

HCV Resistance Primer

Introduction

Understanding principles of the emergence of drug-resistant viruses is critical when using targeted antiviral therapies. The best example of these principles can be gleaned from the study of HIV. Like HIV, HCV is an approximately 9.5 kilobase RNA virus that replicates very rapidly (billions of viruses daily). The production of each new virus is performed by an enzyme that results in 1 to 3 errors per replication cycle, on average. Many of these errors either have no effect on the progeny virus product or result in progeny viruses that are nonreplication competent (ie, dead viruses). For some newly produced viruses, however, the transcription errors result in changes in critical coding regions that may, by chance, change the susceptibility of the virus to 1 or more drugs used to treat the virus. The emergence of such drug-resistant viruses most often occurs when drug levels are subtherapeutic, thereby creating selective pressure for the resistant viruses to emerge as the dominant species. These newly formed resistant viruses have a selective growth advantage that allows them to replicate in the presence of antiviral drugs. In a subset of patients with chronic HCV infection, viral variants harboring substitutions associated with resistance to HCV directing-acting antivirals (DAAs) are detectable prior to antiviral therapy and, particularly in the case of NS5A inhibitor-containing regimens, may negatively impact treatment response. These substitutions often are referred to as baseline resistance-associated substitutions (RASs).

In the case of HCV DAAs, resistant viruses are also selected for and/or enriched in patients for whom a DAA regimen fails. These viruses contain substitutions that are designated as treatment-emergent (or treatment-selected) RASs. NS5A and NS3 RASs are frequently selected in patients with failure of NS5A or NS3 inhibitor-containing regimens, respectively. In contrast, NS5B nucleotide RASs are rarely detected (1% of failures) even after exposure to a failing DAA regimen containing a nucleotide inhibitor ([Svarovskaia, 2014](#)); ([Wyles, 2017](#)). This is likely due to the highly conserved catalytic site region that nucleotides bind, making substitutions in this region extremely rare—often referred to as a high barrier to resistance. Additionally, any such substitution would likely render the virus replication incompetent. Compounding the clinical impact of NS5A RASs is their ability to maintain high replication competence (aka, relative fitness) in the absence of continued drug pressure, allowing them to remain the dominant viral quasispecies for prolonged periods (years) relative to NS3 protease or NS5B nucleotide polymerase inhibitor RASs, which are typically less fit and tend to disappear over several months, being overcome by more fit wild-type virus species.

The magnitude of the negative impact of RASs, both baseline and selected, on treatment outcome varies according to regimen (coadministered drugs); patient factors that impact treatment response (cirrhosis); and the fold change decrease in potency conferred by the specific RAS(s). Given these considerations, RAS testing alone will not dictate optimal DAA regimen selection. In addition, a drug predicted to suffer a significant loss of potency in the presence of a RAS still may be used in specific clinical settings/regimens.

Terminology, Thresholds of Clinical Relevance, and Assays

Terminology

1. Naming Convention for Hepatitis C Proteins

The hepatitis C genome codes for approximately 5 HCV-specific proteins, which are essential to: 1) form the viral structure (core and envelope proteins); 2) cut the HCV polyprotein; 3) provide enzymatic functions for replication and escape from the innate immune response (NS3/NS4A protease); 4) replicate the HCV RNA (NS5B RNA-dependent RNA polymerase); and 5) bind the HCV replication complex during replication and assembly (NS5A).

2. Polymorphism (Substitution)

A reference (or consensus) nucleotide—and therefore amino acid sequence—has been defined for each HCV genotype. A polymorphism (or substitution) is a difference in an amino acid at a defined position of the HCV protein between a patient's HCV and the reference HCV protein. Substitution is the preferred terminology among most experts. However, the US Food and Drug Administration currently uses the term polymorphism.

To define a polymorphism, it is necessary to define: the HCV genotype (eg, genotype 1, 2, 3, etc) and subtype (eg, 1a vs 1b); the HCV protein (eg, NS5A); and the amino acid position (eg, 93). Polymorphisms are reported as letter-number-letter (eg, Y93H). The first letter refers to the amino acid typically expected for that position in the reference protein. The number refers to the amino acid position, and the final letter refers to the amino acid that is found in the patient's HCV isolate. Thus, NS5A Y93H refers to amino acid position 93 of the NS5A protein. The amino acid at this position in the reference strain is Y (ie, tyrosine) and the amino acid in the tested strain is H (ie, histidine). For some patients, multiple variants are present and several amino acids may be found at a given position. Thus, it is possible to have a virus with NS5A Y93H/M. Such a patient would have viruses with the amino acids histidine (H) or methionine (M) at position 93 of the NS5A protein.

3. Resistance-Associated Substitutions

A resistance-associated substitution describes any amino acid change from the consensus sequence at a position that has been associated with reduced susceptibility of a virus to 1 or more antiviral drugs. A specific RAS may or may not confer a phenotypic loss of susceptibility to other/multiple antiviral agents.

4. Drug-Class RASs

Drug-class RASs are amino acid substitutions that reduce the susceptibility of a virus to any (and at least 1) member of a drug class or, alternatively, the viral variants with reduced susceptibility that carry these substitutions. Class RASs may or may not confer resistance to a specific drug in that class.

5. Drug-Specific RASs

Drug-specific RASs are amino acid substitutions that reduce the susceptibility of a virus to a specific drug. When assessing the potential clinical impact of RASs on a given regimen, drug-specific RASs should be used. In an HCV-infected population not previously exposed to a DAA drug or class, drug-specific RASs will be found less frequently than class RASs.

Thresholds of Clinical Relevance

HCV resistance to DAAs is a rapidly evolving field with demonstrated clinical impact in specific situations with currently available DAA regimens. Presently, the most clinically significant RASs are in the NS5A position for genotypes 1a and 3.

Data from clinical trials have demonstrated that RASs are commonly, but not always, found at the time of virologic failure. Viruses that are resistant to NS3/4A protease inhibitors seem to be less fit and may disappear from peripheral blood within a few weeks to months, whereas NS5A inhibitor-resistant viruses may persist for years, which could have implications for treatment and retreatment.

In general, drug-specific RASs need to be present in at least 15% of the viruses of a given patient to reduce the likelihood of achieving SVR ([Pawlotsky, 2016](#)). Drug-specific RASs that are found at a lower frequency may not convey sufficient resistance to reduce SVR with currently available DAA regimens.

Assays

Methods to detect RASs include population sequencing (aka, Sanger sequencing) and deep sequencing (aka, next generation sequencing [NGS]). Both methods depend on sequencing the HCV RNA, calculating the amino acid sequence, and then inferring the presence of RASs. The methods differ in their sensitivity for detecting RASs. For the purposes of clinical care and decisions regarding which DAA regimen to use, both methods can be considered equivalent if a $\geq 15\%$ cut point is used for determination of RASs by NGS. Recent studies have shown that NGS at a 1% level of sensitivity often result in the identification of additional RASs that are not associated with clinical failure ([Jacobson, 2015b](#)); ([Sarrazin, 2016](#)); ([Zeuzem, 2017](#)).

1. Genotypic Analysis

a. Population-Based Sequencing (Sanger)

Population sequencing of the HCV coding region of interest may be performed using reverse transcription polymerase chain reaction (PCR) and standard Sanger sequencing of the bulk PCR product. The sensitivity for detection of resistance substitutions varies but is generally 15% to 25%. As a standard, substitutions are reported as differences compared with a genotype-specific, wild-type strain.

b. Deep Sequencing Analysis

NGS (deep sequencing approaches) can increase the sensitivity of detection for minor variants. After sequencing HCV coding regions using PCR, a software algorithm is used to process and align sequencing data via a multistep method to identify the substitutions present at a predetermined level. This level, or threshold, can vary but is often set as low as $>1\%$ for research purposes. To approximate results obtained by population sequencing, NGS thresholds are often set to $\geq 10\%$.


2. Phenotypic Analysis

Phenotypic analysis involves laboratory techniques whereby the degree of drug resistance conferred by an amino acid change as well as the replicative capacity (fitness) of a particular RAS can be estimated in the presence of a wild-type or consensus strain. These research techniques are not routinely used for clinical practice. To assess the level of resistance, RASs are typically introduced as point mutations into the backbone of an existing standard HCV genome within an existing cell culture/replicon or enzyme-based assay. Isolates harboring these RASs are then challenged by appropriate antiviral agents at increasing concentrations and fold changes—based on EC_{50} or IC_{50} and EC_{90} or IC_{90} values—are determined for inhibition of replication or enzyme activity, respectively, in comparison to wild-type virus. Comparison of replication levels for variants and wild-type constructs in the absence of drug allows for estimation of fitness.


3. Assay Summary Points

- Either population sequencing or deep sequencing can be used to detect the presence of RASs in NS3, NS5A, and NS5B.
- For clinical decisions, population sequencing or deep sequencing with at least 15% prevalence of RASs as the cutoff is recommended. The presence of RASs with $<15\%$ prevalence should not be considered clinically significant.
- When assessing the potential clinical effect of RASs, it is important to determine the drug-specific RASs.

Resistance Testing in Clinical Practice

Regimen-Specific Recommendations for Use of RAS Testing in Clinical Practice	
RECOMMENDED	RATING 
<p>Elbasvir/grazoprevir</p> <p>NS5A RAS testing is recommended for genotype 1a-infected, treatment-naive or -experienced patients being considered for elbasvir/grazoprevir. If present, a different regimen should be considered.</p>	I, A
<p>Ledipasvir/sofosbuvir</p> <p>NS5A RAS testing can be considered for genotype 1a-infected, treatment-experienced patients without cirrhosis being considered for ledipasvir/sofosbuvir. If clinically important^a resistance is present, a different recommended therapy should be used.</p> <p>NS5A RAS testing can be considered for genotype 1a-infected, treatment-experienced patients with cirrhosis being considered for ledipasvir/sofosbuvir. If clinically important^a resistance is present, a different recommended therapy should be used.</p>	I, A
<p>Sofosbuvir/velpatasvir</p> <p>NS5A RAS testing is recommended for genotype 3-infected, treatment-naive patients with cirrhosis and treatment-experienced patients (with or without cirrhosis) being considered for 12 weeks of sofosbuvir/velpatasvir. If Y93H is present, weight-based ribavirin should be added or sofosbuvir/velpatasvir/voxilaprevir should be used.</p>	I, A
<p>Daclatasvir plus sofosbuvir</p> <p>NS5A RAS testing is recommended for genotype 3-infected, treatment-experienced patients without cirrhosis being considered for 12 weeks of daclatasvir plus sofosbuvir. If Y93H is present, weight-based ribavirin should be added.</p> <p>NS5A RAS testing is recommended for genotype 3-infected, treatment-naive patients with cirrhosis being considered for 24 weeks of daclatasvir plus sofosbuvir. If Y93H is present, treatment should include weight-based ribavirin, or a different recommended therapy used.</p>	I, B
<p>^a Clinically important = greater than 100-fold resistance</p>	

Regimen-Specific Clinical Practice Situations in Which RAS Testing Is Not Recommended

NOT RECOMMENDED	RATING 
<p>Elbasvir/grazoprevir RAS testing is not recommended for any genotype 1b-infected patients being considered for elbasvir/grazoprevir therapy.</p>	I, A
<p>Glecaprevir/pibrentasvir RAS testing is not recommended for patients with genotype 1, 2, 3, 4, 5, or 6 infection being considered for glecaprevir/pibrentasvir for 8, 12, or 16 weeks.</p>	I, A
<p>Ledipasvir/sofosbuvir NS5A RAS testing is not recommended for any genotype 1b-infected patients being considered for ledipasvir/sofosbuvir therapy.</p>	I, A
<p>NS5A RAS testing is not recommended for genotype 1a-infected, treatment-naive patients being considered for ledipasvir/sofosbuvir therapy.</p>	I, A
<p>NS5A RAS testing is not recommended for genotype 1a- or 1b-infected, treatment-naive patients without cirrhosis and with a viral load <6 million IU/mL being considered for an 8-week course of ledipasvir/sofosbuvir therapy.</p>	I, A
<p>Paritaprevir/ritonavir/ombitasvir with dasabuvir ± weight-based ribavirin, or paritaprevir/ritonavir/ombitasvir + weight-based ribavirin RAS testing is not recommended for genotype 1- or 4-infected, treatment-naive or -experienced patients being considered for therapy with paritaprevir/ritonavir/ombitasvir with dasabuvir ± weight-based ribavirin or paritaprevir/ritonavir/ombitasvir + weight-based ribavirin, respectively.</p>	I, A
<p>Sofosbuvir/velpatasvir RAS testing is not recommended for patients with genotype 1, 2, 4, 5, or 6 infection being considered for 12 weeks of sofosbuvir/velpatasvir therapy.</p>	I, A
<p>Sofosbuvir/velpatasvir/voxilaprevir RAS testing is not recommended for patients with genotype 1, 2, 3, 4, 5, or 6 infection being considered for 12 weeks of sofosbuvir/velpatasvir/voxilaprevir therapy.</p>	I, A

Resistance testing is most important in clinical practice when the results would modify treatment management by impacting the duration of therapy and/or inclusion of ribavirin, or result in selection of alternative therapy. Unfortunately, at this time, the utility of RAS testing varies by both patient characteristics and DAA regimen.

Approaches to Overcome Resistance

Data for currently approved DAAs provide limited insight on optimal retreatment approaches for patients with a previous DAA therapy failure and high fold change RASs, particularly those in NS5A. Until regimens combining multiple drugs predicted to be active (based on the available resistance profile) are available and adequate phase 2/3 studies in DAA

treatment failure populations are accomplished, other aspects of therapy must be optimized in treatment-experienced patients with RASs. In general, optimization involves appropriately characterizing the patient along with use of an extended duration of therapy and the addition of ribavirin (unless an absolute contraindication to ribavirin exists).

Characterizing Patients at Risk

The characteristics that increase the risk of DAA treatment failure are different for each oral regimen. Thus, understanding the population at risk is imperative. Generally, this requires accurate assessment of liver fibrosis and clarification of prior therapy.

Virus

Determination of HCV genotype, subtype, and baseline RASs may be necessary to fully characterize a patient's risk for therapeutic failure and optimize the treatment approach.

Treatment Duration

The duration of therapy should always be optimized to attain a cure. Although short-duration therapy has been associated with a higher chance of relapse, careful selection of patients for shortened therapy may minimize relapse risk and lead to significant cost savings. In contrast, extension of therapy (often to 24 weeks) in conjunction with the addition of ribavirin has been associated with reasonable SVR rates during retreatment of patients with past DAA therapy failure, even in the presence of significant drug-specific RASs prior to retreatment ([Cooper, 2016](#)); ([Gane, 2016](#)).

Ribavirin

The addition of ribavirin increases SVR in patient populations with an increased risk for treatment failure (eg, decompensated cirrhosis). It also improves SVR rates among patients with baseline NS5A RASs and prior DAA treatment failure.

Complementary Therapy

Although data are limited, patients with multiclass RASs can achieve SVR by combining triple or quadruple drug class regimens (see section on [retreatment](#) in prior DAA failure). This approach may become less necessary with the approval of standalone dual- or triple-drug regimens composed of second-generation protease and NS5A inhibitors with improved activity against common RASs.

Considerations With Current Antiviral Regimens

Daclatasvir + Sofosbuvir

Daclatasvir plus sofosbuvir is most commonly used for genotype 3-infected individuals. The phase 3 ALLY-3 study had an overall SVR rate of 89% in treatment-naïve and -experienced, genotype 3-infected patients treated with 12 weeks of daclatasvir plus sofosbuvir without ribavirin. This study demonstrated that lower SVR rates were observed in patients with cirrhosis, irrespective of treatment experience (97% [73/75] SVR without cirrhosis vs 58% [11/19] SVR with cirrhosis). When RAS impact was assessed, the presence of baseline Y93H was associated with a lower SVR rate in those with cirrhosis. Thirteen patients had Y93H at baseline; 67% (6/9) without cirrhosis achieved SVR whereas only 25% (1/4) with cirrhosis achieved SVR ([Nelson, 2015](#)). The subsequent ALLY-3+ study evaluated 12 weeks or 16 weeks of daclatasvir plus sofosbuvir and ribavirin in treatment-naïve or -experienced patients with genotype 3 infection and advanced fibrosis or compensated cirrhosis. The overall SVR rate was 90%. Again, virologic failure was higher in individuals with cirrhosis (86% SVR) compared to those with stage 3 fibrosis (100% SVR). Increased treatment duration did not appear to improve efficacy. Eight patients had a baseline RAS, including 2 with Y93H, 5 with A30K, and 1 with A30A/K. The only relapse occurred in a patient with the Y93H RAS ([Leroy, 2016](#)).

Elbasvir/Grazoprevir

Elbasvir/grazoprevir is indicated for treatment-naïve and -experienced patients with genotype 1 or 4 infection. The presence of NS3 RASs has no significant impact on SVR12 in patients treated with elbasvir/grazoprevir. The presence of NS5A RASs has no significant impact in genotype 1b infection.

In treatment-naïve, genotype 1a-infected patients (with or without cirrhosis) treated with 12 weeks of therapy, the

presence of NS3 RASs has no impact ([Zeuzem, 2015](#)). In treatment-naïve or prior relapse patients treated for 12 weeks with elbasvir/grazoprevir without ribavirin, the presence of high fold change NS5A RASs (at amino acid positions 28, 30, 31, and 93) decreased SVR to 58% (14/24) compared to 98% SVR in those without NS5A RASs. The presence of NS5A RASs had a similar impact on treatment-experienced patients (with or without cirrhosis) who received 12 weeks of elbasvir/grazoprevir without ribavirin (SVR12 29% vs 97%, respectively) ([Jacobson, 2015b](#)).

Glecaprevir/Pibrentasvir

In a study of the resistance profiles of glecaprevir and pibrentasvir using cell cultures ([Ng, 2017](#)), selection of genotypes 1a, 1b, 2a, 3a, 4a, and 6a replicons for reduced susceptibility to glecaprevir resulted in the emergence of RASs at A156 or D/Q168. The A156 RAS resulted in the greatest reductions (>100-fold) in glecaprevir susceptibility. The D/Q168 RAS had varying effects on glecaprevir susceptibility depending on genotype/subtype and specific amino acid change; the greatest reductions (>30-fold) were observed in genotypes 1a (D168F/Y), 3a (Q168R), and 6a (D168A/G/H/V/Y). However, these RASs are rarely detected clinically. Pibrentasvir selected no viable colonies in genotype 1b, 2b, 4a, 5a, and 6a. Of the few RASs selected by pibrentasvir, Y93H/N conferred <7-fold resistance.

The presence of RAS at baseline had minimal impact on SVR rates with glecaprevir/pibrentasvir in registration trials, that predominantly enrolled non-cirrhotic subjects. In a pooled analysis of NS3/4A protease inhibitor- and NS5A inhibitor-naïve patients who received glecaprevir/pibrentasvir in phase 2 and 3 studies ([Forns, 2017](#)); ([Foster, 2017](#)); ([Asselah, 2018b](#)); ([Zeuzem, 2016](#)); ([Kwo, 2017b](#)), baseline RASs in patients with genotype 1, 2, 4, 5, or 6 infection had no impact on SVR12 ([Krishnan, 2018](#)). Among treatment-naïve genotype 3-infected patients without cirrhosis who received glecaprevir/pibrentasvir for 8 weeks, the A30K polymorphism was detected in 10%, of whom 78% achieved SVR12. There are insufficient data to characterize the impact of A30K in genotype 3-infected patients with cirrhosis or prior treatment experience. All genotype 3-infected patients with Y93H prior to treatment achieved SVR12.

Ledipasvir/Sofosbuvir

Several comprehensive analyses of genotype 1-infected patients treated with ledipasvir/sofosbuvir in phase 2 and phase 3 studies have helped clarify the impact of baseline RASs on SVR rates with this regimen ([Sarrazin, 2016](#)); ([Zeuzem, 2017](#)). In a pooled analysis of patients with genotype 1a or 1b infection who received ledipasvir/sofosbuvir, 93.5% (316/338) of those with baseline NS5A RASs achieved SVR12 compared to an SVR12 rate of 98.4% (1,741/1,770) in patients without baseline NS5A RASs ([Sarrazin, 2016](#)). In this analysis, the reduction in SVR rate was driven predominantly by patients with genotype 1a NS5A RASs. The SVR12 rates for genotype 1a-infected patients with and without NS5A RASs were 92.3% and 98.3%, respectively. A slightly lower SVR12 rate of 90% was observed for genotype 1a-infected patients with NS5A RASs using a 15% deep sequencing cutoff value.

Notably, other factors further delineated populations at risk for relapse in this analysis, including high-level baseline NS5A RASs (>100-fold resistance with Q30H/R, L31M/V, and Y93C/H/N in genotype 1a) and a shorter duration therapy (8 weeks or 12 weeks vs 24 weeks). SVR12 rates were 97.4% to 100% in treatment-experienced patients without NS5A RASs or with RASs with <100-fold resistance treated with ledipasvir/sofosbuvir for 12 weeks or 24 weeks. However, when RASs with >100-fold resistance were present, SVR12 rates dropped to 64.7% (11/17) with 12 weeks of therapy compared to 100% (6/6) with 24 weeks of therapy. In this small subset of patients, the addition of ribavirin did not appear to offer the same benefit as extension of therapy to 24 weeks in this pooled analysis. SVR12 rate was 81.8% in those with >100-fold NS5A resistance who received 12 weeks of ledipasvir/sofosbuvir with ribavirin. In contrast, in the SIRIUS trial, all 8 treatment-experienced cirrhotic patients with >100-fold resistance treated for 12 weeks with ledipasvir/sofosbuvir plus ribavirin achieved SVR12.

Paritaprevir/Ritonavir/Ombitasvir + Dasabuvir ± Ribavirin

Paritaprevir/ritonavir/ombitasvir plus dasabuvir is currently indicated for genotype 1-infected patients. Paritaprevir/ritonavir/ombitasvir is indicated for genotype 4-infected patients, including those with prior peginterferon/ribavirin therapy failure. Patients with genotype 1a or 4 infection receive the addition of ribavirin whereas genotype 1b-infected patients do not. RAS testing has not been demonstrated to impact SVR rates, partially due to the addition of ribavirin in those patients at higher risk for treatment failure in the setting of RASs. Use of paritaprevir/ritonavir/ombitasvir plus dasabuvir alone in patients with a history of prior DAA treatment failure is not recommended.

Sofosbuvir/Velpatasvir

Sofosbuvir/velpatasvir is a pangenotypic therapy indicated for treatment-naïve and -experienced patients with or without cirrhosis. The presence of NS5A RASs had no impact on SVR12 for patients with genotype 1, 2, 4, 5, or 6 infection treated with sofosbuvir/velpatasvir for 12 weeks in the ASTRAL studies ([Hézode, 2018](#)). The presence of Y93H in genotype 3-infected patients decreased the SVR12 rate to 84% (21/25 patients) compared to 97% (242/249) in those without this RAS ([Foster, 2015a](#)). This appeared to be more impactful in patients with cirrhosis and/or prior treatment experience with an interferon-based regimen. Ribavirin was not used in these trials and thus, an evidence-based strategy to improve efficacy in those with genotype 3 infection and the NS5A Y93H RAS is not known.

Sofosbuvir/Velpatasvir/Voxilaprevir

Sofosbuvir/velpatasvir/voxilaprevir fills an important role as a pangenotypic regimen for patients who have experienced treatment failure with DAA therapy. The presence of NS3, NS5A, or NS5B RASs prior to treatment did not influence the likelihood of SVR12, and 12 weeks of treatment produced high SVR12 rates (96%) in DAA-experienced patients. RAS testing has not been demonstrated to impact SVR rates with sofosbuvir/velpatasvir/voxilaprevir therapy ([Bourlière, 2017](#)).

Table 1. Most Common, Clinically Important RASs by DAA, Genotype, and Fold Change

DAA	Genotype 1a				Genotype 1b	
	M28T	Q30R	L31M/V	Y93H/N	L31V/I	Y93H/N
Ledipasvir	20x	>100x	>100x / >100x	>1000x / >10,000	>100x / >50x	>100x / --
Ombitasvir	>1000x	>100x	<3x >100x	>10,000x / >10,000x	<10x	20x / 50x
Daclatasvir	>100x	>1000x	>100x / >1000x	>1000x / >10,000x	<10x	20x / 50x
Elbasvir	20x	>100x	>10x >100x	>1000x / >1000x	<10x	>100x / --
Velpatasvir	<10x	<3x	20x / 50x	>100x / >1000x	<3x	<3x / --

Color Key: light green = <3-fold change; dark green = <10-fold change; orange = >10- to 100-fold change; pink = >100-fold change

Table 2. Clinically Important RASs by DAA Regimen and Genotype

DAA Regimen	Genotype		
	1a	1b	3
Ledipasvir/sofosbuvir	Q30H/R L31M/V Y93C/H/N	L31V ?Y93H	n/a
Elbasvir/grazoprevir	M28A/T Q30H/R L31M/V Y93C/H/N	Y93H	n/a
Paritaprevir/ritonavir/ombitasvir with dasabuvir ± ribavirin	n/a	n/a	n/a
Sofosbuvir/velpatasvir	n/a	n/a	Y93H

Table 3. NS5A RAS Testing Recommendations Prior to Initiation of DAA Treatment Among Genotype 1 Patients by DAA Regimen, Virus Subtype, Prior Treatment Experience, and Cirrhosis Status

DAA Regimen	1b TN ^a or TE ^b	1a TN	1a TE No Cirrhosis	1a TE Cirrhosis
Ledipasvir/sofosbuvir	No	No	Yes	Yes
Elbasvir/grazoprevir	No	Yes	Yes	Yes
Sofosbuvir/velpatasvir	No	No	No	No
Paritaprevir/ritonavir/ombitasvir with dasabuvir ± ribavirin	No	No	No	No
^a TN = treatment naive ^b TE = treatment experienced				

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