ribavirin in the absence of absolute contraindications. Twelve weeks of therapy with daclatasvir plus sofosbuvir is recommended for individuals without cirrhosis, and 24 weeks of therapy is recommended for those with cirrhosis, given the suboptimal response of 86% with 12 to 16 weeks of daclatasvir plus sofosbuvir with ribavirin. The role of RAS testing for treatment-naïve individuals with HCV genotype 3 infection without cirrhosis is unclear.

Sofosbuvir/Velpatasvir

Velpatasvir has an improved resistance profile compared with early generation NS5A inhibitors, although the Y93H variant in HCV genotype 3 infection still confers a high level of resistance (> 100 × EC₅₀) to velpatasvir.¹¹ In the ASTRAL-3 study, which evaluated 12 weeks of treatment with sofosbuvir/velpatasvir, SVR12 was 88% (28/32) in participants with baseline velpatasvir-specific RASs (A30H/K, L31F/M, and Y93H), compared with 97% in those without NS5a RASs. Of

the 4 participants whose treatment failed, 3 had the Y93H RAS and 1 had the A30K RAS before treatment. The overall SVR12 rate was 84% (21/25) for participants with a baseline Y93H RAS (1% cutoff used with identification of 1 additional Y93H RAS). Given the lower SVR12 (89%) in treatment-experienced individuals with cirrhosis than in treatment-naïve individuals without cirrhosis (97%), the addition of ribavirin is recommended a priori for those with both treatment experience and cirrhosis.¹⁶ Therefore, resistance testing is not advised for this population, as it would not change the recommended treatment.

However, resistance testing should be considered for treatment-experienced individuals without cirrhosis and treatment-naïve individuals with cirrhosis. Ribavirin should be added if the Y93H RAS is detected, or therapy can be extended to 24 weeks if ribavirin cannot be added.

RAS Testing at Time of Failure of NS5A Inhibitor-Based Treatment

Cure rates with NS5A inhibitor-based regimens are remarkably high. However, when NS5A inhibitor-based treatment fails, NS5A RASs frequently emerge. More than 90% of individuals have NS5A resistance mutations at the time of failure of treatment with ledipasvir-, elbasvir-, or ombitasvir-containing therapies. Such mutations may persist for years (86% were still detectable 96 weeks after the failure of ledipasvir-containing treatment²⁴). Therefore, NS5A resistance testing is recommended at the time of failure of NS5A inhibitor-based treatment and still may be useful months to years afterward, given the persistence of NS5A RASs. Current guidance also recommends HCV NS3 PI resistance testing to inform treatment options for individuals pursuing retreatment,¹⁶ with particular attention to the Q80K mutation, which impacts response to simeprevir in those with cirrhosis.²⁵ Given that HCV NS3 PI RASs wane more quickly than NS5A RASs,⁷ it may be advisable to obtain NS3 PI RAS testing at the time of treatment failure even if retreatment is not planned, to document the presence of PI mutations that could impact future

PI-containing treatment. NS3 PI RAS testing for sofosbuvir is not recommended, as sofosbuvir-associated RASs are rare and, generally, have not impacted the efficacy of subsequent sofosbuvir use.

Retreatment After Failure of an NS5A Inhibitor–Based Regimen

Timing and options for retreatment after failure of an NS5A inhibitor–based regimen are challenging, given the limited options and data supporting retreatment. Guidance currently suggests deferring HCV treatment for individuals without cirrhosis and when there is not another indication for urgent retreatment,¹⁶ in anticipation of the availability of better retreatment options currently in development. For individuals who are retreated with currently available regimens, tailoring retreatment based on results of NS5A and NS3 resistance testing is recommended as follows: if no NS5A RASs are present, treat with sofosbuvir plus an NS5A inhibitor (ledipasvir or velpatasvir) plus ribavirin for 24 weeks; if NS5A RASs are present but no NS3 PI RASs (specifically Q80K) are present, treat with simeprevir plus sofosbuvir plus ribavirin for 24 weeks; and if NS5A and NS3 RASs are present, defer therapy or consider a clinical trial or a regimen as outlined below.

Retreatment After Failure of an NS5A Inhibitor–Based Regimen: Tailoring to the NS5A RAS Profile

Ledipasvir/Sofosbuvir

Several studies have examined retreatment with ledipasvir/sofosbuvir after failure of an NS5A inhibitor–based regimen. Among individuals whose treatment with 8 to 12 weeks of ledipasvir/sofosbuvir failed, efficacy of retreatment with ledipasvir/sofosbuvir for 24 weeks (without ribavirin) was impacted by the presence of NS5A RASs.²⁶ SVR12 was 100% if no NS5A RASs were present and 60% if they were present. The presence of 2 or more RASs was associated with a 50% SVR12, and the presence of Y93H/N RASs was associated with a 33% SVR12.

The addition of ribavirin may mitigate the impact of NS5A RASs. Among 9 HIV/HCV-coinfected individuals whose treatment with 12 weeks of ledipasvir/sofosbuvir failed, 7 of 9 had ledipasvir RASs at the time of treatment failure, including 4 with Y93H/N RASs, and 8 of 9 attained an SVR12 with 24 weeks of treatment with ledipasvir/sofosbuvir plus ribavirin.²⁷ The single treatment failure occurred in an individual who had an L31M RAS before retreatment. Based on the limited data available, ledipasvir/sofosbuvir plus ribavirin for 24 weeks may be considered for retreatment after failure of an NS5A inhibitor–containing regimen, but is not recommended if ledipasvir RASs are present.

Sofosbuvir/Velpatasvir

In a single-arm study of individuals treated with 4 to 12 weeks of sofosbuvir/velpatasvir or sofosbuvir/velpatasvir plus the investigational HCV PI GS-9857 during phase II studies,

retreatment with 24 weeks of sofosbuvir/velpatasvir plus weight-based ribavirin led to a 91% SVR (59/65).²⁸ Individuals with HCV genotype 1, 2, or 3 infection were included. Pre-treatment NS5A RASs (detected using a 1% UDS cutoff) were present in 18% of individuals with genotype 1 infection, 62% of those with genotype 2 infection, and 81% of those with genotype 3 infection. The presence of NS5A RASs did not impact SVR in participants with genotype 1 (33/34; SVR12, 97%) or 2 (13/14; SVR12, 91%). Among 13 participants with genotype 3 infection, 11 (85%) had the Y93H NS5A RAS, and 9 (82%) attained an SVR12. These results indicate that the presence of the Y93H RAS may reduce HCV cure rates among individuals with genotype 3 infection retreated with sofosbuvir/velpatasvir. However, the majority of participants in this study with genotype 3 infection and the Y93H RAS were cured. Individuals with HCV genotype 3 infection in whom NS5A inhibitor–based treatment has failed remain a population for whom improved retreatment options are needed.

Simeprevir Plus Sofosbuvir

Simeprevir plus sofosbuvir has been studied in treatment-experienced (with peginterferon alpha–based regimens) individuals with cirrhosis and led to a 79% SVR12.²⁵ Data on simeprevir plus sofosbuvir for individuals experienced with NS5A inhibitor–based treatments are limited.

In a small observational study, 14 of 16 participants with HCV genotype 1 infection attained SVR12 with 12 weeks of retreatment with simeprevir plus sofosbuvir after failure of a prior daclatasvir-based regimen (most included peginterferon alpha); 13 of 16 participants had NS5A RASs, and 8 of 16 participants had NS3 RASs (2 with Q80K).¹⁹ Of the 2 participants whose retreatment with simeprevir plus sofosbuvir failed, 1 had the Q80K RAS and 1 had the R155K RAS (which confers a high level of resistance to simeprevir). Of note, simeprevir plus sofosbuvir was only given for 12 weeks and without ribavirin. Current guidelines recommend 24 weeks of therapy with simeprevir plus sofosbuvir plus ribavirin for individuals whose prior treatment with an NS5A inhibitor failed, to optimize treatment success in this hard-to-treat population for whom options are limited.¹⁶ Testing for the NS3 PI mutation Q80K is recommended before simeprevir use in individuals with HCV genotype 1a infection only in the presence of cirrhosis, due to reduced response when Q80K is present (74%) versus when it is absent (92%).

3- and 4-Class Combination Therapies for Retreatment

Retreatment with elbasvir/grazoprevir plus sofosbuvir and ribavirin led to a 100% SVR in 25 individuals whose prior treatment with elbasvir/grazoprevir plus sofosbuvir for 4 to 8 weeks failed; 52% had elbasvir RASs before retreatment (15% cutoff), which did not impact SVR12, nor did the presence of NS3 PI RASs.²⁹ Therapy with paritaprevir/ritonavir/ombitasvir plus dasabuvir plus sofosbuvir (and ribavirin for those with genotype 1a infection) led to a similarly strong SVR rate of 95% in 22 individuals with HCV genotype 1a infection retreated after failure of a variety of regimens. Of 20 individuals

with genotype 1a infection, 16 (80%) had NS5A RASs before retreatment and 19 (95%) attained an SVR12; 2 of 2 (100%) individuals with genotype 1b infection attained an SVR. Of note, 13 of 14 individuals with the HCV PI Q80K mutation also attained an SVR.³⁰

The high SVR rates achieved with these combination therapies despite the presence of baseline NS5A resistance are encouraging. However, combining drugs from different classes made by different pharmaceutical manufacturers creates additional barriers to treatment access and increases cost. Triple-class combination regimens currently in development may be more accessible if provided as coformulations from the same manufacturer.

Future Therapies for Retreatment After Failure of an NS5A Inhibitor-Based Regimen

Investigational, ribavirin-free combination therapies have shown tremendous promise for retreatment after failure of an NS5A inhibitor-based regimen,³¹⁻³³ regardless of whether NS5A resistance preexists. In a study of 44 DAA treatment-experienced individuals without cirrhosis (50% NS5A experienced, 84% PI experienced), the combination of the investigational NS3 inhibitor glecaprevir and the investigational NS5A inhibitor pibrentasvir yielded a 100% SVR in those without NS5A RASs and 83% to 96% SVR12 in those with NS5A resistance with 12 and 16 weeks of treatment, respectively. There was a high cure rate even in the presence of NS5A resistance, including 100% SVR in those with the NS5A RASs Y93H/N.³¹

The multiclass combination of sofosbuvir, velpatasvir, and the investigational NS3 inhibitor voxilaprevir given for 12 weeks without ribavirin led to cure in 96% of HCV-infected individuals (all genotypes) who had a prior NS5A treatment failure.³² Seventy-nine percent of individuals had NS5A resistance before retreatment, and 96% attained an SVR. The presence of NS3 and NS5B RASs did not impact SVR. Six virologic failures occurred, all of which were among individuals with cirrhosis.

Similarly, treatment with the multiclass combination of grazoprevir, the investigational NS5A inhibitor ruzasvir, and the investigational NS5B polymerase inhibitor uprifosbuvir given for 16 weeks with ribavirin or for 24 weeks without ribavirin led to a 100% SVR4 in individuals with HCV genotype 1 infection whose prior NS5A inhibitor-containing regimens failed; 84% had NS5A RASs and 65% had NS3 RASs at baseline.³³ Collectively, these data suggest that ribavirin-free combination therapies in development should be highly effective for most individuals whose prior NS5A inhibitor-based regimen failed, regardless of the presence of preexisting NS5A, NS5B, or NS3 RASs.


When Resistance Testing Is Not Available

In the scenarios outlined above, RAS testing may help guide treatment choices. However, RAS testing is not recommended for the majority of individuals initiating HCV treatment.

Further, a lack of RAS testing should not be a barrier to HCV treatment. Practitioners may not have access to RAS testing because of a lack of insurance coverage or limited access in their practice setting. In the absence of RAS testing, an effective DAA regimen can almost always be constructed using the patient's clinical history and the expected efficacy of the available regimens.

Summary

When available, NS5A resistance can have important clinical implications for treatment-naïve and -experienced individuals with HCV infection. NS5A resistance testing is recommended for individuals who have not received prior NS5A inhibitor-based treatment if treatment with elbasvir/grazoprevir is planned (for those with genotype 1a infection), if treatment with ledipasvir/sofosbuvir is planned (only for those with genotype 1a infection and cirrhosis or a prior treatment failure), and in the case of genotype 3 infection in the presence of cirrhosis or a prior treatment failure. NS5A resistance testing is also recommended for all individuals whose prior NS5A inhibitor-based treatment failed.

Future HCV treatments combining next-generation antiviral drugs that have improved resistance profiles, in some cases from as many as 3 different drug classes, are effective even in the setting of previous DAA-based treatment failure with drug resistance. Although additional data are needed, such regimens may obviate the need for resistance testing in most situations. 

Financial affiliations the past 12 months: Dr Wyles has received research grants awarded to his institution from AbbVie, Gilead Sciences, Inc, Merck, and Tacere Therapeutics, Inc. He has also served as a consultant for Gilead Sciences, Inc, AbbVie, and Merck. Dr Luetkemeyer has received research grants awarded to her institution from AbbVie, Gilead Sciences, Inc, and Merck.

References

- Rong L, Dahari H, Ribeiro RM, Perelson AS. Rapid emergence of protease inhibitor resistance in hepatitis C virus. *Sci Transl Med*. 2010;2(30):30ra32.
- Wyles DL, Gutierrez JA. Importance of HCV genotype 1 subtypes for drug resistance and response to therapy. *J Viral Hepat*. 2014;21(4):229-240.
- Svarovskaia ES, Dvory-Sobol H, Parkin N, et al. Infrequent development of resistance in genotype 1-6 hepatitis C virus-infected subjects treated with sofosbuvir in phase 2 and 3 clinical trials. *Clin Infect Dis*. 2014;59(12):1666-1674.
- Wyles D, Dvory-Sobol H, Svarovskaia ES, et al. Post-treatment resistance analysis of hepatitis C virus from phase II and III clinical trials of ledipasvir/sofosbuvir. *J Hepatol*. 2017;66(4):703-710.
- Kwo P, Gitlin N, Nahass R, et al. Simeprevir plus sofosbuvir (12 and 8 weeks) in hepatitis C virus genotype 1-infected patients without cirrhosis: OPTIMIST-1, a phase 3, randomized study. *Hepatology*. 2016;64(2):370-380.
- Lahser F, Galloway A, Hwang P, et al. Interim analysis of a 3-year follow-up study of NS5A and NS3 resistance-associated variants (RAVs) after treatment with grazoprevir-containing regimens in patients with chronic hepatitis C virus (HCV) infection. *Hepatology*. 2016;64(suppl 1):32A.
- Krishnan P, Tripathi R, Schnell G, et al. Long-term follow-up of treatment-emergent resistance-associated variants in NS3, NS5A and NS5B with paritaprevir/r-, ombitasvir- and dasabuvir-based regimens. *J Hepatol*. 2015;62(suppl 2):S220.

8. Lahser FC, Bystol K, Curry S, et al. The combination of grazoprevir, a hepatitis C virus (HCV) NS3/4A protease inhibitor, and elbasvir, an HCV NS5A inhibitor, demonstrates a high genetic barrier to resistance in HCV genotype 1a replicons. *Antimicrob Agents Chemother*. 2016;60(5):2954-2964.
9. Krishnan P, Tripathi R, Schnell G, et al. Resistance analysis of baseline and treatment-emergent variants in hepatitis C virus genotype 1 in the AVIATOR study with paritaprevir-ritonavir, ombitasvir, and dasabuvir. *Antimicrob Agents Chemother*. 2015;59(9):5445-5454.
10. Sarrazin C, Zeuzem S. Resistance to direct antiviral agents in patients with hepatitis C virus infection. *Gastroenterology*. 2010;138(2):447-462.
11. Hezode C, Reau N, Svarovskaia E, et al. Resistance analysis in 1284 patients with genotype 1 to 6 HCV infection treated with sofosbuvir/velpatasvir in the phase 3 ASTRAL-1, ASTRAL-2, ASTRAL-3 and ASTRAL-4 studies. The International Liver Congress 2016. April 13-17, 2016; Barcelona, Spain.
12. Ng T, Krishnan P, Kati W, et al. ABT-530 an HCV NS5A inhibitor with potent pangenotypic activity and high barrier to resistance. 21st Conference on Retroviruses and Opportunistic Infections (CROI). March 3-6, 2014; Boston, Massachusetts.
13. Asante-Appiah E, Liu R, Curry S, et al. MK-8408, a potent and selective NS5A inhibitor with a high genetic barrier to resistance and activity against HCV genotypes 1 to 6. The International Liver Congress 2014. November 7-11, 2014; London, United Kingdom.
14. Jacobson I.M., Asante-Appiah E, Wong P, et al. Prevalence and impact of baseline NSA resistance associated variants (RAVs) on the efficacy of elbasvir/grazoprevir (EBR/GZR) against GT1a infection. 66th Annual Meeting of the American Association for the Study of Liver Diseases. November 13-17, 2016; San Francisco, California.
15. Zeuzem S, Mizokami M, Pianko S, et al. NS5A resistance-associated substitutions in patients with genotype 1 hepatitis C virus: Prevalence and effect on treatment outcome. *J Hepatol*. 2017;66(5):910-918.
16. AASLD/IDSA. HCV guidance: recommendations for testing, managing, and treating hepatitis C. <http://hcvguidelines.org/>. Accessed on July 12, 2017.
17. Merck. Zepatier [prescribing information]. 2016. Whitehouse Station, New Jersey.
18. Sarrazin C, Dvory-Sobol H, Svarovskaia ES, et al. Prevalence of resistance-associated substitutions in HCV NS5A, NS5B, or NS3 and outcomes of treatment with ledipasvir and sofosbuvir. *Gastroenterology*. 2016;151(3):501-512.
19. Hezode C, Chevaliez S, Scoazec G, et al. Retreatment with sofosbuvir and simeprevir of patients with hepatitis C virus genotype 1 or 4 who previously failed a daclatasvir-containing regimen. *Hepatology*. 2016;63(6):1809-1816.
20. Wang C, Jia L, O'Boyle DR, et al. Comparison of daclatasvir resistance barriers on NS5A from hepatitis C virus genotypes 1 to 6: implications for cross-genotype activity. *Antimicrob Agents Chemother*. 2014;58(9):5155-5163.
21. Foster GR, Afdhal N, Roberts SK, et al. Sofosbuvir and velpatasvir for HCV genotype 2 and 3 infection. *N Engl J Med*. 2015;373(27):2608-2617.
22. Nelson DR, Cooper JN, Lalezari JP, et al. All-oral 12-week treatment with daclatasvir plus sofosbuvir in patients with hepatitis C virus genotype 3 infection: ALLY-3 phase III study. *Hepatology*. 2015;61(4):1127-1135.
23. Leroy V, Angus P, Bronowicki JP, et al. Daclatasvir, sofosbuvir, and ribavirin for hepatitis C virus genotype 3 and advanced liver disease: A randomized phase III study (ALLY-3+). *Hepatology*. 2016; 63(5):1430-1441.
24. Wyles D, Mangia A, Cheng W, et al. Long-term persistence of HCV NS5A resistance associated substitutions after treatment with the HCV NS5A inhibitor, ledipasvir, without sofosbuvir. *Antivir Ther*. 2017;[Epub ahead of print].
25. Lawitz E, Matusow G, DeJesus E, et al. Simeprevir plus sofosbuvir in patients with chronic hepatitis C virus genotype 1 infection and cirrhosis: A phase 3 study (OPTIMIST-2). *Hepatology*. 2016;64(2):360-369.
26. Lawitz E, Flamm S, Yang JC, et al. Retreatment of patients who failed 8 or 12 weeks of ledipasvir/sofosbuvir-based regimens with ledipasvir/sofosbuvir for 24 weeks. 50th Annual Meeting of the European Association for the Study of the Liver (EASL). April 22-26, 2015; Vienna, Austria.
27. Luetkemeyer A, Cooper C, Naggie S, et al. Retreatment of HCV/HIV co-infected patients who failed 12 weeks of LDV/SOF. 21st International AIDS Conference. July 18-22, 2016; Durban, South Africa.
28. Gane E, Shiffman ML, Etzkorn K, et al. Sofosbuvir/velpatasvir in combination with ribavirin for 24 weeks is effective retreatment for patients who failed prior NS5A containing DAA regimens: results of the GS-US-342-1553 study. *J Hepatol*. 2016;64(2 suppl):S147-S148.
29. Lawitz E, Poordad F, Gutierrez JA, et al. C-SWIFT retreatment (part b): 12 weeks of elbasvir/grazoprevir with sofosbuvir and ribavirin successfully treated GT1-infected subjects who failed short-duration all-oral therapy. *Hepatology*. 2015;62(6):1386A-1387A.
30. Poordad Fea. QUARTZ-1 ombitasvir/paritaprevir/r, dasabuvir, and sofosbuvir treatment of patients with HCV genotype 1-infection who failed a prior course of DAA therapy. Digestive Disease Week. 2016; San Diego, CA.
31. Poordad F, Pol S, Asatryan A, et al. MAGELLAN-1, part 2: glecaprevir/pibrentasvir for 12 or 16 weeks in patients with chronic HCV genotype 1 or 4 and prior direct-acting antiviral treatment failure. 52nd Annual Meeting of the European Association for the Study of the Liver (EASL). April 19-23, 2017; Amsterdam, The Netherlands.
32. Bourliere M, Gordon SC, Flamm SL, et al. Sofosbuvir, velpatasvir, and voxilaprevir for previously treated HCV infection. *N Engl J Med*. 2017;376(22):2134-2146.
33. Wyles D, Wedemeyer H, Reddy R, et al. Safety and efficacy of the fixed-dose combination regimen of MK-3682 / grazoprevir / MK-8408 (ruzasvir) in cirrhotic or non-cirrhotic patients with chronic HCV GT1 infection who previously failed a direct-acting antiviral regimen (C-SURGE). 67th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD). November 11-15, 2016; Boston, Massachusetts.

Top Antivir Med. 2017;25(3):[published online July 14, 2017].

©2017, IAS–USA. All rights reserved