Thematic Miniseries on HCV cure

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Treatment failure with DAA therapy: Importance of resistance

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Summary

Viral resistance is a major reason for virological failure in patients being treated with direct-acting antivirals (DAAs) for chronic HCV infection. However, the importance of viral resistance mainly depends on the DAA regimen and HCV genotype. For first-line therapy with glecaprevir/pibrentasvir (G/P) or velpatasvir/sofosbuvir (VEL/SOF) no general baseline resistance analysis is required because of the high antiviral activity and high barrier to resistance. If available, resistance testing may help to optimise therapy in certain subgroups of patients with HCV genotype 3 and other rare HCV geno/subtypes. Voxilaprevir/velpatasvir/sofosbuvir (VOX/VEL/SOF) is the first choice for the second-line treatment of patients following a previous DAA failure, with rates of viral eradication above 90% irrespective of the presence of resistance-associated substitutions (RASs). However, in resource-limited settings, only first-generation DAAs may be available for second-line therapy. Here, RASs selected during initial antiviral therapy should be considered if testing is available and rescue treatment should include a switch to a regimen with a new DAA class to optimise treatment response. Patients with HCV genotype 3 are overrepresented in the group who experience DAA treatment failure. Limited data are available for third-line therapies, but promising results have been achieved with G/P plus SOF or VOX/VEL/SOF with or without ribavirin for 12 to 24 weeks; these regimens should be administered irrespective of a patient's RAS profile.

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Introduction

Chronic hepatitis C is one of the major global causes of chronic liver disease, cirrhosis, its sequela and liver cancer.[1] The causative agent, HCV, is a positive single-stranded RNA virus. A major prerequisite to survive in the presence of human innate and acquired immune reactions is a high viral turnover with an error prone viral polymerase.[2] Mathematical modelling reveals that with production of 10^{10} to 10^{12} virions per day and an error rate of 10^{-3} to 10^{-5} mutations per nucleotide per genomic replication every possible variant is generated every day.[3] As the virus has no long-lasting reservoir, ongoing replication is required for survival, which supports a number of mechanisms for circumvention of the host immune response.[4] Conversely, efficient suppression of replication will lead to viral eradication. Since 2014, combination therapies comprising different direct-acting antivirals (DAAs) that block HCV replication have been available, with sustained virologic response (SVR) rates above 95% with these therapies.[5] The reasons for virologic failure on DAA therapies are heterogeneous and include non-adherence, drug-drug interactions, insufficient drug concentrations due to intestinal malassimilation or impaired cellular uptake, for example due to cirrhosis and genetic variations in the host immune response, *e.g. IFNL3* (IL28B) polymorphisms among others.[5] However, another important reason for virologic failure on DAAs is resistance-associated substitutions (RASs) that lead to resistance-associated viral variants (RAVs), which by chance are preexisting in patients with chronic hepatitis C or have been selected by previous therapy and which confer resistance to DAAs.[6,7]

Natural prevalence and assessment of resistance

HCV sequence analysis has been performed in multiple studies to determine the natural frequency of preexisting RASs in different HCV genotypes and subtypes.[6,8,9] However, the definition of clinically relevant RASs is difficult. In phenotypic resistance assays, RASs selected in cell culture studies with administration of individual DAAs are not necessarily identical to RASs selected in patients with failure on DAA therapies. Accordingly, not all RASs detected by sequencing for genotypic resistance analysis are relevant for prediction of response to DAA therapies. Moreover, the frequency of RAVs within the HCV quasispecies may be of importance. Population-based sequencing reveals variants at a frequency of 15–20% within the HCV quasispecies, while deep sequencing can detect RASs present at a frequency of approximately 1%.[6] Currently, it seems that variants detectable below 15% within the HCV quasispecies are of minor importance for treatment response.[10] Finally, sequence analysis was only performed on

target regions of different DAA classes in most studies. Thus, the importance of potential RASs in areas outside the binding domain or the target protein are unknown. The prevalence of naturally occurring RASs is different for different HCV genotypes and subtypes, and varies geographically.[9] An overview of important features of HCV resistance analysis is provided in Fig. 1. Regarding sofosbuvir, which is the only available NS5B polymerase inhibitor, S282T was not observed as a preexisting RAS and the importance of other variants is unclear.[9,11,12] Except for Q80K which can be found in approximately 40% of HCV subtype 1a-infected patients, the natural frequency of relevant preexisting NS3 RASs conferring significant resistance to pangenotypic DAAs is below 2%. NS5A RASs conferring significant resistance to DAAs are naturally present at frequencies between 5–15% in the major HCV geno-/subtypes (1a, 1b, 2a, 2b, 3a, 4a, 4d).[6,9] In contrast, the frequencies and importance of RASs for virologic response to DAAs in rare HCV subtypes can be high.[13,14] Generally, with the current highly active pangenotypic DAA regimens velpatasvir/sofosbuvir (VEL/SOF) and glecaprevir/pibrentasvir (G/P), baseline NS3, NS5A or NS5B RASs in DAA treatment-naïve patients are of minor importance, apart from patients with HCV genotype 3 infections (see below). The importance of RASs on treatment with dual DAA regimens, such as ledipasvir/sofosbuvir, grazoprevir/elbasvir or asunaprevir/daclatasvir among others, has been previously shown.[6,7,15,16] Limited data are available regarding the importance of baseline NS5A RASs on the pangenotypic regimen daclatasvir/sofosbuvir±ribavirin. Generally, this regimen shows a high barrier to resistance but as for the other pangenotypic regimens, HCV genotype 3 seems to be overrepresented in patients experiencing virologic failure.[17,18] Given the broad use of daclatasvir in many countries, studies on the importance of resistance would be desirable.

Baseline resistance analysis for pangenotypic DAA regimens

While no general sequencing is required in subgroups of patients infected with HCV genotype 3, baseline resistance analysis may be useful to optimise the selection of DAA regimens for treatment-naïve patients.

VEL/SOF and genotype 3

In phase III approval studies for VEL/SOF, baseline NS5A RASs were not associated with virologic treatment outcome in patients infected with HCV genotypes 1, 2, 4-6. For HCV genotype 3, SVR rates with and without VEL-specific RASs were 93% and 98%, respectively. The frequency of NS5A RASs (15% cut-off by deep sequencing) specific for VEL was 12% overall and 5% for Y93H, respectively.[19] In addition, in the approval study, SVR rates in genotype 3-infected patients with and without compensated cirrhosis were 91% and 97%, respectively.[20] Analysis of all HCV genotype 3-infected patients with cirrhosis enrolled in phase II and III clinical trials showed a difference between patients with and without baseline NS5A RASs, with SVR rates of 80% and 97%, respectively (60% for Y93H) (Fig. 2). The potential additional importance of a prior treatment failure in these patients is not well defined.[21] *In vitro* assays showed low to medium level resistance of A30H/K, L31F/M, H58G and high-level resistance of Y93H/S to VEL.[22] A subsequent randomised controlled trial evaluated whether the addition of ribavirin improved the efficacy of this regimen in HCV genotype 3-infected patients with cirrhosis.[23] Ribavirin is known as a non-specific weak antiviral for the treatment of hepatitis C which has been shown to increase SVR rates slightly when given in addition to interferon-free DAA regimens.[24–26] In one randomised study the addition of ribavirin was associated with lower relapse rates and higher SVR rates both overall and in a subgroup of patients with NS5A RASs (Fig. 2).

Key point

Baseline resistance analysis is not generally recommended for the pangenotypic DAA regimens VEL/SOF or G/P.

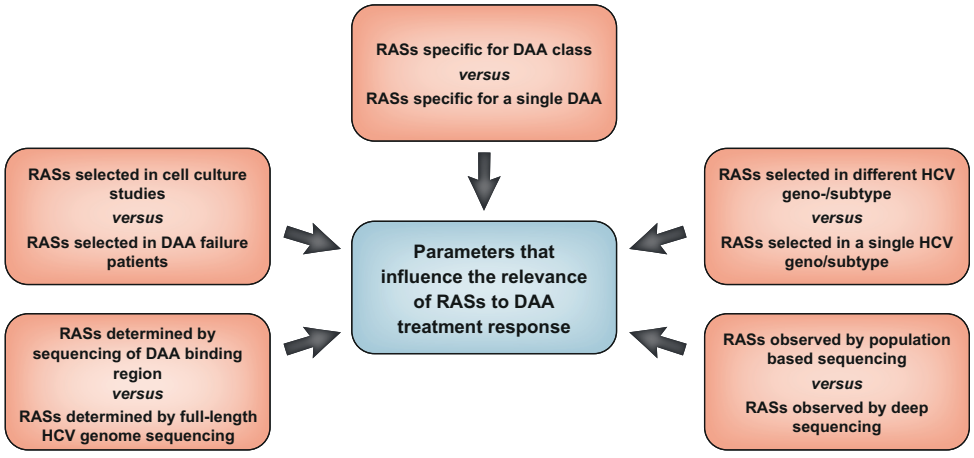

Fig. 1. Important aspects of HCV resistance analysis. Potential parameters which influence importance/detection of RASs for virologic response to DAA therapies. DAA, direct-acting antiviral; RASs, resistance-associated substitutions.

Key point

For certain HCV genotype 3 subtypes, RASs are associated with reduced SVR rates on VEL/SOF or G/P.

Differences were not statistically significant owing to the relatively small number of patients enrolled in the study. Therefore, in experienced centres, baseline resistance analysis can be performed in treatment-naïve and -experienced patients with HCV genotype 3 and compensated cirrhosis. If RASs are detected, standard dose ribavirin (1,000/1,200 mg according to body weight) should be added to treatment with VEL/SOF (Table 1).

G/P and genotype 3

Early studies for G/P in HCV genotype 3 revealed higher relapse and lower SVR rates in comparison to other HCV genotypes, especially for treatment-experienced patients.[27] Therefore, initially only 8 weeks of G/P was approved for treatment-naïve patients with HCV genotype 3 without cirrhosis.[28] Baseline RASs within NS3 or NS5A in HCV genotype 1, 2, 4-6 were not significantly associated with virologic treatment failure.[29,30] However, for HCV genotype 3, A30K was associated with differences in treatment responses in treatment-naïve patients without cirrhosis treated for 8 and 12 weeks, although this variant does not seem to confer resistance to pibrentasvir *in vitro* (Fig. 3).[29] In a pooled resistance analysis of phase II/III studies, baseline A30K was associated with differences in SVR rates in treatment-naïve patients receiving 8-week therapy (78% *vs.* 99%, respectively) and treatment-experienced (interferon-alfa) patients receiving 12-week therapy (25% *vs.* 96%, respectively).[29,30] These differences disappeared with longer treatment durations of 12 weeks in treatment-naïve and 16 weeks in treatment-experienced patients. However, the number of patients treated for 16 weeks was low and real-world experiences are very limited. As for treatment-experienced patients with HCV genotype 3 the approved treatment duration is 16 weeks, though alternative treatment options may be preferred and no resistance analysis is recommended in this situation. However, for treatment-naïve patients with HCV genotype 3 without cirrhosis baseline resistance analysis may be considered prior to the standard 8-week regimen. A30K naturally occurs at a frequency of approximately 6%.[29] If A30K is detected, slightly lower SVR rates are to be expected and G/P may not be the first choice. No data are available on the potential efficacy of G/P plus RBV for 8 weeks in patients with A30K and the longer duration of 12 weeks is not approved and may lead to reimbursement issues. Thus, baseline resistance analysis in treatment-naïve patients with HCV genotype 3 without cirrhosis may only be used in settings with common access to HCV sequencing to select an optimal alternative treatment regimen and to avoid rescue therapy, which is also associated with higher failure rates in HCV genotype 3.[31] While baseline Y93H is the major RAS associated with treatment failure on VEL/SOF, A30K is for G/P. A combination of a dual A30K/Y93H mutation is associated with failure on both therapies but is an exceedingly rare event (Table 1).[19,29]

More recently, 8 weeks treatment with G/P was also approved for treatment-naïve patients with compensated cirrhosis.[32] In 63 patients with HCV genotype 3 only 1 virologic treatment failure was observed, and this patient had no baseline RASs within NS3 or NS5A. Thus, data are currently insufficient to assess the importance of A30K or other RASs in treatment-naïve HCV genotype 3-infected patients with compensated cirrhosis treated for 8 weeks.

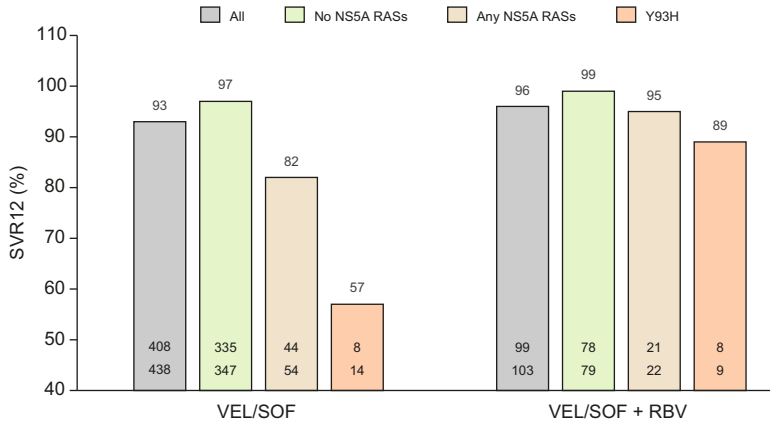

Fig. 2. VEL/SOF±RBV in HCV genotype 3-infected patients with compensated cirrhosis. Comparison of SVR rates following treatment with VEL/SOF *vs.* VEL/SOF+RBV for 12 weeks in all patients, patients without NS5A RASs, any NS5A RASs and Y93H as the most relevant NS5A RAS for VEL. For VEL/SOF, SVR rates in HCV genotype 3-infected patients with compensated cirrhosis were obtained from an integrated analysis of phase II/III approval studies[21] and the VEL/SOF arm of the controlled randomised Spanish study for comparison with VEL/SOF+RBV.[23] For VEL/SOF+RBV, data are shown from the Spanish study only, as these are the only available data from a controlled randomised trial.[23] RASs, resistance-associated substitutions; RBV, ribavirin; SOF, sofosbuvir; SVR, sustained virologic response; VEL, velpatasvir.

Table 1. Importance of RASs for pangenotypic regimens in DAA-naïve HCV genotype 3-infected patients.

HCV genotype 3-infected patients				
Regimen	**Subgroup**	**RAS with reduced SVR**	**Natural RAS prevalence**	**Alternative regimen**
VEL/SOF 12 weeks	Compensated cirrhosis	Y93H	Approx. 5%	VEL/SOF+RBV 12 weeks G/P 8/16 weeks
G/P 8 weeks.	Treatment-naïve, without (with?) cirrhosis	A30K	Approx. 6%	VEL/SOF 12 weeks

Selection of RASs after failure on first-line DAA treatment

Pattern of RASs in DAA failures

In most patients with virologic failure on DAA regimens, RASs are detectable within the target regions of the respective DAAs. Diverse patterns of resistance to NS3, NS5A and NS5B inhibitors have been characterised based on data from approval studies and real-world studies with larger cohorts of patients with virologic treatment failure (Table 2).[16,19,25,29,33–38]

Overall, RAS patterns are partially overlapping for the different NS3 and NS5A inhibitors. However, there are also differences with non-overlapping resistance profiles which may be important for selection of rescue DAA regimens. For example, the first- and second-generation NS3 protease inhibitors (PIs) simeprevir, paritaprevir/ritonavir, grazoprevir *vs.* glecaprevir, voxilaprevir showed different RAS profiles (Table 2). For NS5A inhibitors, RAS profiles are remarkably similar for HCV genotype 1b, but some differences are observed for other HCV geno-/subtypes (Table 2). For dasabuvir, as the only non-nucleoside NS5B polymerase inhibitor, a single variant (S556G) was most commonly selected in HCV genotype 1a/b (Table 2). For sofosbuvir, as a nucleotide NS5A polymerase inhibitor, S282T was selected very rarely, mainly in HCV genotypes 1, 3 and 4. The importance of selection of other variants so far is unclear (Table 2). Moreover, for rare HCV genotypes/subtypes, for HCV genotypes 5 and 6, and for pangenotypic regimens and HCV genotype 2, no or only few valid data are available as no larger cohorts of virologic failure patients have been reported to date (Table 2). Finally, for voxilaprevir/velpatasvir/sofosbuvir (VOX/VEL/SOF), as a regimen targeting 3 HCV proteins with a high genetic barrier to resistance in the majority of virologic failure patients, no RASs have been selected (Table 2). Resistance levels of single RASs are highly variable for the different NS3 and NS5A inhibitors. Pangenotypic DAA regimens generally display higher barriers to resistance. However, several combined NS5A RASs and the P32del variant which was observed mainly in HCV genotype 1b-infected DAA failure patients from Asia also confer high levels of resistance to G/P and VEL/SOF.[39–41]

Key point

Rescue treatment with multiple targeted therapies (VOX/VEL/SOF) is the first choice for failures on first-generation DAAs or G/P.

Persistence of RASs

RASs selected during administration of NS3 PIs and the NS5B inhibitor sofosbuvir will disappear relative rapidly (within weeks to months) during follow-up depending on HCV geno-/subtype and based on population sequencing.[6,7] During long-term follow-up for several years no or only minor NS3 RASs for different PIs were observed.[42–44] An exception is the NS3 variant Q80K in HCV subtype 1a-infected patients, which typically persists during long-term follow-up.[45] In contrast, RASs selected on treatment with NS5A inhibitors seem to persist for more than 2 years in >80% of patients.[46] However, some changes within the HCV quasispecies have been observed by deep sequencing. Also, during long-term follow-up (for

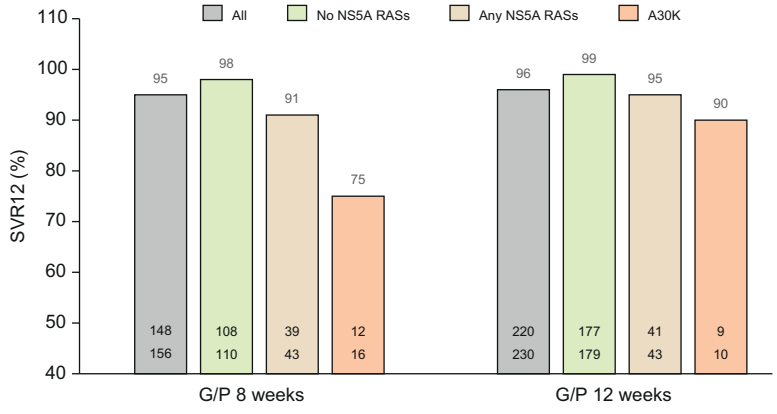

Fig. 3. G/P in treatment-naïve HCV genotype 3-infected patients without cirrhosis. Comparison of SVR rates for treatment with G/P for 8 *vs.* 12 weeks in all patients, patients without NS5A RASs, any NS5A RASs and A30K as the most relevant NS5A RAS for pibrentasvir. Data are obtained from supplementary material to the publication of the approval study.[28] G/P, glecaprevir/pibrentasvir; RASs, resistance-associated substitutions; SVR, sustained virologic response.

Table 2. Typical resistance pattern in patients with virologic failure on DAAs.

Failure on	GT1a	GT1b	GT2	GT3	GT4	GT5	GT6
NS3 inhibitors							
SMV	R155K D168E	D168V	n.a.	n.a.	Q80R, D168E	n.a.	n.a.
PTVr	R155K D168V	Y56H D168V	n.a.	n.a.	Y56H D168V	n.a.	n.a.
GZR	Q80K A156V/T D168A/E	A156G/T D168A/E/G/V	n.a.	n.a.	A156T	n.a.	n.a.
GLE	A156V	Y56F Q80L S122G	no RASs	Y56H Q80R/K A156G Q168L/R	no data	no data	no data
VOX	Q80K	no RASs	no RASs	no RASs	A156S	no RASs	no data
NS5A inhibitors							
DAC	Q30H/R L31M	L31M Y93H	no data	Y93H	L28M L30R Y93C/H	no data	no data
LDV	Q30H/R L31M	L31M Y93H	n.a.	no RASs	L28M Y93C/H	n.a.	n.a.
OMV	M28T/V Q30R	Y93H	n.a.	n.a.	L28V L30R M31L/V Y93H	n.a.	n.a.
EBV	M28T Q30H/R L31M Y93C/H	R30H L31F/M/V Y93H	n.a.	n.a.	L30R M31L/V	n.a.	n.a.
VEL	Q30R/H L31M Y93N/H	L31M Y93H	L31M	A30K L31M Y93H	L28M/V L30R M31L/V Y93C/H	no data	T58S
PIB	Q30R L31M H58D Y93N	L31M/F P32del Y93H	F28C L31M	M28G A30K/G L31F/M Y93H	no data	no data	no data
NonNUC inhibitors							
DSV	S556G	S556G	n.a.	n.a.	n.a.	n.a.	n.a.
NUC inhibitor							
SOF	S282T	L159F C316N S282T	no data	L159F S282T	S282T	no data	no data

Data from DAA combination therapies (SMV/SOF, PTVr/OMV±DSV, GZR/EBR, GLE/PIB, DAC/SOF, LDV/SOF, VEL/SOF, VOX/VEL/SOF with and without RBV. Only variants selected in the majority of patients with virologic treatment failure are shown as presented in different studies and from own unpublished data of the European Resistance Data bank. Other RASs also have been detected in individual patients. For HCV genotypes 2-6 RASs selected depend substantially on HCV subtypes.[16,19,25,29,33–38] DAA, direct-acting antiviral; DAC, daclatasvir; DSV, dasabuvir; EBV, elbasvir; GLE, glecaprevir; GT, genotype; GZR, grazoprevir; LDV, ledipasvir; Non-NUC, non-nucleosidic NS5B polymerase inhibitor; NUC, nucleotide NS5B polymerase inhibitor; OMV, ombitasvir; PIB, pibrentasvir; PTVr, paritaprevir/ritonavir; RASs, resistance-associated substitutions; RBV, ribavirin; SMV, simeprevir; SOF, sofosbuvir; VEL, velpatasvir; VOX, voxilaprevir. n.a., not applicable as no (general) use of respective DAAs in these HCV genotypes.

Key point

There is no evidence that known RASs impact on rescue treatment with multiple targeted therapies (VOX/VEL/SOF).

up to 4 years) a decline in several NS5A RASs has been reported, depending on the NS5A inhibitor and HCV genotype.[6,7,45]

Reselection of RASs during repeated DAA therapy

For HIV infection it is well known that RASs selected during direct-antiviral therapy can remain in the quasispecies below the limit of detection of sequencing assays. During retreatment, these variants then reappear very rapidly leading to immediate development of resistance. For HCV, few studies – with contradictory results – have investigated the persistence and reselection of RASs after DAA treatment. In several patients, persistence and reselection of NS3 RASs was observed during sequential treatment with a PI at short-term intervals.[47] However, in patients who were re-exposed to a NS3 PI after more than 1 year, there was no clear evidence of persistence but *de novo* generation of resistance was observed (by deep sequencing) in most patients.[48,49] As NS5A RASs typically persist at high frequencies and selection of RASs on treatment with sofosbuvir is rare, no comparable data are available for NS5A or NS5B RASs.

Importance of RASs for rescue treatment

A limited number of studies on retreatment after DAA failure have been published. Therefore, evidence for recommendations on suitable retreatment options is limited (Fig. 4).

Retreatment with first-generation DAAs

Retreatment with first-generation DAAs is the only option in many healthcare settings globally; thus, data on the importance of RASs and other

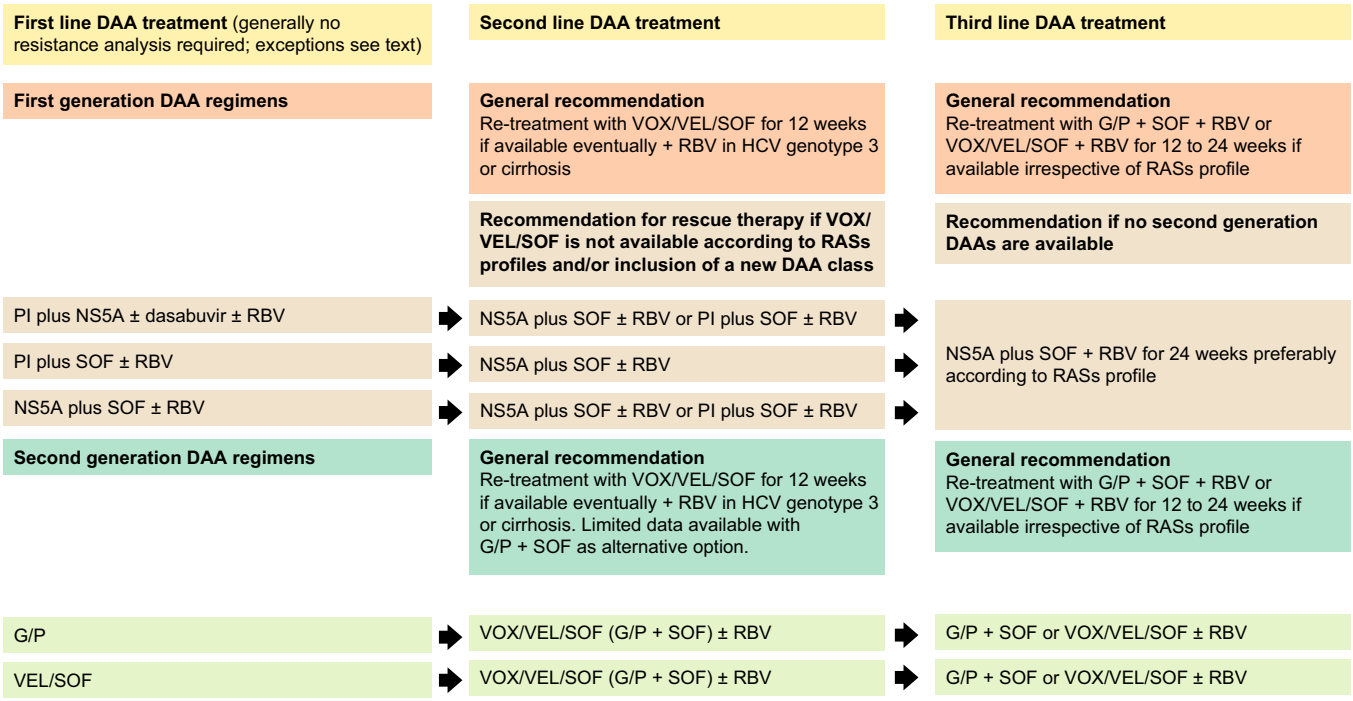

Fig. 4. General concepts and recommendations for retreatment in patients with virologic failure on interferon-free DAA combination therapies. Recommendations according to pretreatment with first- or second-generation DAA regimens and according to availability of first- or second-generation DAA regimens for first- and second-line retreatment. Regarding combinations of a PI together with SOF as rescue treatment, data are only available for simeprevir (which is no longer available) while no data exist for other PIs like Narlaprevir or asunaprevirDAA, direct-acting antiviral; G/P, glecaprevir/pibrentasvir; RASs, resistance-associated substitutions; RBV, ribavirin; SOF, sofosbuvir; SVR, sustained virologic response; VEL, velpatasvir; VOX, voxilaprevir.

predictors for the selection of DAA regimens and the duration of rescue treatment is of importance. Moreover, ribavirin was shown to increase SVR rates in many DAA studies and thus should generally be considered for rescue treatment with first-generation DAAs. However, epidemiological, clinical and virologic characteristics of published cohorts are highly heterogeneous, and no prospective evaluation of retreatment strategies is available. After failure on a combination regimen with first-generation DAAs, RASs selected during the previous DAA therapy are observed in most patients and these RASs seem to influence virologic treatment outcome. For example, retreatment with an identical regimen is generally not recommended, as high rates of virologic failure have been reported.[50] Thus, RAS testing may be helpful to tailor salvage therapy. However, RAS testing is often not available or affordable. In this setting, as a general rule, a new DAA class (NS3-, NS5A-, NS5B-inhibitor) should be introduced for rescue treatment to optimise treatment response if possible.

Failure on PI plus NS5A inhibitor

In patients with virologic failure on a first-generation PI (simeprevir, asunaprevir, grazoprevir, paritaprevir) in combination with a first-generation NS5A inhibitor (daclatasvir, ombitasvir, elbasvir) typically drug-specific NS3 and NS5A RAS patterns are detectable.[33] These regimens have mainly been administered to patients with HCV genotype 1 and less frequently to those with genotype 4 infections. In patients with HCV genotype 1b infection and previous failure on asunaprevir/daclatasvir, retreatment with sofosbuvir plus ledipasvir led to SVR rates of 64–70% in a study from Japan.[51] Rescue therapy with sofosbuvir plus velpatasvir was shown to be associated with equally high SVR rates in a small cohort; the presence of the P32del NS5A variant seems to be associated with reduced efficacy.[41,52] Similarly, sofosbuvir plus an NS5A inhibitor was reported to be highly effective for the retreatment of patients who failed on paritaprevir/ombitasvir plus dasabuvir (3D) with or without ribavirin.[53] Therefore, although no systematic study data are available, switching to a nucleotide inhibitor (sofosbuvir) in combination with a NS5A inhibitor (ideally pangenotypic) seems to be highly effective for the retreatment of patients after virologic failure on a PI plus NS5A inhibitor (with or without a non-nucleosidic NS5B polymerase inhibitor [dasabuvir]). Ribavirin may be administered in addition and treatment duration may be extended from 12 to 24 weeks in difficult to treat patients. A high range of SVR rates between 60% and 100% were

reported with this approach; the presence of NS5A RASs may be associated with reduced SVR rates (Fig. 4).

Failure on PI plus sofosbuvir

After virologic treatment failure on a PI (mainly simeprevir) in combination with sofosbuvir as a first-line DAA therapy, NS3 RASs are typically detectable in most patients. Data on rescue treatment for these mainly HCV genotype 1-infected patients are scarce, but high SVR rates (75–100%) have been reported, irrespective of the existence of RASs, following a switch to a NS5A inhibitor (ledipasvir, daclatasvir, velpatasvir) in combination with sofosbuvir. The addition of ribavirin does not seem to be required for further efficacy in this setting.[53,54] In addition, especially in HCV genotype 1b-infected patients, the 3D regimen (paritaprevir/ombitasvir plus dasabuvir) was highly effective in a small number of patients.[53] Thus, in patients not exposed to NS5A inhibitors, retreatment together with sofosbuvir seems to be highly effective and can be recommended for rescue treatment in resource-limited settings.

Failure on NS5A inhibitor plus sofosbuvir

Retreatment of patients with virologic failure on a NS5A inhibitor (ledipasvir, daclatasvir, velpatasvir) in combination with sofosbuvir is more challenging. Data are mainly available for HCV genotype 1- and 3-infected patients. For HCV genotype 1, retreatment with the same or another NS5A inhibitor plus sofosbuvir was associated with SVR rates ranging from 25% to 100%. Significant differences between patients with (33-67%) and without NS5A RASs (88%) have been observed.[18] A switch to other regimens led to variable SVR rates of 89–92% for simeprevir/sofosbuvir with or without ribavirin, 67% for grazoprevir/elbasvir and 60–88% for the 3D regimen. Generally, SVR rates were higher in HCV genotype 1b *vs.* 1a infected patients and the addition of ribavirin seems to increase efficacy further.[18]

For patients infected with HCV genotype 3, no switch to a new DAA class is possible if only first-generation DAAs are available. In such cases, retreatment of daclatasvir/sofosbuvir failures with the same or another NS5A inhibitor for durations between 12 and 36 weeks led to an overall SVR rate of only 60%. The combination of velpatasvir/sofosbuvir was most effective (50–86% SVR). However, the presence of cirrhosis and Y93H as a major NS5A RAS was associated with reduced efficacy (50% and 56%, respectively).[18] Based on data from a large cohort, resistance analysis was recommended as an efficacious way to guide rescue therapy in patients with NS5A inhibitor/sofosbuvir failures. In patients without NS5A RASs, combination therapy with a NS5A inhibitor plus sofosbuvir with ribavirin led to SVR rates of approximately 90%. For HCV genotype 3, velpatasvir/sofosbuvir plus ribavirin for 24 weeks can be recommended, with similar efficacy if only Y93H is observed.[55] Generally, the addition of ribavirin is also recommended for rescue treatment in genotype 3-infected patients as it seems to enhance efficacy.

However, because patients without NS5A resistance are rare after virologic failure on NS5A inhibitors, and the efficacy of NS5A inhibitor-free regimens is limited, multiple targeted DAA regimens are recommended for rescue treatment in most patients (see below).

Key point

If multiple targeted therapies (VOX/VEL/SOF) are not available or affordable rescue treatment with first-generation DAAs is possible with reduced efficacy. In such cases, resistance analysis should be performed if available to tailor salvage therapy. As general rule a switch to a regimen including a new DAA class is of importance to optimise the efficacy of retreatment.

Retreatment with multiple targeted therapies

Inhibition of the NS3 protease, the NS5A replicase and the NS5B polymerase with DAAs following virologic failure represents a promising retreatment approach, as viral escape may become impossible – despite the selection of highly resistant RASs during initial antiviral therapy – because of the broad antiviral targeting potential. Studies have tested the combinations of different first- and second-generation PIs, NS5A inhibitors and sofosbuvir.[56,57] So far, only the combination of the second-generation pangenotypic PI voxilaprevir, with the pangenotypic NS5A inhibitor velpatasvir and sofosbuvir (VOX/VEL/SOF) has been approved for retreatment of patients following DAA failure.

Retreatment with VOX/VEL/SOF

In the approval studies, patients with and without previous exposure to a NS5A inhibitor were enrolled.[31] Retreatment of patients without

Table 3. SVR rates after VOX/VEL/SOF with and without ribavirin as rescue treatment after DAA failure.

Study	Rescue treatment	Patients, n	Cirrhosis (%)	SVR all	SVR cirrhosis	SVR HCV GT3a	SVR VEL/SOF failure
Bourliere *et al.*	VOX/VEL/SOF	263	46%	96%	93%	95%	no data
Degasperi *et al.*	VOX/VEL/SOF±R	169	44%	96%	91%	92%	94%
Llaneras *et al.*	VOX/VEL/SOF	135	34%	95%	89%	80%	no data
Belperio *et al.*	VOX/VEL/SOF	551	35%	91%	90%	91%	83%
Papaluca *et al.*	VOX/VEL/SOF	91	78%	90%	90%	89%	90%
Vermehren *et al.*	VOX/VEL/SOF±R	102	27%	98%	100%	100%	100%
Smith *et al.*	VOX/VEL/SOF	144	40%	91%	81%	81%	74%
Janjua *et al.*	VOX/VEL/SOF±R	191	15%	95%	88%	92%	89%
Flamm *et al.*	VOX/VEL/SOF	176	79%	94%	no data	no data	95%

DAA, direct-acting antiviral; R, ribavirin; SVR, sustained virologic response; VOX/VEL/SOF, voxilaprevir/velpatasvir/sofosbuvir. SVR data from Bourliere *et al.* based on intention to treat analysis.[31,60–67]

previous administration of NS5A inhibitors led to SVR rates of 90% and 98% after VEL/SOF or VOX/VEL/SOF. The approval study for VEL/SOF, also included HCV genotype 1-infected patients with previous exposure to NS3 PIs in combination with peg-interferon-alfa and ribavirin.[58] Interestingly, for this group, the SVR rate after 12 weeks VEL/SOF in the VEL/SOF approval study was 100% in contrast to 91% after VEL/SOF in the DAA retreatment study.[31,58] A potential explanation for this difference may be the previous exposure to sofosbuvir in all patients in the DAA retreatment study, while in the VEL/SOF approval studies neither nucleotide inhibitors nor NS5A inhibitors were allowed as previous therapy.

In patients who were previously exposed to a NS5A inhibitor, rescue treatment with VOX/VEL/SOF yielded an overall SVR rate of 96%.[31] Virologic treatment failure was observed in 7 patients, of whom 4 were infected with HCV genotype 3a. HCV resistance patterns assessed by deep sequencing were typically identical before and after VOX/VEL/SOF rescue treatment. The frequency of RASs in the HCV quasispecies increased in 2 patients and new NS5A RASs were selected after VOX/VEL/SOF failure in only 2 patients.[59]

In the real-world experience with VOX/VEL/SOF, with and without ribavirin, SVR rates of between 91–96% were reported following rescue treatment in cohorts from different countries.[60–67] Although the different studies are highly heterogeneous with respect to distribution of HCV genotypes and rate of patients with cirrhosis, based on larger numbers of patients it seems that HCV genotype 3a and cirrhosis are weak negative predictors of response, with SVR rates of 81–92% and 81–90%, respectively. Also, pretreatment with VEL/SOF was associated with lower SVR rates in some studies, while this was not observed in others (Table 3). Furthermore, SVR rates in patients with active or previous liver cancer were highly variable in the different studies. Finally, it was also shown that in patients with end-stage renal disease, as well as those with decompensated cirrhosis, administration of sofosbuvir and voxilaprevir, respectively, was safe.[63,68] Due to the potential liver toxicity of PIs in patients with decompensated cirrhosis, a lead-in phase with SOF plus a NS5A inhibitor may be considered to downstage patients to Child-Pugh class A cirrhosis before starting VOX/VEL/SOF.[63]

Patients with failure on G/P were not enrolled in the VOX/VEL/SOF approval study. However, rescue treatment with VOX/VEL/SOF for 12 weeks seems to be highly effective, as shown in a small study in 31 HCV genotype 1- or 3-infected patients, which reported an overall SVR rate of 94% (29/31).[69] Alternatively, G/P plus sofosbuvir and ribavirin may be an effective retreatment option; an SVR rate of 96% (22/23) was reported in a small clinical study with treatment durations of 12 or 16 weeks.[70]

Similar to data from the approval study, no single or combined RASs, including the NS5A P32del variant which confers high level resistance to all NS5A inhibitors, were predictive of treatment response to VOX/VEL/SOF.[39,41]

Taken together, VOX/VEL/SOF for 12 weeks represents a standard treatment for patients with failure on previous DAA regimens, including G/P. SVR rates of around 90% can be expected. The addition of ribavirin may be considered in difficult to treat patients with HCV genotype 3a, cirrhosis or liver cancer.

Retreatment after VOX/VEL/SOF failure as rescue treatment

Key point

G/P plus SOF or VOX/VEL/SOF with or without RBV for 12 to 24 weeks are options for the retreatment of VOX/VEL/SOF failures.

So far only case reports are published on retreatment in patients with failure on VOX/VEL/SOF rescue therapy, with the exception of 1 larger analysis. In this study, a cohort of 40 VOX/VEL/SOF failure patients were collected in 3 European countries for resistance analysis. Retrospective data on retreatment efficacy was included in a subgroup of patients.[37] As in previous studies, resistance analysis within NS3, NS5A and NS5B genes before and after VOX/VEL/SOF failure showed only minor changes in RAS frequencies and profiles.[37,59] In line with data on characteristics of VOX/VEL/SOF failures, most patients were infected with HCV genotype 3 (45%) and had cirrhosis (70%). Rescue treatments included G/P+SOF with or without ribavirin (n = 15), G/P alone (n = 2), VOX/VEL/SOF with or without ribavirin (n = 4) and VEL/SOF plus ribavirin (n = 1), all for 12 to 24 weeks. SVR was reported in 17/21 patients (81%). Two patients who died of decompensated liver disease and 2 virologic failures (1 each after VEL/SOF plus ribavirin and G/P plus SOF and ribavirin) were reported. No clear association of NS5A RASs with failure on retreatment after VOX/VEL/SOF was observed in this small cohort of patients. Taken together, repetition of multiple targeted therapies with second-generation DAAs like VOX/VEL/SOF or G/P+SOF with or without ribavirin for 12 to 24 weeks is effective after virologic treatment failure on VOX/VEL/SOF rescue treatment. However, more data on baseline characteristics and RAS profiles are required before more specific recommendations can be made for this difficult to treat subgroup of patients (Fig. 4).

Conclusions

Virologic resistance is a major cause of failure on direct antiviral therapy. Viral variants, particularly in the NS5A protein, occur naturally and are found in most patients with failure on DAA therapy. For pangenotypic regimens in first-line therapy, RASs play only a minor role. Only in HCV genotype 3a does NS5A resistance lead to lower virologic response rates. This is particularly true for Y93H and VEL/SOF in patients with cirrhosis, and A30K

and G/P. For patients after virologic failure on any DAA therapy, a pangenotypic, multiple targeted regimen (VOX/VEL/SOF) is recommended as second-line treatment. In resource limited settings where only first-generation DAA regimens are available, a new antiviral target should be introduced together with sofosbuvir and eventually ribavirin. In addition, treatment duration should be extended. Few data are available for third-line therapy after failure of VOX/VEL/SOF. However, rescue treatment with VOX/VEL/SOF or G/P/SOF with ribavirin seems to be associated with high SVR rates even in this situation.

Abbreviations

DAA, direct-acting antiviral; G/P, glecaprevir/pibrentasvir; PI, protease inhibitor; RASs, resistance-associated substitutions; RAVs, resistance-associated viral variants; SOF, sofosbuvir; VEL, velpatasvir; VOX, voxilaprevir.

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Conflict of interest

Advisory Committees: Abbvie, Gilead, Merck/MSD,. Grant/Research Support: Abbvie, Gilead, MSD. Speaking and Teaching: Abbvie, Gilead, Merck/MSD.

Please refer to the accompanying ICMJE disclosure forms for further details.

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Supplementary data

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