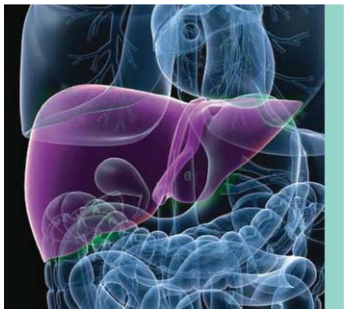

Resistance Testing: Interpretation and Incorporation Into HCV Treatment Algorithms

Jordan J. Feld, M.D., M.P.H.

The introduction of direct-acting antivirals (DAAs) has revolutionized the treatment of hepatitis C virus (HCV) infection. Highly effective and extremely well-tolerated regimens are now available for almost all patients. With such high success rates, patients have come to expect cure when they embark on a course of antiviral treatment. Beyond patients' expectations, with the cost of therapy and restrictions on retreatment, getting it right the first time, or certainly the second time, must be the priority for all clinicians. At least for the time being, maximizing the chance of success requires resistance testing in certain clinical scenarios.

TERMINOLOGY: RESISTANCE-ASSOCIATED VARIANT VERSUS RESISTANCE-ASSOCIATED SUBSTITUTION

There has been a lot of debate about the correct terms to describe resistance. Although somewhat of a semantic argument, it is helpful to use correct terminology. Resistance occurs because nucleotide substitutions occur randomly throughout the HCV genome with every replication cycle. By chance, some substitutions interfere with binding of specific DAAs to their protein targets. *Resistant variants* are viruses that grow better than wild-type virus in the presence of a DAA; the variant is not resistance associated (RAV), it is resistant. However, when virus is sequenced from a patient, specific substitutions may be recognized that are associated with resistance, but whether the specific virion with that substitution actually grows better in the presence of a given DAA is not known. In other words, it is not possible to identify resistant variants in patients. It is possible to identify substitutions that are associated with resistance; hence the term *resistance-associated substitutions* (RASs).[1] As such, *resistant variant* is the preferred term for HCV studied *in vitro*, and *RAS* is the preferred term

Abbreviations: AASLD, American Association for the Study of Liver Diseases; DAA, direct-acting antiviral; EBV/GZV, elbasvir/grazoprevir; HCV, hepatitis C virus; NS5Ai, nonstructural 5A inhibitors; PI, protease inhibitor; RAS, resistance-associated substitution; RAV, resistance-associated variant; SOF/LDV, sofosbuvir/ledipasvir; TE, treatment experienced; TN, treatment naive.

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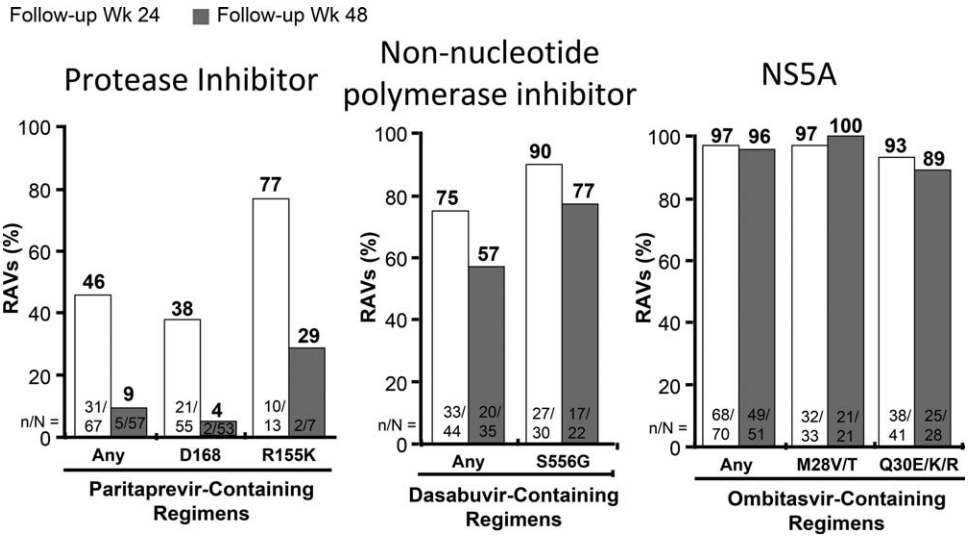

FIG 1 Persistence of RAS after DAA failure. The replication fitness of variants with RASs differs by DAA class. RASs to nucleotide polymerase inhibitors (e.g., sofosbuvir) are very unfit and do not persist long term after treatment (not shown). RASs to PIs have relatively poor fitness and have mostly disappeared by 48 weeks posttreatment. RASs to nonnucleotide polymerase inhibitors vary by specific agent but generally persist >1 year in the majority of patients, whereas RAS to NS5A inhibitors are very fit and persist >1 year in almost all patients. Data are from patients who relapsed after treatment with paritaprevir/ritonavir/ombitasvir and dasabuvir.[3]

for sequencing results from patient samples (Figure 1 and Table 1).

Viral Fitness

It is important to remember that DAAs do not make RASs, they just select for them. Substitutions occur randomly with replication and most will impair viral fitness, that is, how well the virus replicates. Variants with RASs have a fitness advantage in the presence of DAAs, but most will be outcompeted by wild-type virus in the absence of drug. The fitness of specific RASs has a major impact on whether RASs persist after unsuccessful therapy and whether they exist at baseline in treatment-naive patients. RASs to different DAA classes have markedly different fitness. The classical sofosbuvir-associated RAS (S282T) replicates extremely poorly and is quickly outgrown by wild-type virus, explaining why it is almost never seen even after unsuccessful sofosbuvir treatment.[2] High-level protease inhibitor (PI) RASs also have poor fitness and may be detected for some time after treatment with a PI, but rarely occur at baseline and will eventually return to low or undetectable levels off treatment.[3] RASs to nonnucleotide polymerase inhibitors have intermediate fitness, and thus persist after therapy but are uncommon in untreated patients.[3] In contrast, RASs to nonstructural 5A inhibitors (NS5Ai) have very minimal fitness impairment and compete with wild-type virus. They are commonly found in patients naive to NS5Ai (10%-15% genotype 1) and persist long term after a failed course of NS5Ai-based therapy.[4] Because NS5Ai are components of most approved regimens, the effect of baseline and posttreatment NS5Ai RAS is clinically relevant (Figure 2).

TABLE 1. TERMINOLOGY

Term	Meaning/Correct Usage
Variant	Refers to the whole virus
	A specific virion *is* or *is not* resistant; that is, it replicates well or it does not replicate well in the presence of drug
	It is not resistance associated
	Resistant variant is an acceptable term
Substitution	Refers to a change in the RNA sequence
	Some changes are *associated with resistance*
	Hence the term *resistance-associated substitution (RAS)* is preferred
Polymorphism	A change in the RNA sequence that is not necessarily associated with resistance
	All are naturally occurring, that is, not treatment emergent
Mutant	Usually implies selective pressure, which is not present in treatment-naive patients

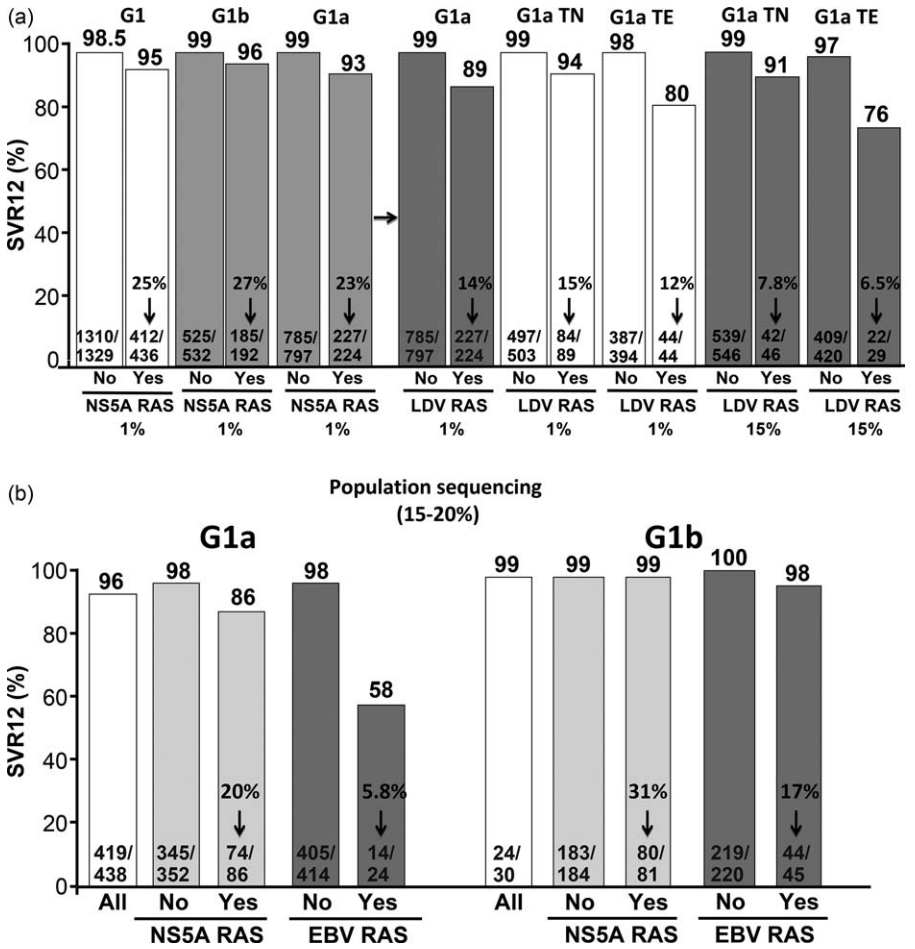

FIG 2 Signficance of RAS reporting for treatment with sofosbuvir/ledipasvir (SOF/LDV) and elbasvir/grazoprevir (EBV/GZV). The significance of baseline RAS on SVR depends very much on how they are reported. (A) As shown, NS5A RASs are common but have little effect on SVR in 1765 patients treated with sofosbuvir/ledipasvir in phase 2/3 trials when all genotype 1 patients are included using a 1% threshold of detection. The effect is more notable in those with genotype 1a infection compared with those with genotype 1b infection. When only ledipasvir-specific RASs are considered and the threshold is raised from 1% to 15%, RAS are less common but have a much greater effect on SVR, particularly in those with unsuccessful responses to a prior course of peginterferon and ribavirin (treatment-experienced [TE]).[6] (B) A similar effect is seen with EBV/GZV. There is no effect of NS5A RASs in genotype 1b infection, and the effect of elbasvir-specific RASs is greater than all NS5A class RASs in those with genotype 1a infection.[5] Percentage with arrow indicates the prevalence of RAS in the specific population. 1% or 15% refers to prevalence of the RAS in the viral population. Abbreviation: TN, treatment naive.

RAS Reporting

Unfortunately, the definitions are not just an issue of RAS versus RAV. Reporting of the effects of RASs in the literature has been very confusing. Some RASs affect response to all members of a DAA class, whereas others have different effects on different DAAs of the same class. The prevalence of RASs also matters. Although deep sequencing is more likely to find RASs, unless they account for at least 15% to 20% of the viral population, they are likely of limited clinical significance.[1] Conveniently, population sequencing, which gives the most common/average sequence of the viruses circulating in a patient, will detect RASs accounting for ~15% to 20% of the viral population. RASs have different effects in different populations (different genotypes/subtypes) and may be more relevant in treatment-experienced patients and those with cirrhosis.

Many studies report class-specific rather than drug-specific RASs, often at low thresholds (1% versus 15%) and in poorly defined patient populations (genotype 1 versus 1a/1b, all versus experienced/naive, all versus cirrhotic/noncirrhotic). Collectively, these approaches tend to downplay the effect of baseline RASs by including patients for whom they are irrelevant, which dilutes the effect size in relevant populations (Figure 2 and Table 2).

TABLE 2. COMPONENTS OF RESISTANCE-ASSOCIATED SUBSTITUTION REPORTING

Component	Significance	Preferred Reporting
Drug- versus class-specific RAS	Whether a RAS confers resistance to a specific DAA or to all members of a class of DAAs	Drug specific
Threshold of detection	The percentage of the viral population with the specific RAS identified Deep sequencing limit of 1% Population sequencing limit 15% to 20%	Population sequencing or deep sequencing with threshold of 15%
Genotype- versus subtype-specific RAS	Most RASs have different effects on the different subtypes within the same genotype	Subtype specific
Patient population	Some RASs have a greater effect in patients with treatment experience and/or cirrhosis	Population specific Treatment history Presence of cirrhosis

Baseline RAS Testing

There is no value in testing (see comment below) for baseline RASs unless the results will change management. Fortunately, for genotypes 1b, 2, 4, and 6, baseline RASs do not appear to affect response to treatment, and thus baseline RAS testing is not warranted.[1] However, for those with genotype 1a and possibly genotype 3, baseline RASs are relevant for certain regimens in certain populations (Table 3). For elbasvir/grazoprevir, the presence of baseline RASs impairs response rates in genotype 1a. Fortunately, extension to 16 weeks and addition of ribavirin appear to overcome the effect of baseline RASs, and thus is label recommended (Figure 3).[5] Data with ledipasvir/sofosbuvir show that certain RASs have relevant effects in genotype 1a patients who did not respond successfully to prior peginterferon/ribavirin therapy and in treatment-naive patients with cirrhosis. Although data are limited, the addition of ribavirin and/or extension of therapy similarly overcome the effect of RASs.[6] For other approved regimens, the effect of baseline RASs in genotype 1a is limited either because of a higher barrier to resistance (sofosbuvir/velpatasvir) or the requirement for the use of ribavirin (paritaprevir/r/ombitasvir/dasabuvir).[7]

In the ASTRAL-3 trial, the sustained virological response (SVR) rate with sofosbuvir/velpatasvir for 12 weeks in patients with genotype 3 fell from 97% to 88% in those with cirrhosis harboring baseline Y93 RASs.[8] A similar effect was not seen in the sofosbuvir/velpatasvir arm of the POLARIS-3 trial despite a similar population.[9] The reason for the difference is not clear. The American Association for the Study of Liver Diseases (AASLD) guidelines recommend testing for the Y93 RAS in patients with cirrhosis and adding ribavirin for patients in whom it is present based on extrapolation from the ASTRAL-4 study of sofosbuvir/velpatasvir in decompensated cirrhosis in which patients with genotype 3 who received ribavirin had the best response rates.[10]

TABLE 3. POPULATIONS FOR WHOM BASELINE RESISTANCE-ASSOCIATED SUBSTITUTION TESTING IS USEFUL WITH CURRENTLY APPROVED REGIMENS

Regimen	Population	RAS Testing	Recommended Approach if RAS Is Present
Elbasvir/grazoprevir	Genotype 1a (all)	NS5A	Extend therapy to 16 weeks and add weight-based ribavirin
Sofosbuvir/ledipasvir	Genotype 1a Peginterferon/Ribavirin experienced (all) Naive with cirrhosis	NS5A	Add weight-based ribavirin and/or extend therapy from 12 to 24 weeks*
Simeprevir + sofosbuvir	Genotype 1a with cirrhosis	PI – Q80K	Use alternative regimen
Sofosbuvir/velpatasvir	Genotype 3 with cirrhosis	NS5A – Y93	Add weight-based ribavirin

*Not specifically recommended by AASLD/Infectious Diseases Society of America guidance or product label, but supported by results of clinical trials.

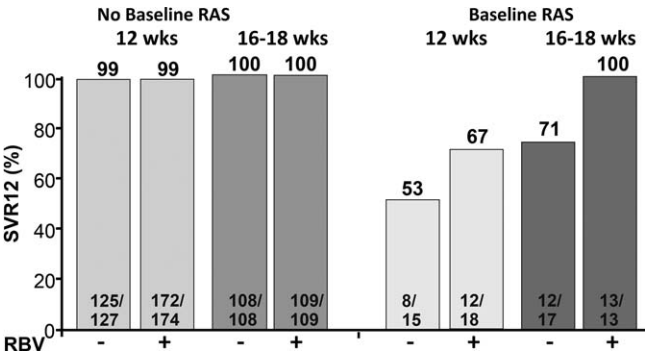

FIG 3 Overcoming the effect of baseline RAS with elbasvir/grazoprevir. Baseline elbasvir RASs have a significant effect on rates of SVR in patients with genotype 1a infection. As noted, in patients without baseline RASs, SVR rates were 99% to 100% with 12 weeks and no ribavirin. In contrast, the SVR rate was only 53% in those with baseline elbasvir RASs who received 12 weeks of therapy. The addition of ribavirin and extension to 16 to 18 weeks improved response rates, and the combination of both longer therapy and ribavirin resulted in 100% SVR.[5]

The Counterargument

Some argue that baseline RAS testing is not necessary even for the specific populations mentioned because the number with baseline RASs who will not respond represents a relatively small percentage of the overall population, and testing may be a barrier to treatment access/uptake. Cost, limited access, and hard-to-interpret reports are also cited as arguments against testing. For the individual patient, the presence of RASs may have a major effect on the chance of SVR. It is true that reports should be improved. Reporting that a specific RAS is ''probably'' or, worse, ''possibly'' resistant to a specific drug is not helpful. It would be more useful to link to practice guidelines to recommend treatment modifications for regimens (e.g., extend/add ribavirin). Fortunately, combinations in late-stage development have improved barriers to resistance, and it is likely that baseline resistance testing will not be required in the near future.

POSTTREATMENT FAILURE

The AASLD guidelines recommend RAS testing in all patients before retreatment after a failed course of DAAs.[11] Importantly, strategies to overcome resistance with current regimens have not been validated in the retreatment setting. Retreatment is rarely an emergency. Given the very promising data with multiple salvage regimens used specifically for retreatment in patients with RASs, it would likely be better for most patients to wait for coming therapies than to be retreated with existing approved therapies.

CONCLUSION

Resistance has added a layer of complexity to HCV management. If properly interpreted, baseline RAS data can add significant clinical value for specific patient populations. Hopefully standardized reporting in the literature and improved clinical reports will make RAS testing easier to use clinically. Fortunately, this is likely a temporary situation. Future regimens are unlikely to require baseline resistance testing and may not even require testing before retreatment. Until these regimens arrive, the biggest resistance that needs to be overcome is the resistance to resistance testing.

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