Curr HIV/AIDS Rep (2017) 14:229–237
https://doi.org/10.1007/s11904-017-0369-5

CO-INFECTIONS AND COMORBIDITY (S NAGGIE, SECTION EDITOR)

# Resistance to DAAs: When to Look and When It Matters

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Published online: 7 November 2017


**Abstract**
*Purpose of Review* This review provides an overview of HCV resistance-associated substitutions (RASs) with a focus on NS3 protease and NS5A inhibitor resistance. Treatment approaches for managing resistance are also covered including the use of newly approved therapies with improved resistance profiles.
*Recent Findings* HCV RASs are frequently selected if the patient is not cured during treatment; NS5A RASs persist for prolonged periods of time (years) after treatment failure and may adversely impact retreatment responses. Newly approved regimens with improved potency and resistance profiles are less impacted by resistance and provide the best retreatment options for patients who previously failed DAA therapy.
*Summary* The clinical impact of HCV RASs has been lessened significantly with the introduction of new DAA treatment regimens. Routine testing for resistance is unlikely to impact retreatment approaches if newer regimens are accessible. Knowledge of factors, such as the presence of cirrhosis and prior treatment regimens, remain as the key to optimizing retreatment approaches.

**Keywords** HCV · Resistance-associated substitutions · Direct acting antivirals

This article is part of the Topical Collection on *Co-infections and Comorbidity*

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## Introduction

The introduction of direct acting antiviral (DAA) therapies for treatment of HCV infection has dramatically improved treatment responses while generally making treatment simpler and much safer. Despite these improvements, several complexities have arisen which are unique to DAA HCV therapies. Resistance-associated substitutions (RASs) are alterations in the HCV amino acid sequence, either naturally occurring or selected, which adversely impact inhibitor activity in vitro and have the potential to impair response to DAA treatment regimens in the clinic.

Genetic testing for HIV antiretroviral (ARV)-associated resistance mutations is an accepted part of the evaluation and treatment of HIV infection [1]. As such, HIV practitioners are familiar with antiviral resistance concepts; however, there are key differences in the impact and management of HCV resistance which those treating HCV, particularly when coming from an HIV background, must be aware of. Perhaps most importantly, our understanding of HCV resistance and its management is still in its infancy and recognizing the limitations of our current knowledge as well as placing management decision surrounding resistance and treatment failure in the context of a continually evolving and improving HCV treatment landscape are crucial to developing best practice approaches.

## Are HCV Resistance Considerations Different than HIV? If so, Why?

HIV-1 and HCV are both RNA viruses and both utilize an error prone polymerase in their replication cycles. Studies estimating the error rate of both polymerases suggest that the HCV NS5B RNA-dependent RNA polymerase (RdRp) has a lower fidelity than HIV reverse transcriptase (~ $10^{-4}$ errors/bp

vs ~ $10^{-5}$ errors/bp, respectively) [2–4]; combined with a higher rate of viral turnover in infected humans (~ $10^{12}$ vs ~ $10^{10}$ virions/day) and an opportunity for the HCV RdRp to act twice in the viral life cyle [5–7], HCV viral diversity is greater both on an intrapatient and global level compared to HIV [8–10]. At a nucleotide level, the diversity between HIV groups is similar to that found between different HCV genotypes (30–40% at the nucleotide level) [2, 11]. Functionally, this difference in diversity is magnified by the fact that the majority of HIV infections worldwide are due to group M viruses (90% of HIV infections), while only approximately 45% of HCV infections globally are due to genotype 1 with a large contribution of genotypes 3 and 4 [10, 12, 13].

These factors favor (and support) the increased prevalence of resistance polymorphisms in the absence of drug exposure in HCV compared to HIV, as well as the rapid development of additional RASs with drug selective pressure. Indeed, both these assertions are supported by observations that up to 50% of viral sequences may harbor DAA RASs [14–16], depending on which polymorphisms are counted and the sensitivity of sequencing techniques, and that DAA-specific RASs are selected in the majority of patients failing therapy [17–19]. On the surface, this would seem to suggest that resistance and clinical resistance testing might be more relevant in HCV. However, two related factors are the key to lessening the impact of HCV resistance compared to HIV: (1) the lack of an integrated or long-lived cellular HCV reservoir and (2) HCV infection is curable without the need for life-long antiviral therapy. Also, transmitted drug resistance, a major contributor to the indication for resistance testing in HIV, has not been a major issue in HCV—in part due to very high cure rates once someone is exposed to current HCV DAA regimens. The absence of a long-lived HCV viral reservoir may also contribute as well as the difficulty in differentiating transmission of natural polymorphism versus transmission of selected drug resistance in HCV [20]. It should be noted, that despite the absence of a long-lived HCV cellular reservoir, data demonstrated the persistence of HCV NS5A RASs for over 2 years after selection in patients failing DAA therapy [19, 21].

## What Determines Whether a RAS Is "Clinically Significant"?

While on the surface, viral characteristics suggest that HCV resistance would be a major consideration in utilizing current DAA therapies—currently available data suggest that the majority of RASs do not have a significant impact on therapeutic responses. Scenarios where clinically significant RASs occur, and thus resistance testing is indicated, occur relatively infrequently as it requires a combination of specific patient and viral characteristics with use of a specific regimen in the setting of a handful of drug-specific RASs.

Some generalizations can help identify populations and regimens most likely to be impacted by pre-existing HCV RASs:

- Patient characteristics associated with poor responses (treatment experience, cirrhosis)
- Viral factors (genotypes 1a and 3)
- Regimen characteristics (NS5A containing vs non-NS5A containing)

Within these populations, RASs of clinical significance generally confer high fold changes in in vitro potency for a given drug. The definition of high fold is somewhat arbitrary and depends on the drug class, intrinsic potency of a specific drug, and the attainable concentration at the biological site of action (e.g., hepatocyte). For NS5A inhibitors, the class most impacted by RASs, high fold change variants often confer more than a 100-fold loss in activity for a given drug in vitro [22].

## Defining Resistance and Clinical RAS Testing

Before RASs testing can be considered a component of patient management—standardized approaches to defining and identifying HCV RASs are crucial. In this regard, much work needs to be done in the HCV resistance arena. This is best exemplified in the case of NS5A genotypic resistance. NS5A variants have been classified using several similar but distinct definitions:

- NS5A class resistance-associated polymorphisms (class RAPs)
- NS5A class resistance-associated variants (class RASs)
- NS5A drug-specific resistance-associated variants (drug-specific RASs)

Within drug-specific RASs, there may be a further distinction between RASs and high fold change RASs which are variously defined as conferring > 5-fold to > 100-fold shift in potency (in vitro).

Currently, it appears that drug-specific NS5A RASs, particularly high fold change variants, are of most clinical import, particularly those that can be detected at a threshold around that of Sanger sequencing techniques (roughly 20% of the viral population) [22–24]. Casting a wide net by considering all identified polymorphisms and using sensitive sequencing techniques (i.e., next generation sequencing (NGS)) remains essential during clinical development programs; however, to guide clinical management, more streamlined criteria are needed so as not to unnecessarily complicate or over-treat patients.

Commercially available genotypic HCV-resistance tests utilize different approaches to sequencing—with one test relying on ultra-deep sequencing but only reporting variant found in > 10% of the sequences while the other is based on traditional population (Sanger)-based sequencing. Both tests offer sequencing for the three major viral drug targets NS3, NS5A, and NS5B across HCV genotypes 1a, 1b, and 3. In order to increase likelihood of a successful test, it is optimal to know the patient's viral genotype and subtype and that the clinical viral load be > 1000–2000 IU/mL. Clinical data suggest that either approaches will detect the majority of clinically significant HCV RASs and thus both approaches are valid for clinical management [23, 24].

Specific situations and approaches to managing HCV drug resistance will be discussed in the following sections. General approaches to managing resistance come down to a couple of simple concepts that apply to most situations, namely:

1. Extension of treatment duration *and/or*
2. Addition of ribavirin (RBV) *or*
3. Use of newly approved regimens with enhanced resistance profiles and multiple mechanisms of action

The recent introduction of two new HCV treatment regimens composed of multiple pangenotypic drugs with improved resistance profiles have dramatically improved expected cure rates when retreating patients who have failed DAA therapy [25, 26]. These regimens are preferred for retreatment of DAA-experienced patients; fulfilling the promise inherent in prior recommendations to defer treatment if possible in this difficult to treat population [27]. These persons should now be prioritized for treatment with one of these new regimens.

## NS3/4a Protease Inhibitor Resistance

Despite being the first class of DAAs approved, resistance to NS3 protease inhibitors (PIs) has not emerged as a major consideration in HCV treatment. This phenomenon stems from several factors including (1) the rare existence of significant resistance variants in the absence of drug-pressure (prior treatment), (2) current DAA regimens which rely on NS5A inhibitors combined with nucleotides or protease inhibitors with improved resistance profiles and/or improved pharmacokinetics mitigating the impact of any variants present, and (3) the relatively rapid loss of selected resistance variants after removal of drug selective pressure [19, 28]. The key PI RASs which clinicians should be aware of include the genotype 1a polymorphism Q80K and resistant variants at positions R155, A156, and D168 in genotype 1 (Table 1). Much less is currently known about PI resistance in non-GT1 genotypes; early generation PIs were much less active, and thus not used clinically, against non-GT1 genotypes such as genotypes 2 and 3 due to polymorphisms at sites such as 168 [29].

With current potent DAA regimens, there are no indications for baseline screening for NS3 PI resistance in the absence of prior PI exposure. In patients with a history of treatment failure and exposure to an NS3 PI, resistance testing may be helpful in select situations though this is limited by the wide availability of efficacious retreatment options which do not contain a PI or utilize a NS3 PI with a higher barrier to resistance [30, 31]. Patients who previously failed an all DAA regimen containing a PI represent a more difficult population to treat; however, the recent approvals of coformulated sofosbuvir, velpatasvir, and voxilaprevir (SOF/VEL/VOX) and glecaprevir with pibrentasivr (GLE/PIB) now provide efficacious retreatment options for these patients as well [25, 26]. NS3 PI RAS testing may be of some benefit in PI and NS5A exposed patients prior to considering retreatment with GLE/PIB and will be discussed later [32].

**The Q80K Variant** The Q80K RAS is almost exclusively seen in patients with genotype 1a, given its frequency in this population (~ 40%) much attention has been paid to its potential impact on protease inhibitor containing therapies. This polymorphism confers a 7–10× fold shift in the 50% effective concentration (EC50) to simeprevir (SMV) in vitro with lesser impact on ombitasvir (3× shift) and no impact on grazoprevir or voxilaprevir [33–36]. Despite this relatively low fold change in activity, the presence of a baseline Q80K in phase 3 studies of SMV plus PEG/RBV resulted in responses that were no better than placebo plus PEG/RBV [37]. Based on this data, the original prescribing information for SMV contained the recommendation to perform baseline testing for this RAS and select alternative therapies for use in combination with PEG/RBV.

The impact of Q80K on interferon-free DAA regimens containing SMV (i.e., SMV + SOF) is much less and, in general, not of clinical significance. Patient factors and treatment duration do appear to modulate this effect. In the phase 3 non-cirrhotic study, no impact of baseline Q80K was found in 1a patients treated with 12 weeks of SOF + SMV (OPTIMIST-1) [38]. However, when a shorter duration of 8 weeks was explored, lower SVR12 rates were seen in patient with Q80K. In a population with cirrhosis treated for 12 weeks with this regimen, the Q80K did again have an adverse impact on treatment outcomes [39]. It is important to emphasize that neither 8 weeks in non-cirrhotic nor 12 weeks in cirrhotic patients are recommended treatment durations with this regimen.

The Q80K RAS does not significantly impact responses to either paritaprevir or grazoprevir containing regimens and baseline testing to identify this variant is not required prior to use of these regimens. Combined these data do not suggest a significant impact of the Q80K polymorphism with modern DAA therapies. In the rare situation of a DAA regimen failure

**Table 1** Select NS3 RASs which result in > 10× FC to compounds listed

| NS3 RASs | SMV | PTV | GZR | GLE | VOX[c] |
|---|---|---|---|---|---|
| GT1a | Q80K[a]<br>**R155K**<br>A156S/T/V<br>**D168any** | **R155K**<br>A156T/V<br>**D168V/any** | **A156G/T/V**<br>**D168A/V** | A156T/**V**[b] | A156T/V |
| GT1b | A156T/V<br>**D168any** | A156T/V<br>D168V/any | **A156T/V** | A156T/V | A156T/V |
| GT3 | N/A | N/A | N/A | **A156G**<br>**Q168R** | A156any |

RASs in bold are most frequently selected with virologic failure. BOLD = selected in > 10% of virologic failures

[a] 7× FC but associated with virologic failure in combination with PEG/RBV

[b] $n = 1$

[c] Treatment-emergent RASs to VOX have not been noted in the context of SOF/VEL/VOX therapy

where treatment with SOF + SMV for 24 weeks is being contemplated, testing for this RAS is reasonable and supported by current guideline recommendations [27]; a paucity of data makes management decisions in the setting of a Q80K unclear.

Interestingly, a signal for an adverse impact of Q80K on patients with genotype 1a infection treated with 8 weeks of SOF/VEL/VOX was noted in the POLARIS-2 study [40]. In vitro this variant has no impact on VOX $EC_{50}$ (1.2×), and therefore, it seems implausible that there is direct biologic effect of this variant alone resulting in the lower response rate seen in this group (88% SVR12; 51/58) [41]. It is worth noting that Q80K is a marker for the North American lineage of genotype 1a distinct from 1a virus clades circulating in other parts of the world [42]. Whether there are more complex genetic characteristics of this lineage which make it more resistant to therapy or if this is simply a marker for an otherwise more difficult to treat population (e.g., all Q80K failures also carried the IL28B T allele) is unknown. The clinical impact is negligible since SOF/VEL/VOX was only approved in the US at the 12 week duration for DAA-experienced populations where no impact of Q80K was noted [43].

**Variants at Position 155** Variants at position 155 are rarely seen in genotype 1 in the absence of drug selective pressure. In surveys, the prevalence of the R155K RAS at baseline is approximately 0.5–1.0% in genotype 1a [14, 15]. This RAS was frequently seen in patient with genotype 1a HCV failing regimens containing telaprevir or boceprevir; currently, this variant is seen in patients failing SMV and, to a lesser extent, paritaprevir (PTV) containing regimens [17, 39]. This variant is not seen in genotype 1b patients after drug exposure due to alternative codon usage at R155 in genotype 1b resulting in a higher genetic barrier to resistance [44]. In vitro (genotype 1a) the R155K resulted in a 90-fold loss in SMV activity, 40-fold for PTV, and minimal (3-fold) shift in GZR activity [33, 34, 36]. No change in in vitro activity is seen for either voxilaprevir or glecaprevir with the R155K substitution in GT1a [41, 45].

R155G/W variants were selected in GT1a/b and 3 during VOX monotherapy and do result in significant fold shift in activity in vitro [35]. These variants have low replication capacity in vitro (< 10%) and thus far they have not been found in virologic failures treated with SOF/VEL/VOX [41, 43].

**Variants at Position 168** Variants at position D168 in genotype 1 are generally not found in the absence of drug selective pressure. They are found after exposure to NS3 PIs in a failing DAA regimen and confer high level resistance to most currently available HCV PIs with exception of VOX and GLE where low to moderate level resistance is seen and GZR where moderate levels of resistance are seen [17, 33–35, 45]. However, following removal of drug selective pressure, these variants tend to be rapidly outgrown by wild-type virus, presumably due to the poor replicative fitness based on many D168 variants in vitro [19].

The 168 position is also a polymorphic site in non-GT1 isolates; in particular, in GT3, position 168 is most often Q and accounts for the limited activity in GT3 of many early generation NS3 PIs [29]. Next generation PIs such as VOX and GLE, and to some extent GZR, have improved pangenotypic activity including against GT3 [35, 45, 46].

**Variants at Position 156** Amino acid 156 of the NS3 protein is located within the active site, and as such, variants at this position have a large impact on enzyme activity and viral fitness. They are rarely seen in the clinic given their poor fitness but do confer broad cross resistance, particularly the A156T/V variants, to all protease inhibitors [19, 35, 45]. As with 168 position variants, their poor fitness also means they are "lost" rapidly following removal of drug selective pressure [19, 35].

## When Should You Look for NS3 RASs?

Protease inhibitor genotypic resistance testing is widely available and historically there were several indications for testing. However, in the current era of DAA therapies, there is no role for baseline NS3 RAS testing (Table 1). Resistance testing was also previously endorsed in the setting of DAA failure, even without prior exposure to a PI, with the idea that such information would allow optimization of subsequent therapies. Following the approval of SOF/VEL/VOX and GLE/PIB, there is no clear role for NS3 RAS testing in DAA-experienced patients (see section on RAS testing and new regimens).

## NS5B Polymerase Inhibitor Resistance

Similar to HIV therapy, there are two types of HCV polymerase inhibitors: nucleoside and non-nucleoside inhibitors. Dasabuvir is the only non-nucleoside inhibitor currently approved and there are no other compounds in this class in late stage clinical trials. Resistance to this class of inhibitors is not of clinical significance and will not be discussed further.

Nucleoside inhibitors are unique among HCV DAAs with an extremely high barrier to resistance. Currently, sofosbuvir, a uridine analog, is the only approved drug in this class. A second member of this class, uprifosbuvir, is in last stage clinical trials. The signature resistance mutation for HCV nucleosides is S282T which confers a modest fold change (~ 10×) in SOF EC50 in vitro with poor replicative fitness (2–8%) [47, 48]. It is not found prior to drug exposure and is only selected in 1% of virologic failures treated with a SOF containing regimen [47]. Upon removal of drug selective pressure, this variant is quickly lost and does not appear to impact retreatment approaches [49]. Based on these facts, there is no major role for NS5B RAS testing in clinical practice.

## NS5A Inhibitor Resistance

NS5A inhibitors are a component of all first-line DAA regimens and are also the class of HCV drugs where resistance is most clinically relevant. In the absence of prior drug exposure, NS5A class RASs are relatively frequent in genotype 1 HCV being found in 13% of GT1a isolates and 18% of GT1b isolates at a 15% threshold [24]. Despite being more prevalent in GT1b, the majority of the clinical impact is in GT1a. NS5A RASs are also of clinical impact in GT3 where they are found in about 9–13% of viral sequences [50, 51].

Outside of GT1 and 3, the prevalence and impact of NS5A RAS is less well studied (Table 2). In genotype 2, the L31M NS5A polymorphisms is prevalent (~ 40–50% of GT2 isolates) but does not have an adverse impact on treatment responses [51, 52]. Subtypes of genotype 6, particularly 6e, possess NS5A polymorphisms that may impact activity of earlier generation NS5A inhibitors such as ledipasvir [53].

While a large number of NS5A class RASs have been described, those that are most clinically relevant include variants at positions M28, Q30, L31, and Y93 in GT1a, L31, and Y93 in GT1b and A30 and Y93 in GT3 (Table 2).

**Variants at Position 28** Of the specific NS5A variants considered, M28 variants (M28A/G/T/V) are most frequently encountered in GT1a prior to drug exposure (4–8% M28T/V) [22, 24]. In vitro M28 variants result in moderate (> 10×) to high level (> 100×) resistance to early generation NS5A inhibitors such as daclatasvir (DCV), ledipasvir (LDV), ombitasvir (OBV), and elbasvir (EBR) with significant variability based on the specific substitution [17, 18, 23, 54]. Lower fold shifts in VEL EC50 are seen (e.g., M28T 7.5×) [43]. Position 28 variants do not result in significant fold shift in PIB EC50 (< 3×) [45].

Clinically, the most important distinction is that the M28V variant does not result in a significant fold shift (< 5×) in EBR in vitro and should not be considered a significant baseline RAS for EBR/GZR [23].

**Variants at Position 30** Multiple variants at position Q30 in GT1a confer resistance to NS5A inhibitors; the most common variants present without drug exposure are Q30R or Q30H which are present in about 3% of 1a isolates [24]. Q30R/H variants results in high level resistance to DCV, LDV, OBV (R only), and EBR (R only) [18, 54–56], while resulting in minimal fold shifts for VEL or PIB in GT1a [45, 57]. Q30 variants (primarily Q30R/H) are the RASs most frequently selected in GT1a patients after failing therapy with several NS5A inhibitors including LDV, OBV, and EBR [17–19]. In GT1a, they are often found in combination with L31 or Y93 variants after DAA failure [21].

A Q30 deletion has been described in GT1a after selection with PIB in vitro and results in high level resistance to PIB(> 1000×); however, this variants replicates very poorly in vitro and has not been described clinically [45].

In GT3, the A30K variant is observed in ~ 5% of isolates and results in a moderate fold shift for VEL (50×) [51, 57]. While it does not result in a significant fold shift in PIB activity in vitro (< 2×), it was enriched in baseline sequences (50%, 9/18) of GT3 patients with subsequent virologic failure after GLE/PIB [45, 58]. In particular, there was a signal for lower SVR rates with 8 weeks of GLE/PIB in GT3 infected, treatment-naïve, non-cirrhotic patients with an A30K RAS at baseline (78% SVR, 14/18) [58]. Given the limited number of patients, high overall SVR rate with 8 weeks in this population and relative rarity of A30K (~ 3–5%) additional data are needed before recommendations on baseline testing can be made.

**Table 2** Select NS5A RASs which result in > 10× FC to compounds listed

| NS5A RASs | DCV | LDV | OBV | EBR | VEL | PIB |
|---|---|---|---|---|---|---|
| GT1a | M28<br>**Q30**E/H/**R**<br>**L31**M/**V**<br>**Y93C**/H/**N** | K24R<br>M28 T<br>**Q30H/R**<br>**L31M**<br>**Y93H/N** | **M28A/T/V**<br>**Q30E/K/R**<br>Y93C/H/N | M28A/T<br>**Q30R**<br>**L31M**<br>Y93H/N | L31M<br>**Y93**H/**N** | None |
| GT1b | **L31I/M**<br>**Y93H** | **L31I/M/V**<br>**Y93H** | Y93H | **L31M**<br>**Y93H** | None[a] | None |
| GT3 | A30K<br>**Y93H** | N/A | N/A | N/A | A30K<br>**Y93H** | **A30K + Y93H**[b] |

RASs in bold are most frequently selected with virologic failure. BOLD = selected in > 10% of virologic failures

[a] L31M/V plus Y93H have been selected ($n$ = 2)

[b] Dual variant results in 69× FC in PIB activity

The effect of this RAS likely stems from the fact that when the A30K is present at baseline only one additional nucleotide change is required to generate the double mutant (A30K + Y93H) which results in a 70-fold increase in PIB EC50 in vitro [58]. Conversely when the Y93H is present at baseline, two additional nucleotide changes are required to generate an A30K + Y93H variant.

**Variants at Position 31** Position 31 in NS5A is polymorphic across genotypes. At baseline, L31M variants are found in 2–3% of GT1a isolates and 4–5% of GT1b isolates [24]. In vitro L31V variants confer high level resistance (> 100×) in GT1a to all earlier generation NS5A inhibitors (DCV, EBR, LDV, and OBV), moderate resistance to VEL, and no impact on PIB [18, 19, 45, 54, 55, 57]. L31M variants have a lesser and more variable impact in vitro. In GT2, the L31M is a frequent polymorphism with limited clinical impact.

Following unsuccessful treatment, L31M/V variants are often found in combination with other RASs such as Q30R/H in GT1a and Y93H in GT1b [21].

**Variants at Position 93** Variants at position 93 are the NS5A RASs of most clinical importance. In vitro the common variants (Y93C/H/N) confer high level resistance in GT1a to all NS5A inhibitors except for PIB (< 7×) [18, 45, 54, 55, 57]. In GT1b, the impact of Y93H/N variants is more variable but still results in moderate to high level resistance to most inhibitors with the exception of VEL and PIB [45, 57]. The Y93H variant in GT3 results in high level resistance to VEL (> 100×) while PIB retains near wild-type activity (< 3×) [45, 57].

Prior to drug exposure, the Y93H variant is found most frequently in GT1b (10%) and GT3 (8–10%) but are rarely present in GT1a (< 1%) [24, 50, 51]. After failure of DAA therapy, the Y93H and N variants are selected in GT1a; the Y93H variant is the most frequent RAS selected after DAA therapy in GT1b and 3 [18, 50, 51].

A host of other NS5A RASs have been described (e.g., H58D), often in combination with one of the above RASs; however, their impact on treatment response and thus clinical significance are uncertain, particularly in isolation.

## When Should You Look for NS5A RASs?

In patients not previously exposed to DAAs, there are only a few instances where testing for RASs is recommended prior to therapy. Recommendations for testing are driven by regimen, patient, and viral characteristics.

### EBR/GZR—Baseline NS5A RAS Testing in All GT1a

This regimen consisting of an NS5A inhibitor plus an NS3 PI is the only one where resistance testing is recommended in all GT1a patients regardless of treatment history or fibrosis stage [27]. In treatment-naïve GT1a patients treated with EBR/GZR for 12 weeks without RBV, SVR12 rates were 98% for those without EBR-specific NS5A RASs and 58% for this with RASs [23]. EBR-specific RASs include M28A/G/T (not V), Q30R/H, L31M/V, and Y93C/H/N/S [23, 56]. Of note, of the 438 patients sequenced, only 5% had EBR-specific NS5A RASs by population sequencing. In treatment-experienced patients, the difference in SVR12 was even larger (97 vs 29%) though the number of patients analyzed was significantly smaller. The recommendation for baseline RAS screening is further strengthened by a multivariate analysis which only identified baseline VL > 800,000 and the presence of NS5A RASs as significant predictors of non-response in GT1a patients [59]. While extension to 16 weeks with the addition of RBV is an option in patients found to harbor baseline EBR-specific RASs [56], in practice, an alternative regimen which does not require RBV and can be given for 8–12 weeks should be chosen.

Baseline RAS testing is not recommended with this regimen for any GT1b patients, regardless or prior treatment or presence of cirrhosis.

### LDV/SOF—Consideration of Baseline NS5A RAS Testing in Treatment Experienced GT1a Patients

In aggregate data across all treatment durations and without regard to RBV use, baseline LDV NS5A RASs adversely impact responses to LDV/SOF therapy in GT1a (98 vs 90% SVR12, $p < 0.001$) while a trend was observed for GT1b (98.7 vs 94.7%, $p = 0.063$) [22]. The effect was most pronounced in treatment-experienced GT1a patients without cirrhosis treated for 12 weeks without RBV (98 vs 75%) and treatment-experienced GT1a patients with cirrhosis treated for 12–24 weeks ± RBV (96 vs 77%) [24]. However, based on limited numbers in the LDV RAS groups (TE, NC $n = 16$; TE, C $n = 13$), it is impossible to draw firm conclusions.

Ledipasvir-specific NS5A RASs were found in 8% of patients at a 15% sequencing threshold [24]; given this prevalence, NS5A RASs should be screened for in treatment experienced, non-cirrhotic GT1a patients provided they can tolerate and are willing to take RBV or an alternative regimen is accessible if resistance is detected. Options for treatment-experienced patients with LDV-specific RASs include adding RBV to 12 weeks of LDV/SOF or switching to alternative therapy such as 12 weeks of SOF/VEL or 8 weeks of GLE/PIB. None of these approaches have been studied prospectively.

No signal for an impact of baseline NS5A RASs in GT1 was found in phase 3 trials with either SOF/VEL or GLE/PIB; virologic failure rates are extremely low (< 1%) for both of these regimens in GT1 [52, 60, 61]. Similarly, no impact of baseline RASs has been identified with OBV/PTV/r + DSV when RBV is included in the regimen for treatment of GT1a [62].

### SOF/VEL—Baseline NS5A RAS Testing in GT3 Interferon Treatment Experienced or Cirrhotic Patients

Sustained virologic response rates are lower in GT3 patients with baseline NS5A RASs (15% threshold) treated with SOF/VEL for 12 weeks (88 vs 97%) [63]. When limited to the Y93H variant at baseline, SVR12 drops to 84% (21/25 patients) [51]. Since guidelines already recommend the addition of RBV in GT3 treatment-experienced patients with cirrhosis, the groups recommended for baseline resistance testing to detect the Y93H are treatment experienced non-cirrhotic patients and treatment-naïve patients with cirrhosis [27]. If the Y93H variant is detected at baseline addition of weight-based RBV is recommended.

### Special Population: Treatment Experienced Patients with Prior Exposure to DAAs

Prior the approvals of SOF/VEL/VOX and GLE/PIB, resistance testing was routinely recommended prior to retreatment for all patients who had previously failed an interferon-free DAA regimen containing an NS3 PI and/or an NS5A inhibitor [27]. However, impressive new data from phase 2 and 3 trials with these regimens in this difficult to treat population suggest that resistant variants present prior to treatment have no impact on retreatment responses—with the exception being DAA failures exposed to both NS3 PI and NS5A inhibitors retreated with GLE/PIB [32, 43].

In POLARIS-1 (NS5A exposed) and 4 (no NS5A exposure) studies of previously DAA-treated patients, the prevalence of baseline NS3 or NS5A RASs was high (83% in POLARIS-1 and 49% POLARIS-4) [26]. Despite the high prevalence of resistance, no impact on SVR12 was found with 12 weeks of SOF/VEL/VOX, 97% SVR12 with RASs in POLARIS-1 and 100% SVR12 with RASs in POLARIS-4. A detailed resistance analysis did not show any effect of RASs by genotype or specific baseline RASs (e.g., Y93H) [43]. Response rates were lower in GT3, NS5A exposed patients who also had cirrhosis (93%, 52/56) [26]. While the lower response rate did not seem to be related to the presence of RASs, the addition of RBV to 12 weeks of SOF/VEL/VOX for NS5A-experienced GT3 patients with cirrhosis should be considered. For all other genotypes, DAA-exposed patients can be treated with SOF/VEL/VOX for 12 weeks. Based on current data, there is no utility in RAS testing prior to treatment of DAA failures with SOF/VEL/VOX.

Although both GLE and PIB are pangenotypic inhibitors which retain potent in vitro activity against most NS3 PI and NS5A inhibitor class RASs [45], respectively, response rates are lower when retreating patients exposed to both drug classes previously. In part 2 of the MAGELLAN-I study evaluating 12 or 16 weeks of GLE/PIB, SVR12 rates were 100% in NS3 PI only experienced patients regardless of duration [25]. Patients with only prior NS5A exposure had lower SVR12 rates, particularly with 12 weeks of GLE/PIB therapy (SVR12: 88% 12 weeks, 94% 16 weeks). Those exposed to both an NS3 PI and an NS5A inhibitor has the lowest SVR12 regardless of duration (79–81%). When assessed by the presence of baseline RASs similar trends emerged—notably a 96% SVR (22/23) with 16 weeks of GLE/PIB in those with only NS5A RASs but a low 56% SVR (5/9) in those with both NS3 and NS5A RASs (12- and 16-week durations combined) [32]. These results are reflected in the label for GLE/PIB where it is not recommended for retreatment of patients previously treated with an NS3 PI plus an NS5A inhibitor. Although based on small number, it appears clinical history alone is sufficient to determine whether GLE/PIB should be used to treat prior DAA failures; 12 weeks can be used if only

prior PI exposure, 16 weeks with prior NS5A exposure, and SOF/VEL/VOX for 12 weeks if there is prior exposure to both drug classes.

## Conclusion

The clinical impact of HCV RASs continues to evolve in step with the rapid pace of HCV drug development and new regimen approvals. Although there are now regimens for which clinical RAS testing has little utility, formulary imposed preferences and restrictions mandate a basic understanding of the impact of HCV resistance, particularly NS5A resistance. Clinicians treating HCV should be familiar with the situations where RAS testing may still impact treatment outcomes and decisions.

### Compliance with Ethical Standards

**Conflict of Interest** The author declares no conflicts of interest relevant to this manuscript.

**Human and Animal Rights and Informed Consent** This article does not contain any studies with human or animal subjects performed by any of the authors.

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