HCV Resistance Primer

Introduction

Understanding principles of the emergence of drug-resistant viruses is critical when using targeted antiviral therapies. HCV is an approximately 9.2 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 or result in progeny viruses that replicate more slowly (reduced fitness) or are entirely 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. Most resistance-associated substitutions (RASs) affect the viral fitness or ability of the virus to replicate but usually do not persist in the absence of antiviral drug pressure. However, in a subset of persons with chronic HCV infection, viral variants harboring substitutions associated with resistance to HCV directing-acting antivirals (DAAs) are detectable prior to antiviral therapy. This occurs more often in the case of NS5A inhibitor-containing regimens because these substitutions generally do not impair viral replication. These substitutions often are referred to as baseline RASs. The presence of certain baseline NS5A RASs before treatment may negatively impact HCV DAA response in very specific patient populations.

In the case of HCV DAAs, resistant viruses are also selected for and/or enriched in persons for whom a DAA regimen fails. These viruses contain substitutions that are designated as treatment-emergent (or treatment-selected) RASs. NS5A and NS3 (protease) RASs are frequently selected in individuals with failure of NS5A or NS3 inhibitor-containing regimens, respectively. In contrast, NS5B (polymerase) nucleotide RASs are rarely detected (1% of failures) even after exposure to a failing DAA regimen containing a nucleotide inhibitor (Wyles, 2018b); (Svarovskaia, 2014). 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. They are not rare because they do not occur but rather because any substitution at this site renders the virus replication incompetent. Indeed, the specific RAS S282T does lead to sofosbuvir resistance but is extremely unfit so it is very rarely detected even after a failed sofosbuvir-containing regimen. When it is found, because of its low fitness level, it quickly becomes rare in the population and effectively disappears. Accordingly, this particular RAS is often considered to be clinically irrelevant, and sofosbuvir may be used for therapy even when it is present. 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.

Although DAA resistance emerges very quickly, currently used combination regimens are so effective that resistance has become much less of a concern over time. As such, the role of resistance testing in clinical practice has also become less relevant. However, there are specific clinical scenarios discussed in this section in which resistance testing may still be helpful.

Terminology, Thresholds of Clinical Relevance, and Assays

Terminology

1. Hepatitis C Proteins of Relevance
   DAA therapy relies on combining drugs that inhibit different viral proteins in order to raise the barrier to

resistance. Current combinations involve the NS3 protease (protease inhibitors), the NS5A protein (NS5A inhibitors) and the NS5B viral polymerase (polymerase inhibitors). These inhibitors can be recognized by the ending of their respective generic names (ie, protease, –previr; NS5A, –asvir; and NS5B/polymerase, –buvir). When considering DAA resistance, it is useful to consider which class of drugs—protease, NS5A, or polymerase inhibitor—is affected by the RAS.

2. Polymorphism (Substitution)
   A reference (or consensus) nucleotide—and therefore amino acid sequence—has been defined for each HCV genotype. A polymorphism 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. Polymorphisms generally do not affect viral fitness (the ability of the virus to replicate) or its susceptibility to antiviral drugs. Substitutions are changes in the amino acid that lead to a change in viral function and/or susceptibility to antivirals.

   To define a polymorphism/substitution, it is necessary to define: the HCV genotype (ie, genotype 1, 2, 3, etc) and subtype (eg, 1a versus 1b); the HCV protein (eg, NS5A); and the amino acid position (eg, 93). Polymorphisms/substitutions 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). Some individuals may harbor multiple variants. Also, several amino acids may be found at a given position. Thus, it is possible to have a virus with NS5A Y93H/M. Such a person would have viruses with the amino acids histidine (H) or methionine (M) at position 93 of the NS5A protein.

3. Resistance-Associated Substitutions
   RAS is the preferred term to describe amino acid changes that are associated with drug resistance. Specifically, RAS refers to a 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 have differing impacts on the susceptibility of different antiviral agents, even of the same class. For example, the Y93H RAS leads to high-level ledipasvir and velpatasvir resistance but minimal pibrentasvir resistance.

RASs of Clinical Relevance
The most clinically significant RASs presently are in the NS5A position for HCV genotypes 1a and 3.

RASs are most commonly found after viral relapse. HCV harboring RASs may not be entirely eliminated during DAA therapy and can subsequently dominate after treatment is withdrawn. Notably, it is rare for people to experience true nonresponse (viral breakthrough during treatment) because of the use of combinations of different DAA classes and given that even resistant HCV is partially suppressed by DAAs. Viruses that are resistant to NS3/4A protease inhibitors seem to have diminished fitness and may disappear from blood within a few weeks to months. However, NS5A inhibitor-resistant viruses may persist for years, which may have implications for treatment and retreatment. Because NS5A RASs generally do not affect the fitness of the virus, relevant NS5A resistance may occur even in people who have never been exposed to DAAs.

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, determining 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 ≥15% cut point is used for determination of RASs by NGS. Studies have shown that NGS at a 1% level of sensitivity often results in the identification of additional RASs that are not associated with clinical failure (Jacobson, 2017b); (Zeuzem, 2017); (Sarrazin, 2016). Most commercial and public health laboratories use NGS for RAS detection with a threshold of 15% for calling the presence of a RAS.

Resistance Testing in Clinical Practice

Regimen-Specific Recommendations for Use of RAS Testing in Clinical Practice

RECOMMENDED	RATING
Sofosbuvir/velpatasvir NS5A RAS testing is recommended for treatment-naive persons with genotype 3 infection and cirrhosis being considered for 12 weeks of sofosbuvir/velpatasvir therapy. If the Y93H resistance-associated substitution is present, weight-based ribavirin should be added or another recommended regimen should be used.	I, A

Resistance testing is most important in clinical practice when the results would lead to a change in management in terms of the choice of therapeutic agent(s) and/or the duration of therapy. The clinical scenarios when RAS testing may provide clinical utility include:

1. Initial treatment of people with HCV genotype 3 infection and cirrhosis who are to receive sofosbuvir/velpatasvir.
2. Retreatment of people with cirrhosis whose first course of DAA treatment fails to achieve SVR, particularly those with HCV genotype 3 infection.
3. Distinction of relapse versus reinfection in people who are viremic after a course of DAA therapy.

RAS Testing Prior to Initial Treatment

In clinical trials, the presence of baseline RAS had no impact on SVR12 for persons without cirrhosis treated with sofosbuvir/velpatasvir (Hézode, 2018) or glecaprevir/pibrentasvir (Zeuzem, 2018). Accordingly, resistance testing is not recommended for treatment-naive persons with any HCV genotype and without cirrhosis who are

to be treated with sofosbuvir/velpatasvir or glecaprevir/pibrentasvir. Treatment regimens should not be altered even if RAS testing was performed and indicates the presence of RASs.

In the ASTRAL studies, the presence of NS5A RASs had no impact on SVR12 for individuals with genotype 1, 2, 4, 5, or 6 infection treated with 12 weeks of sofosbuvir/velpatasvir (Hézode, 2018). The presence of the Y93H RAS in participants with genotype 3 infection decreased the SVR12 rate to 84% (21/25) compared with 97% (242/249) in those without this RAS (Foster, 2015a). This appeared to be more impactful in persons with cirrhosis. Ribavirin was not used in these trials. However, a subsequent trial that randomized participants with genotype 3 and cirrhosis to sofosbuvir/velpatasvir with or without ribavirin demonstrated lower relapse rates in those receiving ribavirin. However, the difference was only relevant in persons with baseline Y93H RASs prior to therapy, which occurred in 21% of the study population (Esteban, 2018). Accordingly, for individuals with HCV genotype 3 and cirrhosis, baseline RAS testing is recommended if sofosbuvir/velpatasvir is to be used—with the addition of ribavirin for those who harbor the Y93H RAS. RAS testing is preferred to using ribavirin in all persons with genotype 3 and cirrhosis receiving sofosbuvir/velpatasvir because only 10% to 20% of people have the Y93H RAS at baseline. As such, most people do not require ribavirin with its added toxicity (Pawlotsky, 2016). Notably, RAS testing with the addition of ribavirin is recommend in this scenario rather than relying on retreatment in the case of relapse because predictors of failure with retreatment with sofosbuvir/velpatasvir/voxilaprevir include genotype 3 infection, cirrhosis, and prior treatment with sofosbuvir/velpatasvir (Onofrio, 2021); (Belperio, 2019).

The Y93H RAS does not affect SVR rates in people with genotype 3 and cirrhosis treated with glecaprevir/pibrentasvir (Brown, 2020). Thus, for people eligible to take glecaprevir/pibrentasvir (ie, no signs of decompensated cirrhosis and no relevant drug-drug interactions), treatment with glecaprevir/pibrentasvir without baseline RAS testing is a preferred option.

RAS Testing Prior to Retreatment

Fortunately, retreatment studies have shown the presence of treatment-emergent RASs to have little or no impact on treatment efficacy. Among persons who relapsed after a previous course of an NS5A inhibitor-containing regimen, treatment with sofosbuvir/velpatasvir/voxilaprevir was effective, independent of the presence of RASs (Sarrazin, 2018); (Bourlière, 2017). In individuals with detectable RASs, 96% (199/208) achieved SVR compared with 98% (42/43) with no pretreatment detectable RASs (Sarrazin, 2018). Similar results have been reported in real-world studies, with SVR rates above 95% with retreatment (Graf, 2024). Therefore, RAS testing is not required prior to retreatment with sofosbuvir/velpatasvir/voxilaprevir for people without cirrhosis.

RASs may have more impact among people with cirrhosis, particularly those with HCV genotype 3 infection who do not achieve SVR with their first course of therapy (Graf, 2024). Extending the duration of therapy and using combination regimens may be helpful to overcome complex resistance profiles. However, studies are limited to small samples of patients due to the rarity of these outcomes.

Distinguishing Relapse From Reinfection

Among persons with ongoing risk factors for HCV acquisition who are found to have HCV viremia after a course of DAA treatment, it may be difficult to determine whether a relapse occurred or if the person was reinfected. The question has clinical relevance because reinfection can be successfully treated with first-line therapy whereas relapse requires treatment with a salvage regimen. Documented SVR12 with subsequent viremia indicates reinfection. However, confirmation of SVR12 is often missed (Valencia, 2019). A change in genotype at the time of recurrent viremia may indicate reinfection (eg, genotype 1a infection prior to initial

treatment but genotype 3a at time of recurrent viremia). However, people may be reinfected with the same genotype and even from the same source (eg, injecting partner) (Rose, 2018).

RAS testing may help distinguish relapse from reinfection. The presence of NS5A RASs after an unsuccessful treatment with an NS5A inhibitor-containing regimen is nearly universal (Krishnan, 2018); (Bourlière, 2017), whereas only 10% to 20% of people without prior treatment harbor NS5A inhibitor RASs (Pawlotsky, 2016). Hence, in someone with recurrent viremia and ongoing risk factors after a course of therapy, RAS testing showing the presence of NS5A RASs could indicate a likely relapse. Similarly, RAS testing showing no NS5A RASs could be assumed to indicate reinfection. Presumed relapse would be treated with a salvage regimen whereas presumed reinfection could be treated with a first-line therapy. Note that this approach has not been validated.

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

	Genotype 1a				Genotype 1b		Genotype 3a	
DAA	**M28T**	**Q30R**	**L31M/V**	**Y93H/N**	**L31V/I**	**Y93H/N**	**A30K**	**Y93H**
Ledipasvir	20x	>100x	>100x / >100x	>1000x / >10,000x	>100x >50x	>100x / --	NA	NA
Elbasvir	20x	>100x	>10x >100x	>1000x / >1000x	<10x	>100x / --	50x	>100x
Velpatasvir	<10x	<3x	20x / 50x	>100x / >1000x	<3x	<3x / --	50x	>100x
Pibrentasvir	<3x	<3x	<3x	<10x	<3x	<3x	<3x	<3x

Color Key: light green, <3-fold change; dark green, <10-fold change; orange, >10-fold 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	NA
Elbasvir/grazoprevir	M28A/T Q30H/R L31M/V Y93C/H/N	Y93H	NA
Sofosbuvir/velpatasvir	NA	NA	Y93H
Glecaprevir/pibrentasvir	NA	NA	A30K

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