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Antiviral resistance testing in the management of hepatitis C virus infection

PHE Hepatitis C Virus Resistance Group

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Summary

A recent NHS England dataset including 15,000 people with HCV naïve to direct acting antivirals (DAA) identified a high (>95%) cure rate for many DAA regimens across genotypes 1-3, and for a smaller number with genotypes 4-6ⁱ. Although information on treatment history and impact of resistance-associated substitutions (RAS) on treatment outcomes was not presented, this data provide reassurance that many patients will be cured without access to RAS testing.

The aim of this document is to support clinicians treating people with HCV, where the issue of resistance may be a factor in clinical-decision making. Based on its expert opinion and following review of evidence from *in vitro* data, clinical trials and real world cohorts, the PHE HCV Resistance Group suggests the following pragmatic approach to the use of RAS testing in the current NHS landscape. 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, resistance testing may be performed in the scenarios below.

Recommendations for RAS testing

- 1. NS5A RAS in GT1a prior to Elbasvir/Grazoprevir.**
Where elbasvir RAS are identified, patients should receive 16 weeks of therapy with ribavirin or an alternative regimen.
- 2. NS5A RAS in GT3 with compensated cirrhosis prior to Sofosbuvir/Velpatasvir.**
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 is currently lacking.
- 5. NS3 and NS5A RAS in all patients with previous exposure to NS3 and/or NS5A inhibitors, prior to re-treatment.**
This is either to determine duration of therapy, in some cases, or to guide future treatment decisions.

i. Drysdale *et al*, BASL, 2018, York

Although RAS testing may not influence the immediate treatment decision in all cases, it may be helpful either to the particular patient or to others in the future. Clinicians are referred to Tables 1-5 on pages 6-10 for a summary of the relevant evidence.

Some of the available data, in particular analyses of the NHS England registry, has been presented in preliminary form, without peer review, and this must be considered a limitation in interpreting clinical significance. As a result, the Group acknowledges that its recommendations may change as more detailed analysis of existing data, as well as newer data, becomes available.

It is hoped that this document will be useful to NHS clinicians, and we would welcome any feedback you may have.

Table 1. HCV resistance testing in treatment-naïve individuals

GT1a	SOF/LDV +/- RBV	PrOD + RBV	ELB/GZR +/- RBV	SOF/VEL +/- RBV	GLE/PIB	SOF/ELB/GZR	SOF/VEL/VOX	SOF/GLE/PIB
Non Cirrhotic	Regimen recommended	Regimen recommended	(1)	Regimen recommended	Regimen recommended	Regimen not recommended	Regimen not recommended	Regimen not recommended
Cirrhotic	Regimen recommended	Regimen not recommended	(1)	(2)	Regimen recommended	Regimen not recommended	Regimen not recommended	Regimen not recommended

GT3a

Non Cirrhotic	Regimen not recommended	Regimen not recommended	Regimen not recommended	Regimen recommended	Regimen recommended	Regimen not recommended	Regimen not recommended	Regimen not recommended
Cirrhotic	Regimen not recommended	Regimen not recommended	Regimen not recommended	(3)	Regimen recommended	Regimen not recommended	(4)	Regimen not recommended

Green – data available supporting use of the regimen without pre-treatment RAS testing

Yellow – data available supporting pre-treatment RAS testing in some or all groups of patients

Red – regimen not recommended in international guidelines

1. Where elbasvir RAS are identified, 16 weeks of ELB/GZR/RBV or an alternative regimen is advised [1].
2. Data is available supporting the use of RAS testing in individuals with decompensated cirrhosis. Where NS5A RAS are identified, 12 weeks of SOF/VEL/RBV or 24 weeks of SOF/VEL are advised [2].
3. Where the NS5A RAS Y93H is identified, 12 weeks of SOF/VEL/RBV, 24 weeks of SOF/VEL, or an alternative regimen is advised [3].
4. If the NS5A RAS Y93H is absent, 12 weeks of SOF/VEL is advised [3].

ELB, elbasvir; GLE, glecaprevir; GZR, grazoprevir; LDV, ledipasvir; PIB, pibrentasvir; PrOD, ritonavir-boosted paritaprevir, ombitasvir, dasabuvir; RBV, ribavirin; SOF, sofosbuvir; VEL, velpatasvir; VOX, voxilaprevir

Table 2. HCV resistance testing in individuals previously treated with pegylated interferon and ribavirin

GT1a	SOF/LDV +/- RBV	PrOD + RBV	ELB/GZR +/- RBV	SOF/VEL +/- RBV	GLE/PIB	SOF/ELB/GZR	SOF/VEL/VOX	SOF/GLE/PIB
Non Cirrhotic	Regimen recommended	Regimen recommended	(1)	Regimen recommended	Regimen recommended	Regimen not recommended	Regimen not recommended	Regimen not recommended
Cirrhotic	Regimen recommended	Regimen not recommended	(1)	(2)	Regimen recommended	Regimen not recommended	Regimen not recommended	Regimen not recommended
GT3a								
Non Cirrhotic	Regimen not recommended	Regimen not recommended	Regimen not recommended	Regimen recommended	Regimen recommended	Regimen not recommended*	(4)	Regimen not recommended
Cirrhotic	Regimen not recommended	Regimen not recommended	Regimen not recommended	(3)	Regimen recommended	Regimen recommended	(4)	Regimen not recommended

*See section B7

Green – data available supporting use of the regimen without pre-treatment RAS testing

Yellow – data available supporting pre-treatment RAS testing in some or all groups of patients

Red – regimen not recommended in international guidelines

1. Where elbasvir RAS are identified, 16 weeks of ELB/GZR/RBV or an alternative regimen is advised [1].
2. Data are available supporting the use of RAS testing in individuals with decompensated cirrhosis. Where NS5A RAS are identified, 12 weeks of SOF/VEL/RBV or 24 weeks of SOF/VEL are advised [2].
3. Where the NS5A RAS Y93H is identified, 12 weeks of SOF/VEL/RBV, 24 weeks of SOF/VEL, or an alternative regimen is advised [3].
4. Where the NS5A RAS Y93H is absent, 12 weeks of SOF/VEL is an alternative [3].

ELB, elbasvir; GLE, glecaprevir; GZR, grazoprevir; LDV, ledipasvir; PIB, pibrentasvir; PrOD, ritonavir-boosted paritaprevir, ombitasvir, dasabuvir; RBV, ribavirin; SOF, sofosbuvir; VEL, velpatasvir; VOX, voxilaprevir

Table 3. HCV resistance testing in individuals previously treated with sofosbuvir and ribavirin with or without pegylated interferon

GT1a	SOF/LDV + RBV	PrOD	ELB/GZR +/- RBV	SOF/VEL +/- RBV	GLE/PIB	SOF/ELB/ GZR	SOF/VEL/ VOX	SOF/GLE/ PIB
Non Cirrhotic	Regimen recommended	Regimen not recommended	(1)	Regimen recommended	Regimen recommended	Regimen not recommended	Regimen recommended	Regimen not recommended
Cirrhotic	Regimen not recommended	Regimen not recommended	(1)	(2)	Regimen recommended	Regimen not recommended	Regimen recommended	Regimen not recommended
GT3a								
Non Cirrhotic	Regimen not recommended	Regimen not recommended	Regimen not recommended	(3)	(5)	Regimen not recommended	Regimen recommended	Regimen not recommended
Cirrhotic	Regimen not recommended	Regimen not recommended	Regimen not recommended	(4)	(5)	Regimen not recommended	Regimen recommended	Regimen not recommended

Green – data available supporting use of the regimen without pre-treatment RAS testing

Grey – insufficient data available on the use of pre-treatment RAS testing

Red – regimen not recommended in international guidelines

1. No current data exists for SOF-exposed individuals but the regimen is recommended in treatment guidelines [4] . By extension of data showing an impact of baseline elbasvir RAS in PEG/RBV +/-PI -experienced individuals, 16 weeks of ELB/GZR/RBV or an alternative regimen is advised where elbasvir RAS are identified [1].
2. Where NS5A RAS are identified in individuals with decompensated cirrhosis, 12 weeks of SOF/VEL/RBV or 24 weeks of SOF/VEL are advised. This is by extension of findings from PEG/RBV+/-PI experienced individuals in the absence of other available data [2].
3. There is currently insufficient data to inform interpretation. By extension of data from therapy-naïve and PEG/RBV-experienced patients, RAS are unlikely to impact on SVR12, although further data is required [3].
4. There is currently insufficient data to inform interpretation. By extension of data from therapy-naïve and PEG/RBV-experienced patients, where the NS5A Y93H RAS is identified in patients with cirrhosis, 12 weeks of SOF/VEL/RBV, 24 weeks of SOF/VEL, or an alternative regimen is advised [3].
5. There is currently insufficient data to inform interpretation. By extension of data from therapy-naïve and PEG/RBV-experienced patients, baseline NS5A RAS are unlikely to impact SVR12, although further data is required [5, 6].

ELB, elbasvir; GLE, glecaprevir; GZR, grazoprevir; LDV, ledipasvir; PIB, pibrentasvir; PrOD, ritonavir-boosted paritaprevir, ombitasvir, dasabuvir; RBV, ribavirin; SOF, sofosbuvir; VEL, velpatasvir; VOX, voxilaprevir

Table 4a. HCV resistance testing in individuals previously treated with pegylated interferon, ribavirin and a protease inhibitor

GT1a	SOF/LDV +/- RBV	PrOD	ELB/GZR +/- RBV	SOF/VEL +/- RBV	GLE/PIB	SOF/ELB/GZR	SOF/VEL/VOX	SOF/GLE/PIB
Non Cirrhotic	Regimen recommended	Regimen not recommended	(1)	Regimen recommended	(3)	Regimen not recommended	Regimen recommended	Regimen recommended
Cirrhotic	Regimen recommended	Regimen not recommended	(1)	(2)	(3)	Regimen not recommended	Regimen recommended	Regimen recommended

Table 4b. HCV resistance testing in individuals previously treated with a protease inhibitor and sofosbuvir

GT1a	SOF/LDV	PrOD	ELB/GZR	SOF/VEL +/- RBV	GLE/PIB	SOF/ELB/GZR	SOF/VEL/VOX	SOF/GLE/PIB
Non Cirrhotic	Regimen not recommended	Regimen not recommended	Regimen not recommended	Regimen not recommended	(3)	Regimen not recommended	Regimen recommended	Regimen recommended
Cirrhotic	Regimen not recommended	Regimen not recommended	Regimen not recommended	(2)	(3)	Regimen not recommended	Regimen recommended	Regimen recommended

Green – data available supporting use of the regimen without pre-treatment RAS testing

Yellow – data available supporting pre-treatment RAS testing in some or all groups of patients

Red – regimen not recommended in international guidelines

1. Where elbasvir RAS are identified, 16 weeks of ELB/GZR/RBV or an alternative regimen is advised [1].
2. Data is available supporting the use of RAS testing in individuals with decompensated cirrhosis. Where NS5A RAS are identified, 12 weeks of SOF/VEL/RBV or 24 weeks of SOF/VEL are advised [2].
3. Where NS3 or NS5A RAS are identified, GLE/PIB is advised for a duration of 12 or 16 weeks, respectively. Where both NS3 and NS5A RAS are identified, an alternative regimen is recommended [7, 8].

ELB, elbasvir; GLE, glecaprevir; GZR, grazoprevir; LDV, ledipasvir; PIB, pibrentasvir; PrOD, ritonavir-boosted paritaprevir, ombitasvir, dasabuvir; RBV, ribavirin; SOF, sofosbuvir; VEL, velpatasvir; VOX, voxilaprevir

Table 5. HCV resistance testing in individuals previously treated with an NS5A inhibitor-containing regimen

GT1a	SOF/LDV	PrOD	ELB/GZR	SOF/VEL + RBV	GLE/PIB	SOF/ELB/GZR	SOF/VEL/VOX +/-RBV	SOF/GLE/PIB +/- RBV
Non Cirrhotic	Regimen not recommended	Regimen not recommended	Regimen not recommended	Regimen not recommended	(2)	Regimen not recommended	Regimen recommended	Regimen recommended
Cirrhotic	Regimen not recommended	Regimen not recommended	Regimen not recommended	Regimen recommended*	(2)	Regimen not recommended	Regimen recommended	Regimen recommended
GT3a								
Non Cirrhotic	Regimen not recommended	Regimen recommended	Regimen recommended					
Cirrhotic	Regimen not recommended	Regimen not recommended	Regimen not recommended	(1)*	Regimen not recommended	Regimen not recommended	Regimen recommended	Regimen recommended

**only if decompensated cirrhosis*

Green – data available supporting use of the regimen without pre-treatment RAS testing

Yellow – data available supporting pre-treatment RAS testing in some or all groups of patients

Grey – insufficient data available on the use of pre-treatment RAS testing

Red – regimen not recommended in international guidelines

1. Therapy with 24 weeks of SOF/VEL/RBV is recommended in individuals with decompensated cirrhosis and prior NS5A inhibitor containing therapy. Data is available suggesting reduced SVR12 with 24 weeks of SOF/VEL/RBV in genotype 3a-infected individuals, with or without compensated cirrhosis, with prior NS5A inhibitor treatment and baseline NS5A RAS [9] . However, there is no currently available alternative regimen in individuals with decompensated cirrhosis [2]. NS5A RAS testing is advised to obtain data to inform future clinical decision making in these individuals.
2. Where NS3 or NS5A RAS are identified, GLE/PIB is advised for a duration of 12 or 16 weeks, respectively. Where both NS3 and NS5A RAS are identified, an alternative regimen is recommended [7, 8, 10].

ELB, elbasvir; GLE, glecaprevir; GZR, grazoprevir; LDV, ledipasvir; PIB, pibrentasvir; PrOD, ritonavir-boosted paritaprevir, ombitasvir, dasabuvir; RBV, ribavirin; SOF, sofosbuvir; VEL, velpatasvir; VOX, voxilaprevir

A. Introduction to HCV resistance testing

1. 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 result in sustained virological response (SVR) rates of >95%. SVR is typically assessed at 12 weeks after cessation of antiviral therapy and is referred to as 'SVR12'. Many of the DAA regimens currently available in the UK exert differential activity according to the viral genotype and the presence of viral resistance, and an understanding of the principles of resistance testing is therefore required.

The aim of this document is to support clinicians treating people with HCV in the UK, where the issue of resistance may be a factor in clinical-decision making. Where guidance is presented, this represents the opinion of the 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 has been included where the presence of resistance may result in increased virological failure with an SVR12 of less than 95%.

The Group also recognises the increasing availability of second-generation DAA regimens, which exert pan-genotypic activity 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, particularly in countries with limited access to specialised tests.

It is emphasised that this working document 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 [4, 11, 12] or policy documents available from other sources [13].

2. 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) [14, 15], result in a large number of viral variants, harbouring different mutations, which coexist within the same host. These are commonly termed 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 RAS. Such RAS-harboured variants are preferentially selected for, or enriched from baseline, following unsuccessful DAA therapy, in view of the growth advantage they confer.

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. RAS in these genes are found in therapy-naïve populations at a prevalence of up to 50% and 15% for NS3 and NS5A, respectively [16, 17]. By contrast, NS5B RAS are rarely observed (1-3%), probably reflecting the loss in replicative capacity (or fitness) that they impart. In particular, nucleotide substitutions in the highly conserved active site of RdRp may effectively halt viral replication [18].

Of the three drug classes, resistance to NS5A inhibitors is clinically the most important. 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. NS3 RAS may reduce susceptibility to NS3 protease inhibitors in some settings, but tend to become undetectable within months of stopping NS3 inhibitor-containing therapy [19]). By contrast, variants harbouring NS5B RAS rarely emerge following exposure to an NS5B inhibitor (1% of virologic failures) and are quickly replaced by fitter, wild-type virus [18].

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 ≥ 2 agents in the same class although not necessarily reducing susceptibility to all drugs of that class).

3. Recommended types of resistance test

Two types of tests predict susceptibility to antiviral drugs based on an analysis of the viral genotype or phenotype. Genotypic susceptibility testing is fast and easier to set up, and is performed at several centres in the UK. Phenotypic resistance testing is laborious and complicated, and is currently only available in research settings.

Genotypic resistance testing

In genotypic antiviral susceptibility testing, either a fragment or the full length HCV genome is 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. Both Sanger (direct) sequencing and next generation sequencing (NGS) platforms are now available.

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. The method can reliably detect RAS if present in $\geq 15\text{-}25\%$ of the total virus population.

Most NGS 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. Minor variants present at 1% of the total population may be detected on this platform. For clinical purposes, a cut-off of 15% is recommended, which approximates to the results of Sanger sequencing. Below this cut-off, RAS are less likely to be clinically significant and susceptibility will be reported [4, 11, 12, 16]. Where data from clinical trials was generated through an NGS platform, this 15% threshold has been applied in the DAA-specific guidance of **Sections B and C**, unless otherwise specified.

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 resistance testing

In phenotypic testing, RAS are introduced as point mutations into a sub-fragment of the HCV genome that has the capability of replicating viral RNA in tissue culture cells (the sub-genomic 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 EC₅₀, 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.

4. Indications for resistance testing

Resistance testing may be indicated in the following scenarios:

RAS testing in DAA-naïve individuals is recommended prior to initiation of therapy for some treatment regimens in view of the reduction in SVR12 that may be associated with particular baseline RAS. See **section B** for details of specific DAA regimens.

In cases of failure following DAA-based therapy, the results of RAS testing may inform the choice of the retreatment 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-harboring variant becoming a minor population over time with the dominant population having wild-type characteristics. See **sections B and C** for resistance testing recommendations prior to retreatment with specific DAA regimens.

5. Interpretation of results

Clinical context

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

- patient characteristics, such as the presence of hepatic cirrhosis (particularly if decompensated) and prior treatment history
- proposed DAA regimen, such as regimen composition and interactions with concomitant medications
- viral characteristics, such as the genotype (particularly genotype 3), subtype (where not commonly prevalent in high income countries, such as 1l 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

Given these multiple factors, treatment decisions should not be made solely on the basis of resistance testing. It is recommended that such decisions are made by a multi-disciplinary team (MDT), which is likely to include hepatologists, infectious disease physicians, pharmacists, clinical nurse specialists and virologists.

Management approaches following identification of RAS

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

- prolongation of DAA therapy
- intensification of therapy with an additional drug, such as weight-based ribavirin
- avoidance of a particular DAA regimen

If resources permit, ribavirin-sparing strategies may be preferable in many cases to avoid possible ribavirin-associated toxicities.

The decision to offer DAA treatment to a patient who has baseline RAS also needs to consider the predicted reduction in SVR12 rate (if known), the liver disease fibrosis stage and the degree of urgency for viral elimination. Thus, the role of the MDT is to consider the merits of immediate versus deferred exposure of the patient to treatment with DAA. For patients with advanced liver disease, the decision for treatment needs to consider the likely suitability of the patient for liver transplantation and the possible impact of transplantation on future timing and responsiveness to antiviral treatment.

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 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.

6. Genotyping

HCV is currently classified into seven genotypes (1-7), which differ by as much as 30% in their nucleotide sequence. Each genotype is further subdivided into subtypes, which may differ by up to 15% in their nucleotide composition, with >80 currently recognised [20]. 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 [11, 12]. 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 [21, 22].

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 other genotypes and subtypes, particularly those more prevalent in low income countries ('rare' subtypes). More data on the sensitivity of these HCV subtypes to current DAA regimens (including but not limited to genotypes 1l, 4r, 3b and 6a-x) is needed and this represents an unmet need.

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, in which 5' sequence from genotype 2k is combined with non-structural sequences from genotype 1b. Therefore any specimen provisionally assigned by core sequencing or 5'UTR (a non-recommended method) to genotype 2 should be additionally sequenced for NS5B or for the whole genome, in order to exclude a recombinant form [23].

7. 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 considerably, with most estimates reported at between 1-30% [24-31]. This broad range likely reflects the heterogeneity of the populations sampled, with the highest rates of mixed infection in groups with frequent HCV exposures, such as people who inject drugs (PWID). In addition, identification of mixed infection may be limited by the sequencing method, with studies relying on Sanger sequencing failing 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 in the context of clearance of the dominant infecting genotype. The newly-identified, emergent genotype may be incorrectly interpreted as a new infection and, in addition, may have developed RAS following DAA exposure, the pattern of failure in this scenario is often viral breakthrough, rather than the more frequently-observed relapse. Where mixed infections are identified prior to therapy, use of a pan-genotypic DAA regimen increases the likelihood for successful cure of both infecting genotypes.

8. Reinfection

Reinfection is common amongst certain populations with repeated exposures to HCV, such as men who have sex with men (MSM) living with HIV, and PWID, with reinfection incidence rates of up to 10-15/100 person-years [32-34]. If reinfection is suspected as a possible cause of recurrence of viraemia during or on completion of therapy, viral sequencing should be performed on the failure specimen and compared with the sequence of the pre-treatment specimen to allow distinction between relapse and reinfection. NGS may be preferable in this scenario, in view of the greater sequencing

information it provides. Sequencing is particularly relevant where recurrence of viraemia occurs with the same subtype in individuals with continued exposure to HCV. However, this approach cannot distinguish between treatment failure and reinfection from the same source.

Where sequencing has not been performed prior to DAA therapy, it is recommended that a pre-treatment blood specimen is stored locally for a minimum of six months, which if necessary may be tested retrospectively in parallel to the failure specimen.

9. 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. For example, only around half of PWID sampled in the Unlinked Anonymous Survey in 2016 were aware of their positive HCV antibody status [35, 36].

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 pan-genotypic DAA regimen which retains potency against RAS-harboured virus (ie 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.

10. Assay turnaround time

It is critical that the results of resistance testing are made available in a timely fashion and do not delay treatment initiation. Results should therefore be available within approximately 7-10 working days.

B. 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

1. Sofosbuvir-Ledipasvir

Genotypes 1, 4, 5 and 6. Treatment-naïve individuals or those exposed to pegylated interferon-ribavirin with or without a protease inhibitor, or sofosbuvir-ribavirin with or without pegylated interferon

The NS5A inhibitor ledipasvir in combination with the NS5B nucleotide analogue, sofosbuvir, is coformulated as a fixed drug combination (Harvoni) and recommended for the treatment of HCV genotypes 1a, 1b, 4, 5 and 6.

AASLD and EASL guidelines recommend treatment durations of between 8 and 24 weeks, with or without ribavirin, according to individual patient- and virus-associated characteristics. Factors influencing the treatment duration and/or need for ribavirin are: HCV genotype/subtype, treatment history, cirrhosis status, baseline viral load, and presence of ledipasvir RAS (conferring ≥ 100 fold change in the EC50).

Ledipasvir

In vitro

Through *in vitro* replicon systems, RAS noted to confer high (≥ 100 x EC50) fold changes to ledipasvir for genotype 1a include Q30E/H/K/R, L31M/V, 32del, H58D and Y93C/H/N [37-39].

For genotype 1b, L31V, 32del and Y93H/N were observed to confer high fold resistance [37, 39].

For genotype 4a, Y93H was reported as conferring a 135 fold reduction in susceptibility to ledipasvir *in vitro*, with no other individual RAS conferring high fold change resistance. Of note, however, RAS impact on ledipasvir susceptibility may be different in non-4a/d subtypes (ie subtypes not commonly found in high income countries). For

example, L31M reduced susceptibility by up to 141 fold in genotype 4r. For some subtypes, such as genotype 4b, there may be a high prevalence of multiple NS5A RAS within naïve populations, with averages of 3.3 RAS for genotype 4b versus 1.8 RAS across all genotype 4 subtypes, respectively. In addition, the impact of the same multiple RAS pattern may vary between subtypes, with examples reported of a greater reduction in susceptibility to ledipasvir *in vitro* for non-4a/d subtypes such as 4b or 4r [40].

For genotypes 5a and 6a, L31F/V and L31M/V were observed to confer high fold resistance, respectively [39].

Phase II, III and IV clinical trials

Several pooled analyses have reported SVR12 rates in individuals receiving therapy with sofosbuvir-ledipasvir in the context of baseline RAS [16, 17].

Zeuzem *et al* presented a pooled analysis of data from 15 phase II and III studies* including 1,765 individuals, both cirrhotic and noncirrhotic, who were treated with sofosbuvir-ledipasvir, with or without ribavirin, according to 2015 AASLD or EASL guidelines [16]. A slight impact of baseline high FC (≥ 100) ledipasvir RAS on SVR12 in genotype 1a therapy-naïve individuals was noted: SVR12 91% (42/46) versus 99% (539/546) in those with and without ledipasvir RAS, respectively. By contrast, there was no impact of baseline ledipasvir RAS on SVR12 in genotype 1b therapy-naïve individuals (SVR12 99% in those with and without ledipasvir RAS).

* GS-US-334-1274, ION-1, ION-3, ION-2, GS-US-337-0113, ION-4, LONESTAR 1, GS-US-337-0121, Electron-2, Solar-1, Solar-2, GS-US-337-0125, GS-US-337-0131, Trilogy-1, GS-US-337-1406

For treatment-experienced individuals infected with HCV genotype 1a (excluding those exposed to NS5A inhibitors), there was a substantial impact of baseline ledipasvir RAS on treatment efficacy, with an SVR12 of 76% (22/29) vs 97% (409/420) in those with and without ledipasvir RAS, respectively. For treatment-experienced individuals infected with HCV genotype 1b (excluding those exposed to NS5A inhibitors), a modest impact of baseline ledipasvir RAS was observed, with SVR12 89% (41/46) vs 98% (267/272) in those with and without ledipasvir RAS, respectively [16].

A separate pooled analysis was performed by Sarrazin *et al*, involving 2144 HCV genotype 1-infected individuals, with and without cirrhosis, who had been recruited to five of the same studies as outlined in the analysis by Zeuzem *et al* (ION-1, ION-2, ION-3, Lonestar-1, Electron-2). The analysis included individuals treated with both guideline-recommended and investigational regimens containing sofosbuvir-ledipasvir +/- ribavirin of 8-24 weeks' duration [17]. Overall, 16% of individuals had baseline NS5A RAS.

Baseline ledipasvir RAS had no effect on SVR12 in individuals with genotype 1b infection. SVR12 was reduced where baseline ledipasvir RAS (FC \geq 100) were present in treatment-naïve individuals infected with genotype 1a receiving 8 weeks of sofosbuvir-ledipasvir (SVR12 83%, 24/29), but with no impact for individuals receiving 12 weeks of therapy (SVR12 96%, 45/47). Of note, 3 of the 5 patients not achieving SVR12 following 8 weeks of therapy had a baseline viral load > 6 million IU/mL. Baseline ledipasvir RAS reduced SVR12 in genotype 1a-infected individuals with previous exposure to pegylated interferon-ribavirin +/- a protease inhibitor who received therapy with 12 weeks of sofosbuvir-ledipasvir, but with no impact for 24 weeks of sofosbuvir-ledipasvir (SVR12 65%, 11/17 and 100%, 6/6, respectively). A reduction in SVR12 was observed in the therapy-experienced group with 12 weeks of sofosbuvir-ledipasvir plus ribavirin in the presence of baseline ledipasvir RAS, but there was minimal impact on SVR12 with 24 weeks of the same therapy (82%, 9/11 and 92%, 11/12, respectively). (The single individual failing therapy in the latter group was non-compliant.) [17].

These results contrast with those of the SIRIUS study, a phase II study which included individuals with cirrhosis and HCV genotype 1a infection who had previously not achieved SVR12 with pegylated interferon-ribavirin and a protease inhibitor. Individuals with baseline ledipasvir RAS achieved an SVR12 of 100% (8/8) and 78% (7/9) with 12 weeks of sofosbuvir-ledipasvir plus ribavirin and 24 weeks of sofosbuvir-ledipasvir, respectively [41]). Overall, the small number of therapy-experienced individuals with RAS in the different therapy arms limits interpretation of differences between the two studies.

A phase II open label study enrolling 30 genotype 1a-infected individuals, with or without cirrhosis, who had previously not achieved SVR12 following therapy with sofosbuvir-ribavirin with or without pegylated-interferon, found no impact of baseline NS5A RAS on SVR12 following 12 weeks of sofosbuvir-ledipasvir with ribavirin [42]. It was not possible to characterise any impact of NS5A RAS on SVR12 with 12 weeks of sofosbuvir-ledipasvir in this cohort, as there was no ribavirin-free arm.

For HCV genotype 4, resistance analyses have been presented for a phase II study including both cirrhotic and non-cirrhotic individuals, who were treatment-naïve or had previously failed (an unspecified) therapy. Most had genotype 4a or 4d infection. SVR12 rates following 12 weeks of sofosbuvir-ledipasvir were 24/27 (89%) and 100% (17/17) in those with and without baseline NS5A RAS [43]. Also of note, in a prospectively-recruited, phase IV, single-arm study in Rwanda of genotype 4 infected, DAA naïve individuals, with and without compensated cirrhosis, only 54% (21/39) of those with subtype 4r achieved SVR12 following 12 weeks of sofosbuvir-ledipasvir, compared to 92% (240/261) for other subtypes (mostly 4k, 4q or 4v), although RAS data was not presented [44].

In a small phase II study including 40 cirrhotic and noncirrhotic individuals infected with genotype 5a, both therapy-naïve and those previously treated with pegylated interferon-ribavirin +/- a protease inhibitor, baseline NS5A RAS did not impact SVR12 following 12 weeks of sofosbuvir-ledipasvir therapy [45]. Similarly, a phase II study including 25 individuals (therapy naïve or pegylated interferon-ribavirin experienced) with HCV genotype 6 of multiple subtypes did not identify an effect of baseline NS5A RAS on SVR12 following 12 weeks of sofosbuvir-ledipasvir therapy [46].

Real world data

A recently presented NHS England dataset of approximately 1800 DAA-naïve, HCV genotype 1a-infected patients, both with and without compensated cirrhosis, who received therapy with sofosbuvir-ledipasvir or sofosbuvir-ledipasvir and ribavirin, identified SVR12 rates of 94-98% and 93-96% respectively [6]. SVR12 was lower in patients with decompensated cirrhosis (94% and 90% for those receiving sofosbuvir-ledipasvir with and without ribavirin, respectively). The majority of treating centres are thought unlikely to have used RAS testing in treatment decisions. Although information on RAS prevalence, treatment history and duration of DAA regimens were not presented, this large dataset suggests that high SVR12 with sofosbuvir-ledipasvir may be achieved for most patients in the NHS setting without routine pre-therapy RAS testing. High SVR12 (93-99%) was also identified for genotype 1b. Few (<200) patients with genotype 4 infection were included [6].

A German real world dataset including 1117 genotype 1a infected patients with and without cirrhosis also identified comparable SVR12 for those receiving 8 versus 12 weeks of sofosbuvir-ledipasvir (SVR12 98% according to the per protocol analysis). Of note, most of those receiving the shorter duration were therapy-naïve patients without cirrhosis [47]. By contrast, a US real world study including 4365 treatment-naïve, genotype 1 infected individuals with and without cirrhosis identified slightly lower SVR12 for patients receiving 8 versus 12 weeks of sofosbuvir-ledipasvir (SVR12 92% vs 94% respectively) . Detailed resistance analyses were not available for either study [48].

Sofosbuvir

There is minimal impact of baseline sofosbuvir RAS on SVR12 following sofosbuvir-ledipasvir-containing therapies. In a pooled analysis of phase II & III studies including 8598 HCV genotype 1-infected individuals exposed to sofosbuvir, with or without ledipasvir, the signature mutation of S282T was detected in only 1% (10/901) of individuals with virological failure [18]. *In vitro*, S282T conferred only a modest (2-19 fold) reduction in susceptibility to sofosbuvir and associated replication capacity was reduced to only 3-22% of wild type [49].

Ledipasvir-sofosbuvir: summary

Outcome data from a large NHS dataset is reassuring that routine pre-therapy RAS testing may not be necessary prior to use of sofosbuvir-ledipasvir with or without ribavirin in genotype 1a [6].

However, where NS5A RAS testing has been performed, there is data supporting the following approach.

Where ledipasvir RAS (≥ 100 FC) are detected in individuals with genotype 1a infection planned to receive 8 weeks of sofosbuvir-ledipasvir, therapy should be extended to 12 weeks or an alternative regimen used [17].

Where ledipasvir RAS (≥ 100 FC) are detected in individuals without cirrhosis who have previously failed therapy with pegylated interferon-ribavirin +/- a protease inhibitor, 12 weeks of sofosbuvir-ledipasvir plus ribavirin, 24 weeks of sofosbuvir-ledipasvir, or an alternative regimen are advised [17, 41].

Where ledipasvir RAS (≥ 100 FC) are detected in individuals with cirrhosis, who have previously failed therapy with pegylated interferon-ribavirin +/- a protease inhibitor, 24 weeks of sofosbuvir-ledipasvir plus ribavirin or an alternative regimen are recommended [17].

In view of the current paucity of data on the impact of RAS in subtypes not commonly found in high income countries, including genotypes 4-6, pre therapy NS5A RAS testing is recommended in these patients, to provide data to guide future practice [43, 45, 46].

Data suggests that NS5A RAS testing does not inform the management of patients in the following scenarios:

Genotype 1a infected therapy-naive individuals being proposed for 12 weeks of sofosbuvir-ledipasvir [16, 17].

Genotype 1a infected individuals with a history of treatment failure on sofosbuvir-ribavirin, with or without pegylated interferon, prior to retreatment with sofosbuvir-ledipasvir and ribavirin [42].

Genotype 1b infected, therapy naïve individuals or those with a history of failure on pegylated interferon-ribavirin +/- a protease inhibitor [16, 17].

Routine baseline NS5B resistance testing is not recommended in individuals being proposed for therapy with sofosbuvir-ledipasvir [18, 49].

2. Ritonavir-boosted paritaprevir, ombitasvir, dasabuvir (PrOD) +/- ribavirin in genotype 1

Ritonavir-boosted paritaprevir, ombitasvir (PrO) + ribavirin in genotype 4

For therapy-naïve individuals and those previously treated with pegylated interferon-ribavirin

The combination of ritonavir-boosted paritaprevir, an NS3/4A protease inhibitor, ombitasvir, an NS5A inhibitor, and dasabuvir, a non-nucleoside NS5B RdRp inhibitor (Abbvie 3D or Viekirax/Exviera) is licensed for genotypes 1a and 1b, with or without ribavirin, respectively. For genotype 4, ritonavir-boosted paritaprevir with ombitasvir (Abbvie 2D or Viekirax) and ribavirin is the licensed combination.

In vitro

In replicon systems, dasabuvir selects for NS5B RAS including the following which confer high fold changes: C316Y and Y448C/H in genotype 1a and C316Y, S368T, C448C and A553V in genotype 1b [50].

In vitro, ombitasvir selects for high fold change NS5A RAS including M28T, Q30R, H58D and Y93C/H/N/S in genotype 1a and L28T in genotype 1b [51]. L28V confers high fold reduction in susceptibility in genotype 4d [52]. Combinations of RAS have also been frequently selected in genotypes 1a/1b and may confer substantial reductions in susceptibility [51, 52].

For ritonavir-boosted paritaprevir, high fold change NS3 RAS selected *in vitro* include D168Y in genotype 1a, D168V/Y in genotype 1b [53] and D168V in genotypes 4a and 4d [52].

Phase II & III clinical trials

A Phase II study reported outcomes for 406 genotype 1a or 1b infected individuals without cirrhosis, who were therapy naïve or had previously received unsuccessful therapy with pegylated interferon-ribavirin, and were treated with PrOD with or without ribavirin for 8-24 weeks. There was no impact of baseline RAS on SVR12 [54]. Similarly, a pooled resistance analysis of phase III randomised controlled trials of PrOD with or without ribavirin (Turquoise-II, Sapphire-II and -IV) has been presented, including individuals with and without cirrhosis, both therapy-naïve and those with prior unsuccessful pegylated interferon-ribavirin therapy. For 736 genotype 1a infected individuals, the presence of baseline RAS did not impact SVR12 where individuals were

treated according to the SPC recommendation [55]. Similarly, baseline RAS did not impact SVR12 for genotype 1b with this regimen (Turquoise-III and Pearl-II) [56].

Regarding genotype 4, high SVR12 rates (97-100%) were reported for the phase II PEARL-I study, which included individuals without cirrhosis who were therapy-naïve or had previously received unsuccessful pegylated interferon-ribavirin containing therapy. Most patients were infected with genotype 4a or 4d. There was no impact of baseline NS5A RAS on SVR12 in those receiving 12 weeks of PrO with ribavirin (SVR12 100%, 56/56) [52]. A phase III randomised controlled study of 120 genotype 4 infected individuals with compensated cirrhosis (Agate-1), predominantly with 4a or 4d subtype, similarly found no impact of baseline NS5A RAS on SVR12 with this regimen [57].

Ritonavir-boosted paritaprevir and ombitasvir, with or without dasabuvir: summary

Data does not support the use of baseline resistance testing prior to initiation of therapy with PrOD for genotypes 1a or 1b or prior to therapy with PrO for genotypes 4a or 4d [54, 55, 57] [52].

3. Elbasvir-grazoprevir

Genotypes 1 & 4 – Therapy-naïve individuals or those previously treated with pegylated interferon-ribavirin with or without a protease inhibitor

The regimen of elbasvir, an NS5A inhibitor, and grazoprevir, an NS3/4A inhibitor (coformulated as Zepatier) with or without ribavirin, is licensed for the treatment of genotype 1a, 1b and 4 infections, in treatment-naïve individuals and those previously treated with pegylated interferon-ribavirin with or without a protease inhibitor.

In vitro

In replicon systems, individual NS5A RAS conferring substantially (≥ 100 fold) reduced susceptibility to elbasvir have been identified for genotype 1a including Q30D/R, L31F/V, del32 and Y93C/H/N. For genotypes 1b and 4, single NS5A RAS may be associated with only slight reductions in susceptibility e.g. Y93H (17 fold) and L30F (15 fold) for genotypes 1b and 4, respectively [1, 39, 58-60].

RAS conferring ≥ 100 fold change in susceptibility to grazoprevir *in vitro* include D168A/V for genotype 1a and A156T and D168K in genotype 1b [58, 61].

For both drugs, combinations of ≥ 2 RAS may have considerably greater impact than individual RAS [39, 58].

Phase II & III clinical trials

A pooled analysis of phase II and III clinical trials of elbasvir and grazoprevir in 1969 cirrhotic and non-cirrhotic, treatment-naïve individuals and those previously treated with pegylated interferon-ribavirin with or without a protease inhibitor, with HCV genotype 1a, 1b or 4 infection, was performed by the US Food and Drug Administration (the C-SURFER, C-EDGE TN, C-EDGE Coinfection, C-EDGE TE, C-WORTHY and C-SALVAGE studies). Following 12 weeks of therapy with elbasvir-grazoprevir, SVR12 was 70% (39/56) versus 98% (441/450) in individuals infected with genotype 1a with and without baseline elbasvir RAS (positions M28, Q30, L31, or Y93), respectively [1]. In a subsequent analysis, a low baseline HCV RNA level ($< 800,000$ IU/mL) appeared to reduce the impact of NS5A RAS on SVR12, with 8/8 individuals with elbasvir RAS and an HCV RNA $< 800,000$ IU/mL achieving SVR12 [62]. Of note, M28V was the commonest variant seen of the four positions, and was associated with a modestly 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 NS5A RAS [1].

In a further pooled analysis confined to individuals with cirrhosis enrolled in the same studies, SVR12 was reduced in genotype 1a infected individuals following 12 weeks of elbasvir-grazoprevir in those with versus those without baseline NS5A RAS (SVR12 73%, 8/11 versus 98%, 96/98, respectively) [63].

For genotype 1b only slightly lower SVR12 was seen in those with versus those without baseline elbasvir RAS (SVR12 94% (48/51) versus 99% (247/248), respectively) with only 4 treatment failures overall [1]. In a separate pooled analysis involving five of the six studies outlined above, individuals infected with HCV genotype 1b with elbasvir RAS who had had no response to pegylated interferon and ribavirin achieved an SVR12 of only 67% (4/6) following 12 weeks of elbasvir-grazoprevir, versus 100% (12/12) following 16-18 weeks of elbasvir/grazoprevir with ribavirin [64]. There was no impact of elbasvir RAS in therapy-naïve individuals or those with relapse on prior therapy. Together, this data suggests that although baseline NS5A RAS rarely impact on SVR12 in genotype 1b-infected patients receiving elbasvir-grazoprevir therapy, a small number of patients with prior non-response to pegylated interferon-ribavirin and elbasvir RAS may benefit from intensified therapy.

For genotype 4 (mostly 4a or 4d), no significant reduction in SVR12 was observed in the context of baseline NS5A RAS in the FDA analysis, where recommended therapy was used [1].

Baseline NS3 RAS did not impact SVR12 in genotypes 1a, 1b and 4 for cirrhotic and noncirrhotic, treatment-naïve individuals, or those with prior exposure to pegylated interferon-ribavirin with or without a protease inhibitor [1].

Elbasvir-grazoprevir: summary

Data supporting use of RAS testing in some or all patient groups.

Data is available supporting the use of baseline NS5A RAS testing in genotype 1a-infected, treatment-naïve individuals and those with prior exposure to pegylated interferon-ribavirin with or without a protease inhibitor, both with and without cirrhosis, prior to starting elbasvir-grazoprevir. Individuals with elbasvir RAS should receive an alternative regimen, or 16 weeks of therapy with ribavirin [1].

Data suggesting RAS testing not required.

Data does not support the routine use of baseline NS5A RAS testing in individuals infected with genotype 1b prior to starting therapy with elbasvir-grazoprevir due to the minor reduction in SVR12 with baseline elbasvir RAS. However, there may be a small number of individuals, particularly those with exposure to pegylated interferon-ribavirin with or without a protease inhibitor, for whom baseline elbasvir RAS impact SVR12 [1, 64].

Data does not support the use of baseline NS5A RAS testing in individuals infected with genotype 4a or 4d prior to starting elbasvir-grazoprevir [1].

Data does not support the use of baseline NS3 RAS testing in individuals infected with HCV genotype 1a, 1b or 4 prior to starting elbasvir-grazoprevir [1].

4. Sofosbuvir-velpatasvir

Treatment-naïve individuals and those previously treated with pegylated interferon-ribavirin with or without a protease inhibitor

This regimen represents the combination of the NS5B nucleotide analogue inhibitor sofosbuvir with the second generation NS5A inhibitor, velpatasvir. The combination is licensed for genotypes 1-6 and prescribed as a single tablet regimen, Epclusa, for 12-24 weeks, with or without ribavirin.

Velpatasvir

In vitro

In vitro, velpatasvir has significant potency against replicons of genotypes 1-6. RAS conferring high (>100x EC₅₀) fold changes include L31V and Y93C/H/N/R/W in genotype 1a, F28S and Y93H in genotype 2a, L31V and Y93H in genotype 3a and L31V in genotype 6a. Combinations of more than one NS5A RAS may also be associated with high fold changes, such as L31M+Y93H in genotype 1b [39, 65, 66].

Phase III clinical trials

Randomised controlled phase III studies have demonstrated high efficacy of sofosbuvir-velpatasvir in cirrhotic and non-cirrhotic, treatment naïve individuals, and those who had received prior treatment, predominantly with pegylated interferon-ribavirin with or without a protease inhibitor. A pooled resistance analysis of the ASTRAL 1-3 and 5, and POLARIS 2 and 3 studies reported no difference in SVR₁₂ for 1300 individuals with and without baseline NS5A RAS for genotypes 1, 2, 4, 5 and 6 (SVR₁₂ 97-100%) [3].

For genotype 3 (predominantly 3a) infected individuals, SVR₁₂ was 93% (53/57) and 98% (411/420) in individuals with and without NS5A RAS, respectively. SVR₁₂ was lowest for those with the Y93H mutation (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, with SVR₁₂ of 89% (33/37) in individuals with both these characteristics. In the small number of individuals with cirrhosis and Y93H, SVR₁₂ was only 67% (4/6) [3, 67].

In the phase III ASTRAL-4 study, 267 individuals with genotypes 1, 2, 3, 4 or 6 with decompensated cirrhosis who were treatment-naïve or had received prior treatment with pegylated interferon-ribavirin with or without a protease inhibitor, were randomised to receiving 12 weeks of sofosbuvir-velpatasvir, 12 weeks of sofosbuvir-velpatasvir and ribavirin or 24 weeks of sofosbuvir-velpatasvir. At baseline, 72/255 (28%) had baseline

NS5A RAS as assessed by NGS with a 1% cut off. SVR12 in genotype 1-infected individuals with and without baseline NS5A RAS was 80% and 96%, respectively in those receiving 12 weeks of sofosbuvir-velpatasvir and 90% and 98%, respectively in those receiving 24 weeks of sofosbuvir-velpatasvir. Individuals receiving 12 weeks of therapy with sofosbuvir-velpatasvir plus ribavirin achieved an SVR12 of 100% and 98% in those with and without baseline NS5A RAS, respectively. Only 6 individuals with genotype 3 harbouring NS5A RAS were included in this study, limiting resistance data interpretation for this genotype [2].

Real world studies

A Spanish real world study reported outcomes in genotype 3-infected individuals with cirrhosis and included those with prior treatment failure, including NS3 or NS5B inhibitor-containing regimens, following 12 weeks of sofosbuvir-velpatasvir with or without ribavirin. SVR12 in those with baseline Y93H who received treatment with and without ribavirin was 89% (8/9) versus 50% (2/4), respectively [68]. In contrast, an analysis of the prospectively-recruited, real world GECCO cohort, which included 293 predominantly therapy-naïve and pegylated interferon-ribavirin experienced individuals, both cirrhotic and noncirrhotic, found no impact of Y93H on SVR12 in genotype 3 infection (subtype not reported) following treatment with 12 weeks of sofosbuvir-velpatasvir with or without ribavirin. However, only 11 individuals (5%) had baseline Y93H, and only one individual with cirrhosis and Y93H was included [69].

In an NHS England dataset of approximately 1500 genotype 3 infected DAA-naïve individuals without cirrhosis, SVR12 was 97-98% following sofosbuvir-velpatasvir and 96-100% following sofosbuvir-velpatasvir plus ribavirin. For 470 genotype 3 infected patients with compensated cirrhosis, SVR12 was 92-93% and 95% in those receiving sofosbuvir-velpatasvir and sofosbuvir-velpatasvir plus ribavirin, respectively [6]. Lower SVR12 (84-86%) was seen for 63 individuals with decompensated cirrhosis receiving therapy with sofosbuvir-velpatasvir +/- ribavirin. Information on RAS prevalence, use of pre-therapy RAS testing, and prior treatment history, were not available.

Sofosbuvir

The presence of baseline NS5B RAS did not impact SVR12 for individuals infected with genotypes 1-6 who received therapy with sofosbuvir-velpatasvir with or without ribavirin [3]. Individuals failing therapy with sofosbuvir-velpatasvir did not develop sofosbuvir-specific NS5B RAS [2, 3].

Sofosbuvir-velpatasvir: summary

Data supporting use of RAS testing in some or all patient groups.

Data is available supporting the use of baseline NS5A RAS testing in genotype 3a infected individuals with cirrhosis, both treatment-naïve and those who have previously received therapy with pegylated interferon-ribavirin, who are being considered for 12 weeks of sofosbuvir-velpatasvir. If Y93H is identified, addition of ribavirin, extension of therapy from 12 to 24 weeks, or an alternative regimen is recommended [3, 67].

Data is available supporting the use of baseline NS5A RAS testing in HCV genotype 1 infected individuals with decompensated cirrhosis who are treatment-naïve or have previously received therapy with pegylated interferon-ribavirin with or without a protease inhibitor prior to treatment with 12 weeks of sofosbuvir-velpatasvir. In those with NS5A RAS, addition of ribavirin is recommended or extension of therapy to 24 weeks [2].

In view of the current paucity of data on the impact of RAS in subtypes not commonly found in high income countries, including genotypes 4-6, pre therapy NS5A RAS testing is recommended prior to therapy with sofosbuvir-velpatasvir, to provide data to guide future practice.

A large real world NHS dataset suggests routine pre-therapy RAS testing may not be necessary prior to use of sofosbuvir-velpatasvir in genotype 3 infected individuals without cirrhosis [6].

However, where RAS testing has been performed, the following approach is suggested.

Individuals who have previously received unsuccessful therapy with interferon-containing therapy who are being considered for 12 weeks of sofosbuvir-velpatasvir : if Y93H is identified, addition of ribavirin, extension of therapy from 12 to 24 weeks, or an alternative regimen is recommended [3, 67]

Data suggesting RAS testing not required.

Data suggests that routine NS5A RAS testing may not be necessary prior to therapy with sofosbuvir-velpatasvir in genotype 3 infected, therapy-naive individuals without cirrhosis [3].

Data does not support the use of baseline NS5A RAS testing prior to therapy in genotype 1 or genotype 2 infected, therapy-naïve individuals, or those with prior treatment with pegylated interferon-ribavirin, both with and without compensated cirrhosis, prior to starting sofosbuvir-velpatasvir [3].

Data does not support the use of baseline NS5B RAS testing prior to therapy with sofosbuvir-velpatasvir for genotypes 1-6 [3].

Areas of unmet need

For genotype 3a infected individuals with cirrhosis the potential impact on SVR12 of substitutions other than H at position Y93 remain to be confirmed

5. Glecaprevir-pibrentasvir

Therapy-naïve individuals and those previously treated with pegylated interferon-ribavirin with or without sofosbuvir.

The DAA agents, glecaprevir and pibrentasvir, are next generation inhibitors of the HCV NS3/4A protease and NS5A protein, respectively. They are coformulated into a fixed dose combination (Maviret), which is prescribed for between 8 and 16 weeks. Both agents demonstrate pangentotypic activity and a high genetic barrier to resistance.

In vitro

In replicon systems, individual NS3 RAS at a limited number of positions were associated with reduced susceptibility to glecaprevir. Those at position A156 conferred the greatest reduction in susceptibility (>100 fold change in EC50): A156T in genotype 1a, A156T/V in genotypes 1b, 2a, 2b and 4a, and A156G in genotype 3a. The RAS D168H was associated with a high fold reduction in susceptibility in genotype 6a. Some

combinations of two or more RAS had a synergistic impact on resistance, such as Y56H+Q168R in genotype 3a [70].

Pibrentasvir rarely selected for RAS in replicon systems and no individual NS5A RAS conferred >100 fold resistance in the EC50 with respect to wild type for genotypes 1a, 1b, 2a, 2b, 3a, 4a, 5a and 6a. In particular, Y93H/N conferred <7 fold resistance in genotypes 1a and 3a. The deletion del32 conferred high fold change in genotypes 1a and 1b [39]. Also, some combinations of RAS appeared to interact synergistically to produce substantial reductions in susceptibility, such as Q30R+Y93H for genotype 1a [71] and F28S+Y93H for genotype 2a [39]. Of note, for genotype 3a, A30K+Y93H conferred 69 fold resistance [5].

Phase II & III clinical trials

A pooled analysis of resistance data from eight phase II and III clinical trials of glecaprevir-pibrentasvir has been presented (Surveyor 1 and 2, Endurance 1, 2, 3 and 4, and Expedition 1 and 2). Cirrhotic and non-cirrhotic individuals, who were infected with genotypes 1-6, and were treatment-naïve or -experienced (prior pegylated interferon -ribavirin with or without sofosbuvir) received 8-16 weeks of therapy at the recommended dose [5].

Overall, there were <1% virological failures (22/2256), with the majority of failures amongst the genotype 3-infected individuals (predominantly genotype 3a) (18/22, 82%). SVR12 treatment outcome was unaffected by presence of baseline NS3 RAS for all genotypes (1-6), or by baseline NS5A RAS for genotypes 1, 2, 4, 5 and 6.

Regarding genotype 3 infected individuals without cirrhosis and the baseline NS5A RAS A30K, SVR12 was achieved in 14/18 (78%) and 13/14 (93%) of treatment-naïve individuals receiving 8 and 12 weeks of glecaprevir-pibrentasvir, respectively, and for 1/4 (25%) treatment-experienced (prior pegylated interferon -ribavirin with or without sofosbuvir) individuals receiving 12 weeks of glecaprevir-pibrentasvir. Inclusion of only one individual per group limited analysis of results for individuals with baseline A30K who were therapy-experienced without cirrhosis receiving 16 weeks of glecaprevir-pibrentasvir, or who had baseline A30K and cirrhosis [5].

For genotype 3 infected, treatment-naïve individuals without cirrhosis and the baseline NS5A RAS Y93H, SVR12 was achieved in 10/10 (100%) and 10/11 (91%) of individuals receiving 8 and 12 weeks of glecaprevir-pibrentasvir respectively, and for 2/4 (50%) treatment-experienced individuals (prior pegylated interferon-ribavirin with or without sofosbuvir) who received 12 weeks of glecaprevir-pibrentasvir. No therapy-experienced individual without cirrhosis who had this RAS at baseline received 16 weeks of therapy. For therapy-naïve individuals with cirrhosis and baseline Y93H, 5/5 (100%) achieved

SVR12 with 12 weeks of glecaprevir-pibrentasvir. Only one therapy-experienced individual with baseline Y93H and cirrhosis was included [5].

For individuals infected with genotype 3 who did not achieve SVR12, the most frequent treatment emergent NS5A RAS overall was Y93H (15/18, 83%). This may reflect the requirement for only one nucleotide substitution to generate Y93H. By comparison, development of A30K from wild type requires two substitutions and was rarely observed as a treatment-emergent RAS [5].

Real world data

An NHS dataset of 642 DAA-naïve, genotype 3-infected patients, with and without cirrhosis, identified SVR12 of 97-98% for individuals receiving therapy with glecaprevir-pibrentasvir. Although data on prior treatment history, HCV subtype and RAS data was not available, this large real world dataset suggest routine pre-therapy RAS testing may not be necessary prior to use of glecaprevir-pibrentasvir [6].

Glecaprevir-pibrentasvir: summary

A large NHS dataset is reassuring that routine pre-therapy RAS testing may not be necessary prior to use of glecaprevir-pibrentasvir in DAA-naïve individuals infected with HCV genotype 3.

However, where RAS testing has been performed, there is data supporting the following approach:

treatment-naïve individuals without cirrhosis infected with genotype 3 and the NS5A RAS A30K, who are being considered for 8 weeks of therapy with glecaprevir-pibrentasvir, should receive an extended duration of 12 weeks of treatment or receive an alternative regimen [5].

In view of the current paucity of data on the impact of RAS in subtypes not commonly found in high income countries, including genotypes 4-6, pre therapy NS5A RAS testing is recommended prior to therapy with glecaprevir-pibrentasvir, to provide data to guide future practice.

Data does not support the use of baseline NS5A RAS testing in treatment-naïve individuals and those with prior exposure to pegylated interferon-ribavirin with or without sofosbuvir, who are infected with genotypes 1 or 2 prior to therapy with glecaprevir-pibrentasvir [5].

Data does not support the use of baseline NS3 RAS testing in treatment-naïve individuals and those previously exposed to pegylated interferon-ribavirin with or without sofosbuvir, who are infected with genotypes 1-6, prior to therapy with glecaprevir-pibrentasvir [5].

6. Sofosbuvir-velpatasvir-voxilaprevir

The combination of sofosbuvir, velpatasvir and voxilaprevir, a second generation inhibitor of the NS3/4A protease, coformulated as Vosevi, has recently been licensed for use in both DAA-naïve and DAA-experienced individuals. The regimen has pan-genotypic activity and a high genetic barrier to resistance. International guidelines currently do not recommend sofosbuvir-velpatasvir-voxilaprevir in treatment-naïve or pegylated interferon and ribavirin-experienced individuals infected with genotypes 1, 2, 4, 5 or 6 with or without cirrhosis, or for genotype 3 infected, therapy-naïve individuals without cirrhosis, owing to the availability of alternative, high efficacy regimens, including sofosbuvir-velpatasvir [4, 12].

In vitro

The only reported individual NS3 RAS conferring a high fold (≥ 100) reduction in susceptibility to voxilaprevir is A156L/T in genotype 1a, A156T/V in genotypes 1b and 3a, A156L/V in genotype 2a and A156L/T/V in genotype 4a with none described for genotypes 5a or 6a. Combinations of two or more RAS may also confer high fold changes, such as Q80K+D168Y for genotype 1a [72, 73].

Phase III clinical trials

A pooled resistance analysis of two phase III studies evaluating the efficacy of 8 weeks of sofosbuvir-velpatasvir-voxilaprevir in 584 DAA-naïve individuals with or without cirrhosis (Polaris-2 and -3), found no impact of baseline NS5A or NS5B RAS on SVR12 for genotypes 1-6, and of baseline NS3 RAS on SVR12 for genotypes 1b, 2, 3, 4, 5 and 6. Overall SVR12 was 94% (257/273) versus 98% (304/311) in those with and without NS3 or NS5A RAS, respectively. However, for genotype 1a, SVR12 was 88% (51/58) versus 94% (101/108) in those with and without the NS3 RAS Q80K, respectively [74].

For HCV genotypes 1-6, baseline RAS did not impact SVR12 in individuals previously treated with DAA-containing therapy who were retreated with 12 weeks of sofosbuvir-velpatasvir-voxilaprevir (**see section C**) [75].

Sofosbuvir-velpatasvir-voxilaprevir: summary

Data does not support the use of baseline resistance testing prior to therapy with sofosbuvir-velpatasvir-voxilaprevir in treatment-naïve individuals [74] or those previously treated with pegylated interferon-ribavirin and/or DAA [75].

7. Sofosbuvir-elbasvir-grazoprevir

The triple combination of sofosbuvir-elbasvir-grazoprevir is currently recommended in genotype 3-infected individuals with compensated cirrhosis who have been treated previously with pegylated interferon-ribavirin [12]. Data from phase II clinical trials also suggest high efficacy in therapy-naïve individuals with genotype 3 infection [76, 77].

Phase II clinical trials

In a phase II, open-label study (C-SWIFT), 29 therapy-naïve individuals without cirrhosis infected with HCV genotype 3 (subtypes not presented), who were treated with sofosbuvir-elbasvir-grazoprevir for 8 or 12 weeks, achieved an SVR12 of 93% (14/15) and 100% (14/14), respectively. Therapy-naïve, genotype 3 infected individuals with cirrhosis received 12 weeks of therapy with an SVR12 of 83% (10/12), although one of the two patients not achieving SVR12 was a study withdrawal. SVR12 was unaffected by the presence of baseline NS3 or NS5A RAS [76]. In the same study, short courses (4-8 weeks) of therapy with sofosbuvir-elbasvir-grazoprevir in treatment-naïve, genotype 1-infected individuals, with or without cirrhosis, yielded a low (32-87%) SVR12 [76].

In the phase II, randomised, open-label C-ISLE study, the efficacy of sofosbuvir-elbasvir-grazoprevir, was further investigated in 100 individuals with compensated cirrhosis infected with HCV genotype 3 (subtypes not presented). Treatment-naïve individuals received 8 weeks of sofosbuvir-elbasvir-grazoprevir plus ribavirin, or 12 weeks of therapy without ribavirin, with SVR12 of 91% (21/23) and 96% (23/24), respectively. Individuals who had previously been treated with pegylated interferon-ribavirin received 12 weeks sofosbuvir-elbasvir-grazoprevir with ribavirin, 12 weeks of the same therapy without ribavirin, or 16 weeks of therapy without ribavirin. SVR12 in these groups was 94% (17/18), 100% (17/17) and 94% (17/18) respectively. The presence of baseline NS3 or NS5A RAS did not impact SVR12 [77].

Sofosbuvir-elbasvir-grazoprevir: summary

Data does not support the use of baseline resistance testing prior to therapy with sofosbuvir-elbasvir-grazoprevir in genotype 3 infected individuals with compensated cirrhosis previously treated with pegylated interferon-ribavirin [76, 77].

Although not currently recommended in international guidelines, data suggests the regimen is effective in genotype 3 infected, therapy naïve individuals, with no impact of baseline RAS on SVR12 [76, 77].

8. Sofosbuvir-glecaprevir-pibrentasvir

The regimen of sofosbuvir-glecaprevir-pibrentasvir is recommended in international guidelines in NS3 and/or NS5A inhibitor experienced individuals [4]. For further details, **see section C**.

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

Background

Approximately 5-10% of individuals receiving DAA therapies in the real world setting do not achieve SVR12 [6, 11, 12, 78].

The majority of these individuals achieve an end-of-treatment response but subsequently experience viral rebound (relapse), which in many cases represents the emergence of a DAA-resistant viral strain. Other possible patterns of failure are viral rebound whilst on therapy (breakthrough) or failure of the viral load to suppress (non-response).

There is currently limited data to inform the optimal strategies for retreatment of individuals experiencing failure with first-line DAA therapies, particularly for non-genotype 1 infections. Treatment failure following NS5A-inhibitor-containing therapy is clinically the most significant. This is due to the high fitness of variants harbouring RAS, which may persist for over 2 years, and the low genetic barrier to developing resistance for this drug class [79].

NS3 RAS also develop frequently following unsuccessful NS3 inhibitor-containing therapy but are less likely to persist, whilst the improved genetic barrier to resistance of newer DAA combinations often overcomes the effect of these RAS [19].

NS5B RAS may also emerge following treatment failure with a regimen containing the non-nucleotide NS5B inhibitor, dasabuvir. However, this currently has little clinical impact on retreatment as dasabuvir remains the only drug in its class and is not recommended in DAA-experienced individuals. NS5B RAS are rarely observed following therapy with sofosbuvir, likely due to the high fitness cost such RAS impart [80]. Retreatment of individuals failing non-NS5A inhibitor containing therapy have largely been considered in previous sections.

Note: In individuals with repeated HCV exposures, such as MSM reporting condomless anal sex with multiple casual partners, or PWID, reinfection rather than 'treatment failure' may account for recurrent viraemia after therapy [33, 81]. Mixed infection may also account for some cases of treatment failure, with emerging dominance of a previously-undiagnosed minor population [24, 82].

Phase II & III clinical trials and real world studies

Sofosbuvir-velpatasvir-voxilaprevir

The pan-genotypic, fixed dose combination of sofosbuvir, velpatasvir and voxilaprevir, is licensed for the retreatment of individuals who have previously failed DAA therapy. Data has recently been presented from POLARIS-4, which enrolled individuals infected with genotypes 1, 2 or 3 and a past history of failure with non-NS5A inhibitor containing therapy and POLARIS-1 that included individuals with genotype 1, and previous failure with an NS5A inhibitor containing regimen (mostly containing ledipasvir, daclatasvir or ombitasvir). Both studies were phase III randomised controlled trials including individuals with and without cirrhosis.

In a pooled analysis (total n= 417), the baseline prevalence of RAS was 69%, of which 83% were NS5A RAS. Overall SVR12 rates of >95% were observed, with no impact of baseline NS3, NS5A and/or NS5B RAS. The lowest SVR12 rate (90%) was seen in HCV genotype 3 infected individuals (subtypes not presented) with cirrhosis and baseline NS5A RAS [75, 83]. An integrated resistance analysis of four phase II studies with this drug combination (n=262), including genotypes 1, 2, 3, 4 and 6, also confirmed the lack of impact of baseline RAS on SVR12 (Lepton, GS-US-367-1168, GS-US-367-1169, Trilog-3) [84]. Notably, however, voxilaprevir and sofosbuvir are contraindicated in individuals with decompensated cirrhosis or an eGFR <30 mL/min/1.73m², respectively.

Glecaprevir-pibrentasvir

In the phase III Magellan-1, Part 2 study, individuals with or without cirrhosis infected with HCV genotype 1 (n=87) or genotype 4 (n=4), who had previously failed NS3- and/or NS5A inhibitor containing therapy, were retreated with glecaprevir-pibrentasvir. Approximately 1/3 (n=30) had received prior therapy with both NS3 and NS5A inhibitors. The median time between first and second DAA regimen was 17 months. For individuals receiving 12 weeks of therapy, 2/2 (100%) with baseline NS3 RAS alone and 20/24 (83%) with NS5A RAS alone achieved SVR12, respectively. Extension of therapy to 16 weeks led to improved SVR12 (22/23, 96%) in the latter group. However, in those with dual class NS3 and NS5A RAS, SVR12 was low for individuals receiving 12 or 16 week therapy (4/5 (80%) and 1/4 (25%), respectively) [8]. A phase II dose-finding study of therapy with 12 weeks of glecaprevir-pibrentasvir with or without ribavirin in 50 genotype 1-infected individuals without cirrhosis who had previously been exposed to an NS3, (n=25) or NS5A inhibitor (n=8) or both classes (n=17) identified lower SVR12 rates (91-93%) in those with baseline NS5A +/- NS3 RAS compared to those with NS3 RAS alone (100%) (Magellan-1, Part 1) [7].

These results contrast with those of another open label, randomised, phase III study, in which individuals infected with HCV genotype 1, who had previously received therapy with an NS5A inhibitor plus sofosbuvir, were retreated with glecaprevir-pibrentasvir. Median time between first and second regimens was 12-16 months, depending on treatment arm. Prevalence of NS5A and NS3 RAS, amongst those with sequencing data available, was 79% (107/136) and 49% (67/136), respectively.

Individuals without cirrhosis received 12 or 16 weeks of glecaprevir-pibrentasvir with 65/68 (96%) and 28/29 (96%) respectively, achieving SVR4 (SVR12 data is awaited). Individuals with compensated cirrhosis received 12 weeks of glecaprevir-pibrentasvir plus ribavirin or 16 weeks of glecaprevir-pibrentasvir alone, with 18/21 (86%) and 21/21 (100%) respectively achieving SVR4. There were only eight individuals who also had prior NS3 inhibitor exposure, all of whom received 16 weeks of therapy. Of note, enrolment to the 12 week glecaprevir-pibrentasvir plus ribavirin group was stopped early due to the low SVR, with a high proportion reporting ribavirin toxicity. Results overall demonstrated a high SVR4 in spite of the high baseline prevalence of NS3 and NS5A RAS [10].

Sofosbuvir-velpatasvir

In a phase II study (GS-US-342-1553) evaluating the effect of 24 weeks of sofosbuvir-velpatasvir and ribavirin in cirrhotic and non-cirrhotic individuals infected with genotypes 1, 2 or 3, who had failed first line NS5A-inhibitor containing therapy, overall SVR12 was 91% (63/69). For those with and without baseline NS5A RAS; SVR12 rates were 5/5 (100%) versus 31/32 (97%) in genotype 1, and 5/5 (100%) versus 8/9 (89%) in genotype 2, respectively, suggesting no impact of baseline NS5A RAS on SVR12. However, for genotype 3a, SVR12 was 77% (10/13) and 100% (4/4) in those with and without baseline NS5A RAS, respectively [9].

Sofosbuvir-elbasvir-grazoprevir

The triple combination of sofosbuvir-elbasvir-grazoprevir is not currently recommended for DAA-experienced patients in international guidelines, but early data suggests it may be highly effective. In the C-SWIFT study (outlined above), genotype 1-infected individuals, with and without cirrhosis, who did not achieve SVR12 with 4-8 weeks of sofosbuvir-elbasvir-grazoprevir were retreated approximately 7 months later with 12 weeks of sofosbuvir-elbasvir-grazoprevir plus ribavirin. SVR12 was 100% (23/23). RAS prior to retreatment were identified by NGS with a 1% cut off: NS3 and NS5A RAS were present in 17 (74%) and 14 (61%) individuals respectively, and, for 11 (48%) individuals, RAS were identified in both viral proteins. There was no impact of pre-retreatment RAS on subsequent SVR12 [76].

In a real world study of patients with HCV genotype 1 or 4 infection, who had failed an NS5A or NS3 inhibitor containing regimen, mostly with advanced fibrosis or compensated cirrhosis, retreatment was provided with sofosbuvir-elbasvir-grazoprevir plus ribavirin for 16 weeks (the ANRS HC34 REVENGE study). Overall 24/26 (92%) had pre-retreatment NS5A RAS and SVR12 was 96% (25/26) [85].

Sofosbuvir-glecaprevir-pibrentasvir

In an open label, phase III study (Magellan-3), individuals without cirrhosis or with compensated cirrhosis, who had previously received therapy with glecaprevir-pibrentasvir, were retreated with sofosbuvir-glecaprevir-pibrentasvir plus ribavirin. Individuals (n=21) received 16 weeks of therapy if they also fulfilled at least one of the following criteria: infection with HCV genotype 3, cirrhosis or prior exposure to any other NS5A and/or NS3 inhibitor. Others (n=2) received 12 weeks of therapy. Most individuals were infected with genotype 3a (n=13) or 1a (n=7). For the 16 week arm, RAS in the NS3 and NS5A genes were present in 0 and 16 (76%) individuals respectively and were present in both genes for 5 (24%) individuals. Overall SVR12 was 96% (22/23) [86].

NS5A inhibitor experienced individuals: summary

Data supporting use of RAS testing in some or all patient groups.

Glecaprevir-pibrentasvir

RAS testing may be performed prior to therapy with glecaprevir-pibrentasvir in genotype 1 infected individuals who have previously received treatment with an NS3 and/or NS5A inhibitor.

In the presence of NS3 RAS alone, the use of 12 weeks of therapy is supported by data from clinical trials, for individuals with and without cirrhosis [7, 8, 10].

In the presence of NS5A RAS alone, 16 weeks of therapy is supported by data from clinical trials, in individuals with and without cirrhosis [7, 8, 10]. One study reported high SVR4 with 12 weeks of therapy in noncirrhotic individuals exposed to an NS5A inhibitor + sofosbuvir [10], but SVR12 outcomes were not available. This data is also conflicting with those of Magellan-1, Part-2, and this shorter duration is not currently recommended in international guidelines [7, 8] [12] [4].

Where both NS3 and NS5A RAS are detected, particularly following exposure to both NS3 and NS5A inhibitors, the writing group advises use of an alternative regimen to glecaprevir-pibrentasvir until further data becomes available, in view of conflicting data from clinical trials involving small numbers of patients and the recommendations of current treatment guidelines [8, 10] [4, 12].

Sofosbuvir-velpatasvir

Data is available suggesting that baseline NS5A RAS in genotype 3 infected individuals, who have previously failed an NS5A inhibitor containing regimen, may reduce SVR12 following retreatment with 24 weeks of sofosbuvir-velpatasvir and ribavirin [9]. For individuals with decompensated cirrhosis who may be recommended this combination, alternative regimens are not currently available. However RAS testing is advised to obtain data to inform future clinical decision-making.

Viral sequencing should be performed in all individuals who have viral recurrence after therapy, where high-risk behaviour means that there is a significant risk of a new infection rather than viral relapse, when re-treatment is being considered.

Viral sequencing should also be performed where treatment failure due to emerging dominance of a minority variant is suspected in the context of an undiagnosed initial mixed infection. Testing should be performed on a stored pre-treatment sample in conjunction with the failure sample.

Next generation sequencing may be preferable, in view of the greater sequencing information it provides.

Data suggesting RAS testing is not required.

Sofosbuvir-velpatasvir-voxilaprevir

Data does not support the routine use of baseline resistance testing prior to therapy with sofosbuvir-velpatasvir-voxilaprevir in individuals infected with genotypes 1-6, with and without cirrhosis, who have previously been exposed to DAA-containing therapy [75].

Sofosbuvir-glecaprevir-pibrentasvir

Data does not support the use of baseline resistance testing prior to retreatment with sofosbuvir-glecaprevir-pibrentasvir in individuals infected with HCV genotype 1a or 3a who have received prior NS3 and/or NS5A inhibitor containing therapy [86].

Sofosbuvir-elbasvir-grazoprevir

Note: this regimen is not currently recommended in DAA-exposed individuals in international guidelines [4, 12].

Data does not support the use of baseline resistance testing prior to retreatment with sofosbuvir-elbasvir-grazoprevir in genotype 1-infected individuals, both with and without cirrhosis, who have received previous NS3 and/or NS5A inhibitor containing therapy [76].

Areas of unmet need

- Further data is needed to assess the impact of RAS on retreatment, according to the duration between first and second DAA regimens.
- Further data is required on the optimal treatment strategy for individuals exposed to second-generation DAA, who are infected with virus harbouring NS5A and/or NS3 RAS.
- Further data is needed to clarify the optimal duration of therapy with glecaprevir-pibrentasvir in DAA-exposed individuals with virus harbouring NS3 and/or NS5A RAS.
- Further options are desirable for retreatment strategies for NS5A-inhibitor experienced individuals, particularly in the following groups: genotype 3 infection, decompensated cirrhosis and severe renal impairment.

Abbreviations

DAA	direct acting antiviral
EC50	half maximal effective concentration
eGFR	estimated glomerular filtration rate
ELB	elbasvir
FC	fold change
FDA	Food and Drug Administration (USA)
GLE	glecaprevir
GT	genotype
GZR	grazoprevir
HCV	hepatitis C virus
HIV	human immunodeficiency virus
LDV	ledipasvir
MDT	multi-disciplinary team
MSM	men who have sex with men
NGS	next generation sequencing
PCR	polymerase chain reaction
PEG	pegylated-interferon
PI	protease inhibitor
PIB	pibrentasvir
PWID	people who inject drugs
PrO	ritonavir-boosted paritaprevir and ombitasvir
PrOD	ritonavir-boosted paritaprevir, ombitasvir and dasabuvir
RAS	resistance-associated substitution
RdRp	RNA-dependent RNA polymerase
RNA	ribonucleic acid
RBV	ribavirin
SOF	sofosbuvir
SPC	Summary of Product Characteristics
SVR	sustained virologic response
SVR12	sustained virologic response at 12 weeks post end of treatment
TE	treatment-experienced
TN	treatment-naïve
VEL	velpatasvir
VOX	voxilaprevir

References

1. Komatsu, T.E., et al., *Regulatory Analysis of Effects of Hepatitis C Virus NS5A Polymorphisms on Efficacy of Elbasvir and Grazoprevir*. *Gastroenterology*, 2017. **152**(3): p. 586-597.
2. Curry, M.P., et al., *Sofosbuvir and Velpatasvir for HCV in Patients with Decompensated Cirrhosis*. *N Engl J Med*, 2015. **373**(27): p. 2618-28.
3. Hezode, C., et al., *Resistance Analysis in Patients with Genotype 1-6 HCV Infection Treated with Sofosbuvir/Velpatasvir in the Phase 3 Studies*. *J Hepatol*, 2018. **68**(5): p. 895-903.
4. *EASL Recommendations on Treatment of Hepatitis C 2018*. *J Hepatol*, 2018. **69**(2): p. 461-511.
5. Krishnan, P., et al., *Pooled Resistance Analysis in HCV Genotype 1-6 Infected Patients Treated With Glecaprevir/Pibrentasvir in Phase 2 and 3 Clinical Trials* *Antimicrob Agents Chemother*, 2018. **62**(10): p. e01249-18.
6. Drysdale, K., et al., *Real-world effectiveness of directly-acting antiviral treatments in England: data on all treated patients from the national registry*, in *British Association for the Study of the Liver*. 2018: York, UK.
7. Poordad, F., et al., *Glecaprevir and pibrentasvir for 12 weeks for hepatitis C virus genotype 1 infection and prior direct-acting antiviral treatment*. *Hepatology*, 2017. **66**(2): p. 389-397.
8. Poordad, F., et al., *Glecaprevir/Pibrentasvir in Patients with HCV Genotype 1 or 4 and Prior Direct-acting Antiviral Treatment Failure*. *Hepatology*, 2017. **67**(4): p. 1253-1260.
9. Gane, E.J., et al., *Sofosbuvir-velpatasvir with ribavirin for 24 weeks in hepatitis C virus patients previously treated with a direct-acting antiviral regimen*. *Hepatology*, 2017. **66**(4): p. 1083-1089.
10. Lok, A., et al. *A phase 3b, open-label, randomized, pragmatic study of glecaprevir/pibrentasvir +/- ribavirin (RBV) for HCV genotype 1 subjects who previously failed an NS5A Inhibitor + sofosbuvir (SOF) therapy*. in *European Association for the Study of the Liver*. 2018. Paris, France.
11. European Association for the Study of the Liver. Electronic address, e.e.e., *EASL Recommendations on Treatment of Hepatitis C 2016*. *J Hepatol*, 2017. **66**(1): p. 153-194.
12. AASLD-IDSA, *HCV Guidance: Recommendations for Testing, Managing, and Treating Hepatitis C*. *Clin Infect Dis*, 2018. **67**(10): p. 1477-1492.
13. *Global health sector strategy on viral hepatitis, 2016–2021: towards ending viral hepatitis*. 2016; Available from: <http://apps.who.int/iris/bitstream/handle/10665/246177/WHO-HIV-2016.06-eng.pdf?sequence=1>.
14. Neumann, A.U., et al., *Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon-alpha therapy*. *Science*, 1998. **282**(5386): p. 103-7.
15. Cuevas, J.M., et al., *Effect of ribavirin on the mutation rate and spectrum of hepatitis C virus in vivo*. *J Virol*, 2009. **83**(11): p. 5760-4.
16. Zeuzem, S., et al., *NS5A resistance-associated substitutions in patients with genotype 1 hepatitis C virus: Prevalence and effect on treatment outcome*. *J Hepatol*, 2017. **66**(5): p. 910-918.

17. Sarrazin, C., et al., *Prevalence of Resistance-Associated Substitutions in HCV NS5A, NS5B, or NS3 and Outcomes of Treatment With Ledipasvir and Sofosbuvir*. *Gastroenterology*, 2016. **151**(3): p. 501-512 e1.
18. Gane, E.J., et al., *The emergence of NS5B resistance associated substitution S282T after sofosbuvir-based treatment*. *Hepatology*, 2017. **1**(6): p. 538-549.
19. Lahser, F.C., et al. *Interim Analysis of a 3-Year Follow-up Study of NS5A and NS3 Resistance-Associated Variants (RAVs) After Treatment With Grazoprevir-Containing Regimens in Patients With Chronic hepatitis C Virus (HCV) Infection*. in *American Association for the Study of Liver Diseases*. 2016. Boston.
20. Smith, D.B., et al. *International Committee on Taxonomy of Viruses (ICTV). HCV Classification*. 2017 [cited 2018 26/03/2018]; Available from: Available at: https://talk.ictvonline.org/ictv_wikis/flaviviridae/w/sg_flavi/56/hcv-classification.
21. Chevaliez, S., et al., *Hepatitis C virus (HCV) genotype 1 subtype identification in new HCV drug development and future clinical practice*. *PLoS One*, 2009. **4**(12): p. e8209.
22. Mellor, J., et al., *Survey of type 6 group variants of hepatitis C virus in Southeast Asia by using a core-based genotyping assay*. *J Clin Microbiol*, 1996. **34**(2): p. 417-23.
23. Susser, S., et al., *Origin, prevalence and response to therapy of hepatitis C virus genotype 2k/1b chimeras*. *J Hepatol*, 2017. **67**(4): p. 680-686.
24. McNaughton, A.L., et al., *Prevalence of mixed genotype hepatitis C virus infections in the UK as determined by genotype-specific PCR and deep sequencing*. *J Viral Hepat*, 2017.
25. Pham, S.T., et al., *Frequent multiple hepatitis C virus infections among injection drug users in a prison setting*. *Hepatology*, 2010. **52**(5): p. 1564-72.
26. Butt, S., et al., *Mixed genotype infections with hepatitis C virus, Pakistan*. *Emerg Infect Dis*, 2011. **17**(8): p. 1565-7.
27. Walker, M.R., et al., *Incident Hepatitis C Virus Genotype Distribution and Multiple Infection in Australian Prisons*. *J Clin Microbiol*, 2016. **54**(7): p. 1855-1861.
28. Yun, H., et al., *High prevalence of HBV and HCV infection among intravenous drug users in Korea*. *J Med Virol*, 2008. **80**(9): p. 1570-5.
29. Schroter, M., et al., *Multiple infections with different HCV genotypes: prevalence and clinical impact*. *J Clin Virol*, 2003. **27**(2): p. 200-4.
30. Janiak, M., et al., *Next-Generation Sequencing of Hepatitis C Virus (HCV) Mixed-Genotype Infections in Anti-HCV-Negative Blood Donors*. *Adv Exp Med Biol*, 2018. **1096**: p. 65-71.
31. Cunningham, E.B., et al., *Mixed HCV infection and reinfection in people who inject drugs--impact on therapy*. *Nat Rev Gastroenterol Hepatol*, 2015. **12**(4): p. 218-30.
32. Midgard, H., et al., *HCV epidemiology in high-risk groups and the risk of reinfection*. *J Hepatol*, 2016. **65**(1 Suppl): p. S33-S45.
33. Ingiliz, P., et al., *HCV reinfection incidence and spontaneous clearance rates in HIV-positive men who have sex with men in Western Europe*. *J Hepatol*, 2017. **66**(2): p. 282-287.
34. Falade-Nwulia, O., et al., *Understanding and addressing hepatitis C reinfection in the oral direct-acting antiviral era*. *J Viral Hepat*, 2018. **25**(3): p. 220-227.
35. *Public Health England. Hepatitis C in England: 2018 report*. 2018 08/05/2018]; Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/693917/HCV_in_England_2018.pdf.
36. *Public Health England. Hepatitis C in the UK: 2017 report 2017*; Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/632465/HCV_in_the_uk_report_2017.pdf.

37. Cheng, G., et al., *In Vitro Antiviral Activity and Resistance Profile Characterization of the Hepatitis C Virus NS5A Inhibitor Ledipasvir*. *Antimicrob Agents Chemother*, 2016. **60**(3): p. 1847-1853.
38. Wong, K.A., et al., *Characterization of Hepatitis C virus resistance from a multiple-dose clinical trial of the novel NS5A inhibitor GS-5885*. *Antimicrob Agents Chemother*, 2013. **57**(12): p. 6333-40.
39. Gottwein, J.M., et al., *Efficacy of NS5A Inhibitors Against Hepatitis C Virus Genotypes 1-7 and Escape Variants*. *Gastroenterology*, 2018. **154**(5): p. 1435-1448.
40. Camus, G., et al., *Resistance characterization of ledipasvir and velpatasvir in hepatitis C virus genotype 4*. *J Viral Hepat*, 2018. **25**(2): p. 134-143.
41. Bourliere, M., et al., *Ledipasvir-sofosbuvir with or without ribavirin to treat patients with HCV genotype 1 infection and cirrhosis non-responsive to previous protease-inhibitor therapy: a randomised, double-blind, phase 2 trial (SIRIUS)*. *Lancet Infect Dis*, 2015. **15**(4): p. 397-404.
42. Wyles, D., et al., *Ledipasvir-sofosbuvir plus ribavirin for patients with genotype 1 hepatitis C virus previously treated in clinical trials of sofosbuvir regimens*. *Hepatology*, 2015. **61**(6): p. 1793-7.
43. Abergel, A., et al., *Ledipasvir plus sofosbuvir for 12 weeks in patients with hepatitis C genotype 4 infection*. *Hepatology*, 2016. **64**(4): p. 1049-56.
44. Gupta, N., et al. *Direct-acting antiviral treatment in sub-Saharan Africa: A prospective trial of Ledipasvir/Sofosbuvir for chronic Hepatitis C infection in Rwanda (the SHARED study)*. in *European Association for the Study of the Liver*. 2018. Paris, France.
45. Abergel, A., et al., *Ledipasvir-sofosbuvir in patients with hepatitis C virus genotype 5 infection: an open-label, multicentre, single-arm, phase 2 study*. *Lancet Infect Dis*, 2016. **16**(4): p. 459-64.
46. Gane, E.J., et al., *Efficacy of ledipasvir and sofosbuvir, with or without ribavirin, for 12 weeks in patients with HCV genotype 3 or 6 infection*. *Gastroenterology*, 2015. **149**(6): p. 1454-1461 e1.
47. Buggisch, P., et al., *Real-world effectiveness of 8-week treatment with ledipasvir/sofosbuvir in chronic hepatitis C*. *J Hepatol*, 2018. **68**(4): p. 663-671.
48. Backus, L.I., et al., *Real-world effectiveness of ledipasvir/sofosbuvir in 4,365 treatment-naïve, genotype 1 hepatitis C-infected patients*. *Hepatology*, 2016. **64**(2): p. 405-14.
49. Xu, S., et al., *In vitro selection of resistance to sofosbuvir in HCV replicons of genotype-1 to -6*. *Antivir Ther*, 2017. **22**(7): p. 587-597.
50. Kati, W., et al., *In vitro activity and resistance profile of dasabuvir, a nonnucleoside hepatitis C virus polymerase inhibitor*. *Antimicrob Agents Chemother*, 2015. **59**(3): p. 1505-11.
51. Krishnan, P., et al., *In vitro and in vivo antiviral activity and resistance profile of ombitasvir, an inhibitor of hepatitis C virus NS5A*. *Antimicrob Agents Chemother*, 2015. **59**(2): p. 979-87.
52. Schnell, G., et al., *Hepatitis C virus genotype 4 resistance and subtype demographic characterization of patients treated with ombitasvir plus paritaprevir/ritonavir*. *Antimicrob Agents Chemother*, 2015. **59**(11): p. 6807-15.
53. Pilot-Matias, T., et al., *In vitro and in vivo antiviral activity and resistance profile of the hepatitis C virus NS3/4A protease inhibitor ABT-450*. *Antimicrob Agents Chemother*, 2015. **59**(2): p. 988-97.
54. Krishnan, P., et al., *Resistance analysis of baseline and treatment-emergent variants in hepatitis C virus genotype 1 in the AVIATOR study with paritaprevir-ritonavir, ombitasvir, and dasabuvir*. *Antimicrob Agents Chemother*, 2015. **59**(9): p. 5445-54.

55. Sarrazin, C., et al., *Analysis of Baseline Variants in GT1a-Infected Patients Treated With 3D With and Without RBV, and Cirrhotic GT1a Patients Treated With 3D Plus RBV for 12 or 24 Weeks in European Association for the Study of the Liver Special Meeting*. 2016: Paris.
56. Sarrazin, C., et al. *Effect of Baseline Resistance-Associated Variants on SVR With the 3D Regimen Plus RBV in European Association for the Study of the Liver*. 2016. Barcelona, Spain.
57. Asselah, T., et al., *Ombitasvir, paritaprevir, and ritonavir plus ribavirin in adults with hepatitis C virus genotype 4 infection and cirrhosis (AGATE-1): a multicentre, phase 3, randomised open-label trial*. *Lancet Gastroenterol Hepatol*, 2016. **1**(1): p. 25-35.
58. Lahser, F.C., et al., *The Combination of Grazoprevir, a Hepatitis C Virus (HCV) NS3/4A Protease Inhibitor, and Elbasvir, an HCV NS5A Inhibitor, Demonstrates a High Genetic Barrier to Resistance in HCV Genotype 1a Replicons*. *Antimicrob Agents Chemother*, 2016. **60**(5): p. 2954-64.
59. Asante-Appiah, E., et al., *Antiviral Activity and Resistance Analysis of NS3/4A Protease Inhibitor Grazoprevir and NS5A Inhibitor Elbasvir in Hepatitis C Virus GT4 Replicons*. *Antimicrob Agents Chemother*, 2017. **61**(7).
60. Liu, R., et al., *Susceptibilities of genotype 1a, 1b, and 3 hepatitis C virus variants to the NS5A inhibitor elbasvir*. *Antimicrob Agents Chemother*, 2015. **59**(11): p. 6922-9.
61. Howe, A.Y., et al., *Virologic resistance analysis from a phase 2 study of MK-5172 combined with pegylated interferon/ribavirin in treatment-naive patients with hepatitis C virus genotype 1 infection*. *Clin Infect Dis*, 2014. **59**(12): p. 1657-65.
62. Harrington, P.R., et al., *Impact of hepatitis C virus polymorphisms on direct-acting antiviral treatment efficacy: Regulatory analyses and perspectives*. *Hepatology*, 2018. **67**(6): p. 2430-2448.
63. Jacobson, I.M., et al., *Safety and Efficacy of Elbasvir/Grazoprevir in Patients With Hepatitis C Virus Infection and Compensated Cirrhosis: An Integrated Analysis*. *Gastroenterology*, 2017. **152**(6): p. 1372-1382 e2.
64. Jacobson, I., et al., *Prevalence and Impact of Baseline NS5A Resistance-Associated Variants (RAVs) on the Efficacy of Elbasvir/Grazoprevir (EBR/GZR) Against GT1a Infection - 16 Weeks vs 12 weeks in 66th Annual Meeting of the American Association for the Study of Liver Diseases*. 2015: Boston, USA.
65. Cheng, G., et al. *GS-5816, a Second-Generation HCV NS5A Inhibitor With Potent Antiviral Activity, Broad Genotypic Coverage, and a High Resistance Barrier in European Association for the Study of the Liver*. 2013. Amsterdam, The Netherlands.
66. Lawitz, E.J., et al., *Clinical Resistance to Velpatasvir (GS-5816), a Novel Pan-Genotypic Inhibitor of the Hepatitis C Virus NS5A Protein*. *Antimicrob Agents Chemother*, 2016. **60**(9): p. 5368-78.
67. Foster, G.R., et al., *Sofosbuvir and Velpatasvir for HCV Genotype 2 and 3 Infection*. *N Engl J Med*, 2015. **373**(27): p. 2608-17.
68. Buti, M., et al. *Safety and Efficacy of Sofosbuvir/Velpatasvir with and without Ribavirin in Genotype 3 HCV-Infected Patients with Cirrhosis*. in *European Association for the Study of the Liver*. 2018. Paris, France.
69. von Felden, J., et al., *High efficacy of sofosbuvir/velpatasvir and impact of baseline resistance-associated substitutions in hepatitis C genotype 3 infection*. *Aliment Pharmacol Ther*, 2018. **47**(9): p. 1288-1295.
70. Ng, T.I., et al., *In Vitro Antiviral Activity and Resistance Profile of the Next-Generation Hepatitis C Virus NS3/4A Protease Inhibitor Glecaprevir*. *Antimicrob Agents Chemother*, 2018. **62**(1).

71. Ng, T.I., et al., *In Vitro Antiviral Activity and Resistance Profile of the Next-Generation Hepatitis C Virus NS5A Inhibitor Pibrentasvir*. *Antimicrob Agents Chemother*, 2017. **61**(5).
72. Dvory-Sobol, H., et al., *Susceptibility to voxilaprevir of NS3 resistance-associated substitutions and clinical isolates from direct-acting antiviral-experienced and -naive patients*, in *American Association of the Study of Liver Diseases*. 2017: Washington DC, USA.
73. Lawitz, E., et al. *Characterization of HCV Resistance From a 3-Day Monotherapy Study of GS-9857, a Novel Pangenotypic NS3/4A Protease Inhibitor* in *American Association for the Study of Liver Diseases*. 2015. Boston, USA.
74. Wyles, D., et al. *No Impact of RASs on the High Efficacy of SOF/VEL/VOX for 8 Weeks in DAA-Naïve Patients: an Integrated Resistance Analysis of the POLARIS-2 and POLARIS-3 Studies*. in *European Association for the Study of the Liver*. 2017. Amsterdam, The Netherlands.
75. Sarrazin, C., et al. *No impact of RASs on the high efficacy of SOF/VEL/VOX for 12 weeks in DAA-experienced patients: an integrated resistance analysis of the POLARIS-1 and POLARIS-4 studies*. in *European Association for the Study of the Liver*. 2017. Amsterdam, The Netherlands.
76. Lawitz, E., et al., *Short-duration treatment with elbasvir/grazoprevir and sofosbuvir for hepatitis C: A randomized trial*. *Hepatology*, 2017. **65**(2): p. 439-450.
77. Foster, G.R., et al., *Elbasvir/grazoprevir and sofosbuvir for hepatitis C virus genotype 3 infection with compensated cirrhosis: A randomized trial*. *Hepatology*, 2018. **67**(6): p. 2113-2126.
78. Irving, W.L., J. McLauchlan, and G.R. Foster. *Real world outcomes of DAA therapy for chronic hepatitis C virus infection in the HCV Research UK National cohort*. in *European Association for the Study of the Liver*. 2017. Amsterdam, The Netherlands.
79. Wyles, D., et al., *Long-term persistence of HCV NS5A resistance associated substitutions after treatment with the HCV NS5A inhibitor, ledipasvir, without sofosbuvir*. *Antivir Ther*, 2018. **23**(3): p. 229-238.
80. Svarovskaia, E.S., et al., *Infrequent development of resistance in genotype 1-6 hepatitis C virus-infected subjects treated with sofosbuvir in phase 2 and 3 clinical trials*. *Clin Infect Dis*, 2014. **59**(12): p. 1666-74.
81. Simmons, B., et al., *Risk of Late Relapse or Reinfection With Hepatitis C Virus After Achieving a Sustained Virological Response: A Systematic Review and Meta-analysis*. *Clin Infect Dis*, 2016. **62**(6): p. 683-694.
82. Abdelrahman, T., et al., *Next-generation sequencing sheds light on the natural history of hepatitis C infection in patients who fail treatment*. *Hepatology*, 2015. **61**(1): p. 88-97.
83. Bourliere, M., et al., *Sofosbuvir, Velpatasvir, and Voxilaprevir for Previously Treated HCV Infection*. *N Engl J Med*, 2017. **376**(22): p. 2134-2146.
84. Reau, N., et al. *Integrated Resistance Analyses of HCV-infected Patients treated with Sofosbuvir, Velpatasvir and Voxilaprevir for 8 and 12 weeks from Phase 2 Studies*. in *American Association for the Study of Liver Diseases*. 2016. Boston.
85. de Ledinghen, V., et al., *Retreatment With Sofosbuvir Plus Grazoprevir/Elbasvir Plus Ribavirin of Patients With Hepatitis C Virus Genotype 1 or 4 Who Previously Failed an NS5A- or NS3-Containing Regimen: The ANRS HC34 REVENGE Study*. *Clin Infect Dis*, 2018. **66**(7): p. 1013-1018.
86. Wyles, D., et al. *Retreatment of hepatitis C virus infection in patients who failed Glecaprevir/Pibrentasvir*. in *European Association for the Study of the Liver*. 2018. Paris, France.

Appendix: Membership of Public Health England's HCV Resistance Group

This document was developed and agreed by all members of the PHE HCV Resistance Group.

Membership of the Group is shown below:

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