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Review

Consensus recommendations for resistance testing in the management of chronic hepatitis C virus infection: Public Health England HCV Resistance Group

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SUMMARY

The treatment of hepatitis C virus (HCV) infection has been revolutionised by the advent of oral, well-tolerated, direct acting antiviral therapies (DAA), with high cure rates. However, in some scenarios, HCV resistance to antiviral therapies may have an impact on treatment success. Public Health England's HCV Resistance Group was established to support clinicians treating people with HCV, where the issue of resistance may be a factor in clinical decision-making, and this review includes the Group's current recommendations on the use of HCV resistance testing. The authors describe the principles behind and approach to HCV resistance testing and consider evidence from *in vitro* studies, clinical trials and real world cohorts on the impact of HCV resistance on treatment outcomes for particular DAA regimens. Five scenarios are identified in the UK and similar settings, where, in the Group's opinion, resistance testing should be performed.

Introduction to HCV resistance testing

Background

The management of hepatitis C virus (HCV) infection has been revolutionised by the advent of direct-acting antiviral (DAA) therapies. These all oral, well-tolerated therapies typically result in sustained virological response (SVR) rates of >95%. SVR is usually assessed at 12 weeks after cessation of antiviral therapy and is referred to as 'SVR12'. DAA regimens may exert differential activity according to the viral genotype and subtype and the presence of viral drug resistance, and an understanding of the principles of resistance testing is therefore required.

This review aims to support clinicians treating people with HCV in settings where the issue of resistance may be a factor in clinical-decision making. Where guidance is presented, this represents the opinion of the UK's Public Health England (PHE) HCV Resistance Group, a panel of experts who have considered data from *in vitro* studies, phase II and III clinical trials and real world studies. In general, data supporting the use of resistance testing have been

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included where the presence of resistance may result in increased virological failure with an SVR12 rate of less than 95%.

The Group also recognises the increasing availability of second-generation DAA regimens, which exert activity across major HCV genotypes and may retain high antiviral potency in the presence of viral resistance-associated substitutions (RAS). The use of these newer regimens underpins a treatment strategy that may remove the requirement for viral genotype determination and resistance testing, thereby permitting treatment scale up.[1] However, in many settings, including England, restrictions are currently imposed on the choice of DAA combinations by national organisations responsible for implementing HCV treatment programs, and scenarios in which pre-therapy RAS testing may be appropriate are therefore still common.

It is emphasised that this review does not seek to provide an overarching strategy for HCV elimination or to recommend the use of one DAA regimen over another. Clinicians wishing to access resources on these topics are advised to consult treatment guidelines[1,2] or policy documents available from other sources.[3]

Mechanisms of viral resistance

HCV virology

HCV is an enveloped, single-stranded, positive-sense RNA virus of the family Flaviviridae and has a genome length of approximately 9.6 kilobases. As a consequence of the low fidelity of the viral RNA-dependent RNA polymerase (RdRp), multiple errors are introduced into progeny viruses during virus RNA replication. The high error rate, in conjunction with a high turnover and progeny number (10^{12} virions/day),[4,5] results in a large number of viral variants, harbouring different mutations, which coexist within the same host. This group of genetically-related viruses is commonly termed a quasispecies.

Some of these nucleotide substitutions result in amino acid variants that confer a selective replication advantage in the presence of antiviral drug pressure. Amino acid substitutions thus may change the susceptibility of the virus to one or more drugs and, in this context, the variant amino acid is referred to as a RAS. Such RAS-harbouring variants are preferentially selected for, or enriched from baseline, following unsuccessful DAA therapy.

Antiviral resistance

Currently-available DAA inhibit one of three virally-encoded proteins: the NS3/4A protease complex, the NS5A protein (required for viral replication and assembly) and the NS5B RdRp. Of note, NS5B inhibitors may exert their effect either as competitive nucleotide analogues (NA) or as nonnucleoside allosteric (non-NA) inhibitors. HCV exhibits a high tolerance for RAS in the NS3 and NS5A genes, which can be accommodated without loss of replicative capacity (fitness). These RAS are therefore common in DAA-naïve populations, with a prevalence of up to 50% and 15% respectively, as detected by population sequencing.[6,7] Non-NA NS5B RAS have also been reported in up to 30% of DAA-naïve individuals.[8] By contrast, NA NS5B RAS are rarely observed (1–3%), probably reflecting the loss of fitness which they impart. In particular, nucleotide substitutions in the highly conserved active site of RdRp may effectively halt viral replication.[9]

Of the three drug classes, resistance to NS5A inhibitors is clinically the most important and prevalence in DAA-exposed populations may approach 100%.[10] This reflects the substantial impact of NS5A RAS on drug susceptibility, the high fitness of NS5A RAS-bearing variants, and the ability of such variants to persist for years even in the absence of drug pressure.[11] NS3 RAS may emerge during therapy with NS3 protease inhibitors, but tend to become undetectable within months of stopping NS3 inhibitor-containing therapy.[12] Non-NA NS5B RAS also emerge frequently after unsuccessful DAA therapies, but are not clinically significant, as the only drug in this class is not indicated in DAA-exposed individuals.[8] By contrast, variants harbouring NA NS5B RAS rarely emerge following exposure to an NS5B inhibitor (1% of virologic failures) and are quickly replaced by fitter, wild-type virus.[9]

RAS are typically divided into those that are drug-specific (conferring reduced susceptibility to one particular antiviral agent) and those that are class-specific (conferring reduced susceptibility to $\geq$2 agents in the same class although not necessarily reducing susceptibility to all drugs of that class).

Recommended types of resistance test

Two types of tests predict susceptibility to antiviral drugs based on an analysis of the viral genotype or phenotype (Fig. 1). Genotypic susceptibility testing is recommended as it is fast and widely available in routine diagnostic laboratories (Box 1). Both Sanger (direct) sequencing and next generation sequencing (NGS) may be used. Where NGS is used, a frequency cut-off of 15% is recommended for interpretation, as RAS present at lower frequency are unlikely to impact on SVR.[1,2,6] For either platform, it is critical that results are made available in a timely fashion and do not delay treatment initiation. Phenotypic resistance testing is laborious and complicated, and currently only available in research settings.

Box 1. General recommendations on the use of sequencing in the management of people with HCV

Types of resistance test
Where resistance testing is performed, Sanger sequencing or next generation sequencing (NGS) methods are recommended.

Timing of resistance tests
Where resistance testing is performed prior to re-treatment in individuals previously exposed to DAA, this should be done on a sample taken as close as possible to the planned re-treatment start date.

Methods of genotype assignment
Genotyping should be performed with Sanger sequencing of NS5B or core, or by whole genome sequencing with NGS.

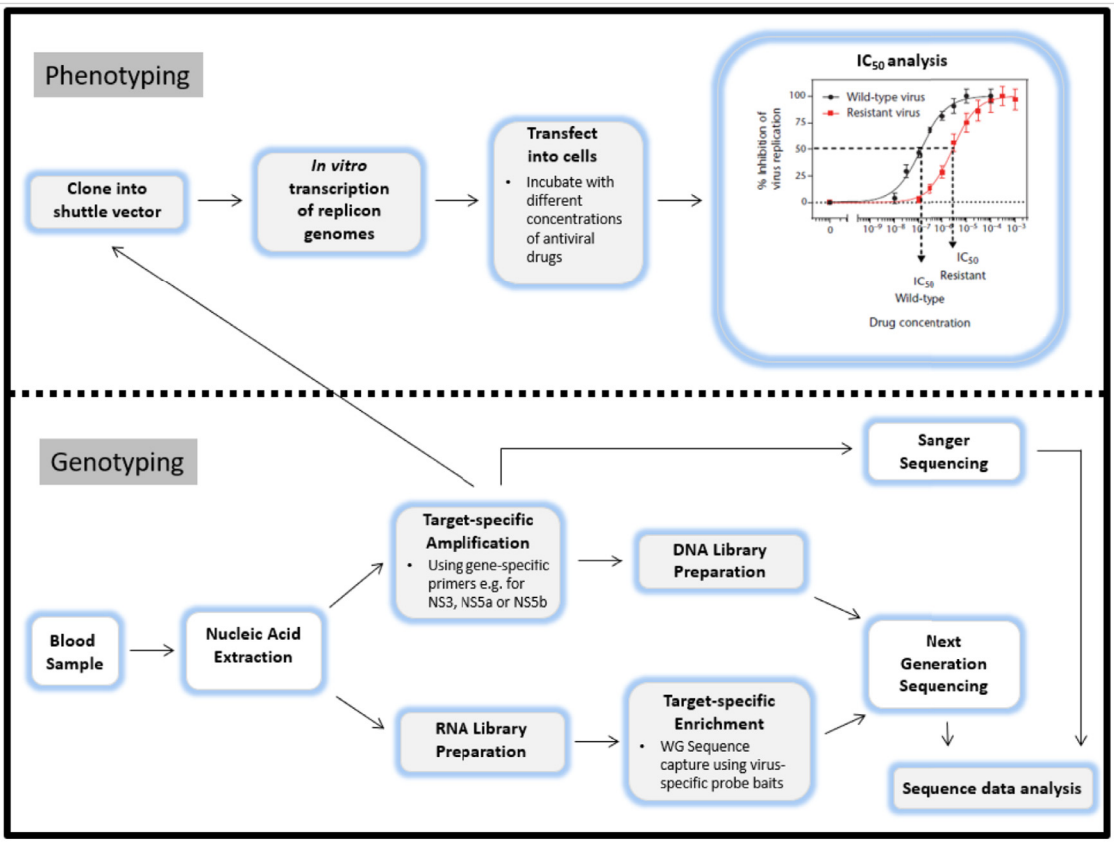
Recombinants
If a sample has been assigned to genotype 2 through sequencing of core, additional sequencing, such as NS5B sequencing or whole genome sequencing should be performed, prior to the use of a genotype-specific DAA regimen, in order to exclude a recombinant form.

Mixed infection
Viral sequencing should be performed where treatment failure is suspected due to an undiagnosed initial mixed infection. Testing of the stored pre-therapy sample should be undertaken in conjunction with the failure specimen.

Reinfection
Viral sequencing should be performed where reinfection is suspected as a cause of recurrence of viraemia during or on completion of therapy. This should be performed on the pre-treatment and failure specimens.

Specimen storage
Pre-treatment specimens should be stored for a minimum of six months to allow retrospective sequencing to be performed when required.

a



b

Drug category	Drug	Resistance-associated substitutions[1]			Resistance detection level[2]
		Category I	Category II	Category III	
NS3/4A protease inhibitors	glecaprevir	-	-	-	No resistance detected
	grazoprevir	-	I170V (80%)	-	Probable resistance detected
	paritaprevir	-	-	-	No resistance detected
	voxilaprevir	-	-	-	No resistance detected
NS5A inhibitors	daclatasvir	L31M (32%)	-	-	Resistance detected
	elbasvir	L31M (32%)	-	-	Resistance detected
	ledipasvir	L31M (32%)	-	-	Resistance detected
	ombitasvir	-	L31M (32%)	-	Probable resistance detected
	pibrentasvir	-	-	-	No resistance detected
	velpatasvir	L31M (32%)	-	-	Resistance detected
NS5B RNA polymerase inhibitors	dasabuvir	-	-	-	No resistance detected
	sofosbuvir	-	-	-	No resistance detected

Fig. 1. (a) Methods for antiviral resistance testing of virus isolated from HCV-infected individuals. (b) The 'virtual phenotype', an example for HCV genotype 1a, from HCV GLUE.[13]

1. Substitutions are assigned to one of three categories according to the strength of evidence for drug resistance. Category I substitutions have the strongest evidence: either (a) *in vitro* resistance level ≥ 5 and found at baseline or treatment-emergent *in vivo*, or (b) both found at baseline and treatment-emergent. Category II: *in vitro* level ≥ 50 or found at baseline or treatment-emergent. Category III: in vitro level ≥ 5.

2. Resistance detection for a given drug is assigned to one of four categories. Resistance detected: any category I substitutions. Probable resistance detected: any category II substitutions. Possible resistance detected: any category III substitutions. No significant resistance detected: none of the above.

Genotypic antiviral resistance testing involves the detection of known drug resistance-associated substitutions in the virus genome using Sanger or next generation (NGS) sequencing methods. Either a fragment or the full length HCV genome may be sequenced. The product is then compared with a database of mutations reported previously as being associated with drug resistance in clinical trials and/or *in vitro* studies. Through this comparison, a 'virtual phenotype' for the sequence of interest is generated with predicted antiviral susceptibilities.

Direct sequencing involves reverse transcription and PCR amplification of usually short (0.2–2.0 kilobase) segments of the NS3, NS5A and/or NS5B genes followed by Sanger sequencing. Most NGS

Table 1
Pre-treatment NS5A RAS testing in DAA-naïve individuals.[14–16]

Drug regimen	Genotype 1a			Genotype 3a		
	Pre-therapy NS5A RAS testing	RAS	Management	Pre-therapy NS5A RAS testing	RAS	Management
Elbasvir-grazoprevir	Yes	Positions 28, 30, 31, 58, 93[a]	Extend to 16 weeks and add WB ribavirin	Not used		
Velpatasvir-sofosbuvir	No			Yes, if cirrhosis	Y93H	Add WB ribavirin
Pibrentasvir-glecaprevir	No			No		
Ledipasvir-sofosbuvir	No			Not used		
Ombitasvir-paritaprevir ritonavir-dasabuvir	No			Not used		

[a] M28A/G/S/T/V, Q30D/E/G/H/K/R/Y, L31F/I/M/V, H58D, Y93C/H/N/S.WB = weight based.

assays use massive parallel sequencing of short fragments, which together encompass the whole or part of the HCV genome. The large number of sequencing products are aligned to a reference genome and software is used to identify RAS. The cut-off for HCV RNA level in the sample selected for resistance testing varies according to assay. Whilst some assays will use a lower limit of 100–300 IU/mL, others may require a HCV RNA level of at least 1000 IU/mL in order for genome amplification to be reliably performed. The cut-off should therefore be confirmed with the testing laboratory. Stored frozen samples can be tested without the need to recall individuals.

Phenotypic testing involves a direct measure of virus susceptibility in cell culture by cloning of parts of the virus genome into a sub-fragment of the HCV genome that has the capability to replicate viral RNA in tissue culture cells (the sub-genomic HCV replicon assay). The extent of viral replication can be measured by expression of a reporter gene and, using this approach, drug susceptibility and viral fitness can be assessed. RAS-bearing isolates are challenged with increasing concentrations of antivirals to establish fold changes, such as IC50, the concentration at which 50% of viral replication is inhibited with respect to wild-type. Of note, HCV cell culture systems for the culture of HCV directly from clinical isolates are still in the early stages of development and are therefore not yet widely used.

Indications for resistance testing

In certain scenarios, RAS testing is recommended in DAA-naïve individuals prior to initiation of therapy, given the reduction in SVR12 rates that may be associated with baseline RAS (see Table 1). In cases of failure of DAA-based therapy, testing may inform the choice of the re-treatment regimen. If RAS testing is performed, this should be carried out using a blood sample taken as close as possible to the planned retreatment start date. This is to take into account the likelihood of the RAS-harbouring variant becoming a minor population over time with the dominant population having wild-type characteristics.

Interpretation of results

The potential impact of RAS on predicted response to DAA therapy may vary according to:

- patient characteristics, including the presence of hepatic cirrhosis (particularly if decompensated) and prior treatment history;
- proposed DAA regimen and interactions with concomitant medications
- viral characteristics, such as the genotype, subtype (where not commonly prevalent within available datasets, such as 1l, 3b or 4r), the decrease in drug susceptibility conferred by specific RAS, the number and pattern of RAS, any associated fitness costs and the pre-treatment HCV RNA level

Treatment decisions should therefore not be made solely on the basis of resistance testing and the interplay between these factors should be taken into account. In particular, RAS are more likely to be of clinical relevance in individuals with additional adverse factors, such as cirrhosis and prior DAA exposure

Management approaches following identification of RAS

Where clinically-relevant RAS are identified, actions may involve one or more of:

- prolongation of DAA therapy
- the inclusion of a class of DAA to which the patient has not been exposed
- intensification with an additional drug, such as weight-based ribavirin
- avoidance of a particular DAA regimen

If resources permit, ribavirin-sparing strategies are preferable to avoid possible ribavirin-associated toxicities[1,2]

The decision to offer DAA treatment to a patient with baseline RAS should involve consideration of these additional factors and the predicted reduction in SVR12 rate, if known. For patients with decompensated liver disease, treatment selection may be impacted by contraindications to certain drugs, such as NS3 protease inhibitors. The suitability of the patient for liver transplantation and the possible impact of transplantation on future timing and responsiveness to antiviral treatment should be considered.

Interpretation of multiple resistance tests

Where more than one resistance test has been performed for the same HCV infection, it may be necessary to consider the cumulative results of both current and prior RAS testing, particularly in the context of NS5A RAS. This may be less relevant for NA NS5B or NS3 RAS, particularly if years have elapsed between the two tests, in view of the lower likelihood of these RAS persisting in the absence of drug pressure.

Genotyping

HCV is currently classified into eight genotypes,[17] which differ by at least 30% in their nucleotide sequence. Each genotype is further subdivided into subtypes, which may differ by greater than 15% in their nucleotide composition, with >80 currently recognised.[18] Accurate determination of genotype/subtype can be critical, as this may impact response to DAA therapies. Recommended methods of genotyping are Sanger sequencing of NS5B or core, and whole genome sequencing by NGS, which have been shown to produce accurate and reproducible results.[2,19] Other platforms, including Line Probe assays and 5′UTR PCR amplification, are not recommended, as they may be insufficiently sensitive to be able to distinguish between subtypes.[20]

Clinical trials have notably focused on HCV genotypes 1a, 1b and 3a, reflecting the distribution of HCV genotypes in high income settings, with limited data presented for other genotypes and

subtypes. There is consequently less evidence to support the interpretation of RAS in "rarer" subtypes such as those prevalent in lower income countries, including but not limited to subtypes 1l (West Africa),[21,22] 3b (China and South East Asia),[23] 4r (all regions of Africa),[24–28] 6a-x (China and South East Asia),[29] 7 (Democratic Republic of Congo)[30] and 8 (India).[17] More data on the sensitivity of these HCV subtypes to current DAA regimens are needed.

Rarely, recombinant strains of HCV have been described, representing HCV genomes comprised of two different viral genotypes. The recombinant 2k/1b is the most frequently observed in real world settings, particularly in countries of the former USSR, in which the 5′ end of genotype 2k is combined with the 3′ end (including the non-structural genes) of genotype 1b. Therefore, where genotype-specific DAA regimens are used, any specimen provisionally assigned by core or 5′UTR (a non-recommended method) sequencing to genotype 2 should be additionally sequenced for NS5B or for the whole genome prior to initiating therapy, in order to exclude a recombinant form.[31]

Mixed infection

A proportion of individuals with HCV are infected with two or more different HCV genotypes or subtypes ('mixed infection'). Mixed infection prevalence varies between 1% and 30%.[32–34] This range likely reflects the heterogeneity of the populations sampled, with the highest rates in groups with frequent HCV exposures, such as people who inject drugs (PWID). Furthermore, identification of mixed infection may be limited by the sequencing method, as Sanger sequencing fails to detect minor populations.

Where individuals with mixed HCV infection receive therapy with a genotype-specific DAA regimen, the response of each infecting genotype may be discordant. Thus, treatment failure may represent the emergence of a previously-undetected minor population following clearance of the dominant genotype. The newly-identified, emergent genotype may be incorrectly interpreted as a new infection and, in addition, may have developed RAS following DAA exposure. Viral sequencing to confirm the genotype should therefore be performed where treatment failure due to an undiagnosed initial mixed infection is suspected, particularly following failure with a genotype-specific DAA regimen, such as in individuals with multiple episodes of risk. Testing of the stored pre-therapy sample should be undertaken in conjunction with the failure specimen. NGS may be preferable in this scenario, given the greater sequencing information it provides. Where mixed infections are identified, use of a DAA regimen, which is active across major genotypes, increases the likelihood for successful cure of both infecting genotypes.

To enable parallel testing of the pre-therapy and failure specimens, pre-treatment samples should be stored for a minimum of six months. They may then be tested retrospectively, where this is indicated.

Reinfection

Reinfection after spontaneous or treatment-induced virus clearance is common amongst populations with repeated exposures to HCV, such as men who have sex with men (MSM) living with HIV, and PWID.[35–37] If recurrence of viraemia is observed during or on completion of therapy, viral sequencing of the failure and pre-treatment specimens can help differentiate between relapse and reinfection, especially where recurrence of viraemia occurs with the same subtype. Clustering of the failure and pre-treatment sequences on a phylogenetic tree is observed in cases of relapse but not reinfection, unless reinfection occurred from the same source. The depth and breadth of coverage of sequencing data provided by NGS may improve the resolution of the tree, with increased ability to differentiate relapse from reinfection.

Difficult-to-engage patient groups

Many people living with HCV are not currently engaged with HCV services. This may be a result of difficulties attending follow-up after a positive HCV diagnosis, or because the diagnosis has not yet been made. Patient groups who may have difficulty accessing HCV services include PWID, people with mental health problems and people who are homeless.[38] For example, only around half of PWID sampled in the UK's PHE Unlinked Anonymous Survey in 2016 were aware of their positive HCV antibody status.[39]

For individuals from these groups, it is particularly critical that treatment be available as soon as possible after diagnosis to minimise the risk of loss to follow-up. Any benefit of performing resistance testing must be weighed against the delay in initiating therapy. Early use of a DAA regimen, which both exerts activity across major genotypes and retains potency against RAS-harbouring virus (*i.e.*, has a high genetic barrier to resistance), avoids the requirement for viral genotyping and resistance testing and facilitates early initiation of treatment in these individuals.

HCV resistance testing prior to the use of specific DAA: treatment-naïve individuals and those for whom non-NS5A inhibitor-containing therapy has previously failed

Sofosbuvir–ledipasvir

Early clinical trials data for treatment outcomes with sofosbuvir plus the first generation NS5A inhibitor ledipasvir suggested ledipasvir RAS may reduce SVR12 for individuals with genotype 1a infection in some scenarios, including those receiving a shortened 8 week treatment course,[7] those previously exposed to pegylated interferon-ribavirin, or those with cirrhosis[7,40] receiving 12 weeks of therapy. Addition of ribavirin and/or extension of therapy was suggested to restore a high SVR12 rate. However, in a large cohort from England's National Health Service (NHS) including HCV genotype 1a-infected patients, high SVR12 rates for both sofosbuvir–ledipasvir (94–98%) and sofosbuvir–ledipasvir with ribavirin (93–96%) were reported. RAS testing is thought not to have been widely used in determining DAA regimens in this cohort. These data suggest routine pre-therapy resistance testing may not be necessary.[41]

Of note, data on the effect of RAS on SVR12 outcomes with sofosbuvir–ledipasvir in 'rare' subtypes such as genotypes 4–6, are few, and mostly from studies with small numbers of individuals.[21,42–45] Early reports suggest a greater prevalence of multiple NS5A RAS in therapy-naïve individuals in these subtypes, which may account for lower SVR12 rates. For example, SVR12 in a Rwandan population was 54% for subtype 4r with 12 weeks of sofosbuvir–ledipasvir.[24] The combination of NS5A RAS L28V+L30R+/−L31M, frequently identified pre-treatment, together with minor populations of NS5B RAS, such as the sofosbuvir mutation S282C/T, may underpin the reduced SVR in this subtype.[26]

Elbasvir–grazoprevir

The regimen of elbasvir, an NS5A inhibitor, and grazoprevir, an NS3/4A inhibitor, with or without ribavirin, is licensed for the treatment of genotype 1a, 1b and 4 infections. In replicon systems, NS5A RAS conferring ≥100 fold reduction in elbasvir susceptibility in genotype 1a include Q30D/R, L31F/V, del32 and Y93C/H/N.[15,46–48] A pooled analysis of phase II and III clinical trials reporting outcomes following 12 weeks of elbasvir–grazoprevir, including treatment-naïve individuals and those previously treated

with pegylated interferon-ribavirin +/− protease inhibitor infected with HCV genotype 1a, both with and without cirrhosis, identified an SVR12 of 70% (39/56) versus 98% (441/450) for those with and without baseline elbasvir RAS, respectively.[15] Of note, M28V was the commonest variant, and was associated with a reduced SVR12 (86%). Based on data from only 6 individuals, prolongation of the regimen to 16 weeks and intensification with ribavirin appeared to increase efficacy in those with baseline elbasvir RAS. Subsequent analyses showed that a low baseline HCV RNA level (<800,000 IU/mL) may reduce the impact of NS5A RAS, with 8/8 individuals with elbasvir RAS and an HCV RNA <800,000 IU/mL achieving SVR12.[49] Of note, however, use of baseline viral load in place of RAS testing for treatment decisions may lead to unnecessary use of ribavirin.[50] An effect of NS5A RAS on SVR12 was not seen for genotypes 1b or 4.[15]

Sofosbuvir–velpatasvir

The combination of sofosbuvir with the second generation NS5A inhibitor, velpatasvir, is licensed for treating genotypes 1–6. *In vitro*, RAS conferring high (>100× EC50) fold changes to velpatasvir include L31V and Y93C/H/N/R/W in genotype 1a, and L31V and Y93H/S in genotype 3a.[46,51,52]

Randomised controlled studies found no impact of RAS on treatment outcomes for genotypes 1, 2, 4 and 6 in individuals with and without compensated cirrhosis, including those with prior exposure to pegylated interferon-ribavirin with or without a protease inhibitor.[16] However, for genotype 3 (predominantly subtype 3a), SVR12 was 86% (19/22) and 98% (445/454) for those with and without Y93H, respectively. Previous interferon-containing treatment and/or cirrhosis were additional factors in reducing treatment efficacy; in particular, SVR12 was only 67% (4/6) in the small number with cirrhosis and Y93H.[16,53] Further pooled analyses of phase II and III studies in genotype 3a-infected patients with compensated cirrhosis identified an SVR12 of only 80% where baseline NS5A RAS were present *vs* 94% overall.[54]

By contrast, results from real world trials of sofosbuvir–velpatasvir in patients with genotype 3 infection, compensated cirrhosis and baseline Y93H are conflicting as to its impact on SVR.[55,56]

However, data from England's NHS ($n = 470$) identified an SVR12 rate of 95% *vs* 92–93% for those with compensated cirrhosis and genotype 3 receiving sofosbuvir–velpatasvir with and without ribavirin, respectively[41] and, although resistance data were not available, this may further support the approach for NS5A RAS testing in the UK setting, with addition of ribavirin or use of an alternative regimen where Y93H is identified.

Treatment outcomes with sofosbuvir–velpatasvir may also be affected by pre-treatment RAS in other patient groups with adverse characteristics. In the ASTRAL-4 study ($n = 267$), SVR12 was reduced in patients with decompensated cirrhosis infected with genotype 1 (subtypes 1a or 1b) and harbouring NS5A RAS, who received 12 weeks of sofosbuvir–velpatasvir (80% *vs* 96% with and without NS5A RAS). This effect of NS5A RAS could be partially overcome by extension to 24 weeks of treatment (SVR12 90%) or fully overcome by addition of ribavirin (SVR12 98%).[57] In the NHS England cohort, the SVR12 rate in individuals with genotype 3 infection (subtype not reported) was only 84% and 86% for those with decompensated cirrhosis receiving therapy with sofosbuvir–velpatasvir with and without ribavirin, respectively.[41] The impact of RAS on SVR in this group remains to be determined.

A high prevalence of pre-treatment NS5A RAS in 'rarer' subtypes, such as A30K+L31M in genotype 3b, may also reduce SVR12 with sofosbuvir–velpatasvir, particularly in individuals with cirrhosis.[58,23] However, the optimal treatment approach in these individuals has not yet been identified.

Other regimens

For ritonavir-boosted paritaprevir, ombitasvir and dasabuvir, and for glecaprevir–pibrentasvir available data suggest routine pre-therapy RAS testing is unlikely to offer benefit in DAA-naïve individuals[59] although further data are required on treatment outcomes with glecaprevir–pibrentasvir in individuals infected with rare subtypes, such as 3b, where multiple pre-treatment NS5A RAS are common and SVR12 rates reduced.[60] NS3 and NS5A RAS testing is recommended in individuals with prior exposure to NS3 protease inhibitors who are retreated with glecaprevir–pibrentasvir, as discussed below.[61,62]

Retreatment of individuals for whom prior NS5A inhibitor-containing therapy has failed

Background

Approximately 5% of individuals receiving DAA therapies in the real world setting do not achieve SVR12.[1,2,41] All currently recommended regimens include NS5A inhibitors, with the consequent high probability of NS5A RAS in DAA-exposed patients.

Most of these individuals achieve an on-treatment response with undetectable virus at end-of-treatment, followed by viral rebound (relapse), which in many cases represents the emergence of a DAA-resistant viral strain. Other possible patterns of failure are viral rebound during therapy (breakthrough) or failure of the viral load to suppress (non-response). Reinfection or initial mixed infection may be alternative explanations for recurrence of viraemia.

There are currently limited data to inform the optimal strategies for retreatment of individuals experiencing failure particularly with second generation DAA therapies, However, the improved genetic barrier to resistance of triple class, second generation DAA combinations, often overcomes the effect of RAS.

Sofosbuvir–velpatasvir–voxilaprevir

In two phase III randomised controlled studies in individuals with and without compensated cirrhosis, infected with genotypes 1–3 and previously exposed to DAA, including first generation NS5A inhibitors (POLARIS 1 and 4), SVR12 following 12 weeks of therapy with sofosbuvir–velpatasvir–voxilaprevir was >95%. There was no impact of baseline RAS, despite a high pre-treatment prevalence of NS3 and NS5A RAS.[63] Treatment-emergent RAS were also uncommon (1/7 individuals with relapse). The lowest SVR12 rate (90%) was seen in HCV genotype 3a infected individuals with cirrhosis and baseline NS5A RAS. An integrated resistance analysis of four phase II studies with this drug combination in DAA-naïve and –experienced individuals infected with HCV genotypes 1–4 and 6, also confirmed the lack of impact of baseline RAS on SVR12.[64]

Real world cohorts have confirmed SVR12 > 90% with this regimen, mainly for genotypes 1–4, following unsuccessful therapy with first generation NS5A inhibitors[65-68] or unspecified NS5A inhibitors.[69,70] However, a lower SVR12 (9/13, 69%) was reported for genotype 3 infected patients (subtypes not presented) with cirrhosis.[71] Regarding second generation NS5A inhibitor exposed individuals, lower SVR12 rates (83–86%) were reported in individuals with genotypes 1–3 previously exposed to sofosbuvir–velpatasvir,[72] but with no impact of this prior combination in other studies (SVR 94–100%)[73,74] emphasising the need for further retreatment outcome datasets in sofosbuvir–velpatasvir exposed patients, including resistance data. High SVR (94%) was reported in glecaprevir–pibrentasvir exposed individuals who received retreatment with sofosbuvir–velpatasvir–voxilaprevir.[75] Notably, however, voxilaprevir and sofosbuvir are contraindicated in individuals with decompensated cirrhosis or an eGFR <30 mL/min/1.73m^2, respectively.

Glecaprevir–pibrentasvir

Glecaprevir–pibrentasvir has also been used to treat NS5A inhibitor-experienced individuals. One phase III study (Magellan-1, Part 2) found reduced SVR12 (83%) in genotype 1 infected individuals with pre-therapy NS5A RAS receiving 12 weeks of therapy, which could be overcome by extension to 16 weeks of treatment (SVR12 96%). The presence of NS3+NS5A RAS led to low SVR12 even with the extended duration[76]. Consistent with these findings, a phase II trial of 12 weeks of glecaprevir–pibrentasvir (Magellan-1, Part-1) reported lower SVR12 (91–93%) in those with NS5A+/-NS3 RAS compared to those with NS3 RAS alone (100%).[61]

A further study including genotype 1a-infected individuals with and without compensated cirrhosis, who were previously exposed to an NS5A inhibitor plus sofosbuvir, compared 12 versus 16 weeks of glecaprevir–pibrentasvir +/− ribavirin. Higher SVR12 (97% and 94% for those with and without cirrhosis) was found in the 16 week arm compared to those receiving the shorter course (86–90%). SVR12 was also lower in those with NS5A RAS pre-treatment (88% *vs* 97%), although this difference was not statistically significant.[77] Treatment-emergent NS3 and/or NS5A resistance mutations were frequently observed in those not achieving SVR12, including the deletion in NS5A at position 32, which is associated with >1000 fold resistance to all NS5A inhibitors.[46] This deletion was also observed in DAA-exposed genotype 1b cohorts and conferred resistance to glecaprevir–pibrentasvir.[78,79]

Sofosbuvir–velpatasvir

A phase II study evaluated outcome with 24 weeks of sofosbuvir–velpatasvir and ribavirin, in individuals with and without compensated cirrhosis, infected with genotypes 1, 2 or 3, for whom first line NS5A-inhibitor containing therapy failed. Overall SVR12 was 91% (63/69) with no impact of baseline NS5A RAS for genotypes 1 and 2. However, for genotype 3a, SVR12 was 77% (10/13) and 100% (4/4) in those with and without baseline NS5A RAS, respectively.[80] In a real world study of 24 weeks of sofosbuvir–velpatasvir +/− ribavirin, mostly in individuals with cirrhosis (of unknown stage) infected with genotypes 1–4, including those exposed to NS5A inhibitors, an SVR12 of 84% (26/31) was reported overall, but was 57% (4/7) in those with baseline L31F/I/M. RAS impact by genotype was not reported.[81] Owing to lower SVR compared to other regimens, sofosbuvir–velpatasvir is not recommended in NS5A inhibitor exposed patients in international guidelines, except in individuals with decompensated cirrhosis, in whom protease inhibitors are contraindicated. However, resistance data in this setting are not yet available.

Sofosbuvir–elbasvir–grazoprevir

Although the combination of sofosbuvir–elbasvir–grazoprevir, with or without ribavirin, is not currently recommended for DAA-experienced patients in guidelines, early data suggest it may be effective. SVR rates of 96–100% have been reported in trials, including individuals with genotypes 1–4 infection, compensated cirrhosis and with no impact of NS5A +/− NS3 RAS on outcomes.[82–84] However, a larger cohort with this combination, including genotype 1a-infected individuals previously exposed to ledipasvir–sofosbuvir, reported an SVR12 of 90% *vs* 100% in those with and without pre-retreatment elbasvir RAS, respectively.[68]

Sofosbuvir–glecaprevir–pibrentasvir

In a phase III study (Magellan-3), individuals with or without compensated cirrhosis, who had previously received therapy with glecaprevir–pibrentasvir, were retreated with sofosbuvir–glecaprevir–pibrentasvir plus ribavirin. Most individuals had genotype 1a or 3a infection and received 16 weeks of therapy. Despite a high baseline prevalence of NS5A RAS, SVR12 was 96% (22/23).[85] Pibrentasvir has the highest genetic barrier to resistance of the NS5A inhibitors[46] and therefore the use of this regimen, with extended duration +/− inclusion of ribavirin, may become an important option for retreatment of patients exposed to second generation and/or triple class DAA regimens. However, this requires evaluation in clinical studies.

Discussion

In the current UK landscape, DAA regimens are recommended, for which pre-treatment RAS may impact SVR12 in certain scenarios. In this and similar settings, the writing group suggests a pragmatic approach to the use of resistance testing. Where access to resistance testing is limited, or where the clinical setting requires rapid initiation of therapy without waiting for the results of specialised testing, RAS testing may be omitted. Where RAS testing is accessible, this should be performed in selected scenarios (Box 2). Although RAS testing may not influence the immediate treatment decision in all cases, results may be helpful either to the particular patient or to others in the future. Some of the data, in particular analyses of the NHS England registry, are only available in preliminary form, without peer review, and this must be considered a limitation in interpreting clinical significance.

Box 2. Scenarios where resistance testing is recommended prior to DAA therapy

1. **NS5A RAS in GT1a prior to elbasvir-grazoprevir.**

   Treatment-naïve individuals and those exposed to Pegylated interferon-ribavirin +/− protease inhibitor
   Where elbasvir RAS are identified, patients should receive 16 weeks of therapy with ribavirin or an alternative regimen.

2. **NS5A RAS in GT3a with compensated cirrhosis prior to sofosbuvir-velpatasvir.**

   Treatment-naïve individuals and those exposed to pegylated interferon-ribavirin +/− protease inhibitor
   Where Y93H is identified, patients should receive 12 weeks of therapy with ribavirin, 24 weeks of therapy, or an alternative regimen.

3. **NS5A RAS in all patients with decompensated cirrhosis prior to DAA therapy.**

   This is to identify patients who may benefit from ribavirin or extension of therapy, or, in some cases, to guide future treatment decisions.

4. **NS5A RAS in subtypes not commonly found in high income countries, including genotypes 4, 5 and 6.**

   This is to guide future treatment decisions, as resistance data are currently lacking.

5. **NS3 and NS5A RAS in all patients with previous exposure to NS3 and/or NS5A inhibitors, prior to retreatment.**

   This is either to determine duration of therapy, in some cases, or to guide future treatment decisions.

The writing group acknowledges that these recommendations differ from those of international bodies, including the European

Association of the Study of the Liver (EASL) and the American Association for the Study of Liver Diseases–Infectious Diseases Society of America (AASLD–IDSA).[1,2] EASL guidelines do not recommend RAS testing in therapy naïve individuals and favour the use of ribavirin-free DAA regimens, citing the efficacy of newer regimens against RAS-harbouring virus, the lack of access to resistance testing and the absence of a consensus on interpretation of results. This is an attractive strategy, but not entirely applicable in the current UK context, given the widespread use of regimens in circumstances where there may be a benefit in including ribavirin, the more restricted access to newer agents, including sofosbuvir–velpatasvir–voxilaprevir, and the availability of resistance testing at multiple centres. Baseline RAS testing therefore enables certain groups to be spared unnecessary ribavirin exposure, such as those with genotype 3a infection and compensated cirrhosis receiving sofosbuvir–velpatasvir, who do not have the NS5A Y93H mutation.

AASLD-IDSA guidelines differ from those of EASL on the indications for RAS testing, with recommendations for NS5A testing prior to elbasvir–grazoprevir in therapy-naïve patients with genotype 1a infection and in those with genotype 3a infection and cirrhosis or prior pegylated-interferon-ribavirin exposure prior to sofosbuvir–velpatasvir use, based on data outlined above.[15,53] Thus, the recommendations of PHE's writing group align more closely with those of AASLD-IDSA for DAA-naïve individuals, reflecting similarities in treatment landscapes.

By contrast, EASL does recommend RAS testing in DAA- experienced individuals prior to re-treatment to assist in optimising the re-treatment regimen, referring to the current lack of data to guide selection and duration of treatment, particularly in those with NS5A inhibitor exposure. Conversely, AASLD-IDSA guidelines do not recommend RAS testing in this setting, citing successful outcomes from POLARIS-1 and POLARIS-4. PHE's recommendations thus align more closely with those of EASL for this cohort.

For individuals with decompensated cirrhosis there is similarly a paucity of data to guide the use of pre-therapy RAS testing. Neither EASL nor AASLD-IDSA recommend baseline resistance testing and advise that ribavirin, if tolerated, should be included in all regimens.[57,86] By contrast, PHE's writing group favours RAS testing based on limited data which suggest that certain individuals with decompensated cirrhosis, who do not have resistant virus, may not require ribavirin[57] as well as the need to gather further data across genotypes to guide best practice. The requirement for additional data also informs the writing group's view that RAS testing should be performed in those with 'rare' subtypes, a group which is not specifically addressed in the EASL or AASLD-IDSA guidelines.

Recommendations on RAS testing are likely to change as more detailed analyses of existing data, as well as newer data, become available. In particular, further studies are needed to address current unmet needs, including the impact of resistance on SVR outcomes in individuals with 'rare' subtypes, the impact of RAS on re-treatment outcomes according to the timeline between first and second DAA regimens and the optimal re-treatment regimens both for individuals exposed to second generation DAA as well as for NS5A inhibitor-exposed individuals with decompensated cirrhosis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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