Response to DAA Therapy in the NHS England Early Access Programme for Rare HCV Subtypes from Low and Middle Income Countries

Ana da Silva Filipe[1], Vattipally Sreenu[1], Joseph Hughes[1], Elihu Aranday-Cortes[1], William L Irving[2], Graham R. Foster[3], Kosh Agarwal[4], William Rosenberg[5], Douglas Macdonald[5], Paul Richardson[6], Mark A. Aldersley[7], Martin Wiselka[8], Andrew Ustianowski[9], John McLauchlan[1], Emma C. Thomson[1]

1. MRC-University of Glasgow Centre for Virus Research, Glasgow, UK.
2. Gastrointestinal and Liver Disorders Theme, NIHR Nottingham Biomedical Research Centre at the Nottingham University Hospitals NHS Trust and the University of Nottingham, UK.
3. Blizard Institute, Queen Mary University of London, London, UK.
4. Institute of Liver Studies, King's College Hospital Foundation Trust, London UK.
5. Institute of Liver and Digestive Health, University College of London, London, United Kingdom.
6. Royal Liverpool University NHS Trust, Liverpool, UK.
7. Liver Unit, St James's University Hospital, Leeds, UK.
8. Infectious Diseases Unit, University Hospitals Leicester NHS Trust, Leicester, UK.
9. Infectious Diseases, North Manchester General Hospital, Manchester, UK.

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Correspondence: Prof J McLauchlan, MRC-University of Glasgow Centre for Virus Research, Sir Michael Stoker Building, Garscube Campus, Glasgow G61 1QH, UK; E-mail, john.mclauchlan@glasgow.ac.uk; Tel no. +44 141 330 4028.

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Author Contributions: The study was designed and led by AS, JM and ECT. EAC, VS and JH formed NGS and host genetic analysis. WLI collated and provided the clinical data from HCV Research UK. GRF, KA, WR, DM, PR, MAA, MW and AU consented the patients, provided clinical data and provided samples. All authors contributed to and commented on the drafting of the final manuscript.

To the Editor:

We read with interest "NS5A resistance-associated substitutions in patients with genotype 1 hepatitis C virus: Prevalence and effect on treatment outcome" by Zeuzem *et al.* in the *Journal of Hepatology*, which indicated lower SVR rates for Gt1a and Gt1b strains carrying multiple resistance-associated substitutions (RASs) in NS5A [1]. Their study is very comprehensive but, as the authors note, it is largely restricted to HCV subtypes Gt1a and Gt1b, and does not represent subtypes from large geographical regions, including Africa. Indeed, current guidelines for treatment with direct-acting antivirals (DAAs) [2] are based on trial data for a limited selection of HCV genotypes, typically found in high-income countries. However, HCV is highly variable with natural polymorphisms at sites of DAA resistance. Our analysis of patients originating from low- and middle-income countries (LMICs), recruited into the NHS England Early Access Programme (EAP), has identified HCV subtypes, which contain naturally-occurring single and double RAS in NS5A that are associated with apparent poor response to approved DAAs.

Previously, we described clinical outcomes from the EAP cohort, which provided IFN-free, DAA therapy to >800 HCV-infected patients with decompensated liver disease [3, 4]; all patients received sofosbuvir±ribavirin (SOF±RBV) combined with either ledipasvir (LDV) or daclatasvir (DCV). Among the Gt1-infected group, 28 patients did not achieve a SVR. From next generation sequence (NGS) analysis, 3 of these patients, designated as Gt1- or Gt1a-infected by the clinical sites, were infected with Gt1l. All 3 patients originated from Nigeria. The patients were retreated with DAAs for a further 24 weeks but again relapsed after therapy. In two Gt1l-infected patients (P1 and P3) where pre- and post-treatment samples were available, there were no sequence changes in either NS5A or NS5B. By contrast, NS5A RASs emerged in Gt1a-infected patients between pre- and post-treatment. For the 3rd Gt1l-infected patient, NGS data was only available at pre-treatment.

Typically, RAS in NS5A are located at positions 24, 28, 30, 31 and 93 as well as residue 58 (for Gt1a) [1]. However, these positions are polymorphic among HCV genotypes/subtypes. The Gt1l sequences in patients P1 and P3 between residues 28-31 were identical (MPRM; Table 1). Thus, at pre-treatment, both patients contained two documented RAS (Q30R+L31M). The

3rd gt1l-infected patient, P2, had one RAS (L31M). In a recent report, introducing Q30R+L31M into a Gt1a replicon confers high *in vitro* resistance for LDV and DCV (>23,400 and >4,452 increased EC_{50} values, respectively); for Velpatasvir, there is a 198-fold rise in EC_{50} for these substitutions [5]. In the context of Gt1a, L31M also increases EC_{50} values for these DAAs but to a lesser extent compared to Q30R+L31M.

In the EAP cohort, there were also one non-responder and 3 responder-relapsers in the Gt4-infected group. From NGS analysis, the non-responder (P4) and one of the three responder-relapsers were infected with Gt4r (P5; Table 1); both patients originated from Somalia. The remaining responder-relapsers were Gt4d-infected. Similar to Gt1l-infected patients P1 and P3, no amino acid alterations were detected in NS5A between pre- and post-treatment for the Gt4r non-responder. The sequence between NS5A residues 28-32 in this patient was identical to that for patients P1 and P3 and thus contained a Q30R+L31M RAS doublet. However, a T282 RAS in NS5B, which is associated with SOF resistance [6], emerged in 100% of sequences at 1 week post-therapy (Figure 1). T282 remained dominant at week 5 post-treatment but at 49 weeks, T282 was not detected and all sequences encoded S282. A similar pattern of emergence and loss of T282 after failed SOF treatment has been reported in a Gt2b-infected patient [7]. The GT4r responder-relapser (P5) had a single NS5A RAS at pre-treatment (Q30R) and a further Y93H RAS emerged upon relapse. No amino acid changes were observed in NS5B.

From the limited Gt1l sequences available (n=3), both double (Q30R+L31M) and single RAS (L31M) are present in published strains [8]. Combined with our data, NS5A position 30 is apparently polymorphic in Gt1l and thereby generates strains with double and single RAS, which could increase NS5A DAA resistance. As with Gt1l, there is a paucity of Gt4r sequence data. However, in a recently reported Gt4 cohort (n=44), Gt4r was detected in three subjects, two of whom did not achieve SVR [9]. The non-SVR Gt4r subjects had baseline RAS (M28M/V+Q30R+L31M), which remained the dominant sequences post-treatment. Moreover, a S282T mutation in NS5B emerged at post-treatment for one Gt4r patient. These data complement our findings and, given that the SOF-resistant S282T variant has emerged in 2/4

Gt4r-infected patients who did not achieve SVR, this substitution may arise with higher frequency in rare subtypes such as Gt4r.

We conclude that certain Gt1l and Gt4r strains contain naturally occurring polymorphisms, which could contribute to resistance to NS5A inhibitors in patients with advanced liver disease. Since decompensated cirrhosis can lead to reduced response to DAAs [10], it is unclear whether extended duration of initial therapy might be advantageous in such patients nor whether patients with less liver damage would respond differently. Nevertheless, with the growing availability of DAAs, it is important that recommendations and guidance reflect the global situation to effectively control HCV infection. Since many DAA combinations for pan-genotypic HCV therapy include NS5A inhibitors, robust evidence is critical to evaluate their effectiveness against the global diversity of HCV subtypes and further trials may be needed in such cohorts to evaluate alternative regimens, perhaps including emerging protease inhibitor combinations. The absence of data in exotic HCV strains and the presence of variants associated with reduced treatment response pose a potential barrier to HCV eradication. Further studies investigating DAA efficacy in these and other strains found in LMICs in cirrhotic and non-cirrhotic patients are required.

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Table 1. Outcomes of DAA treatment/retreatment and sequences at sites of NS5A RAS before and after treatment.

						Position of NS5A RAS						
HCV Gt	Subjects/ Reference	DAA Rx	Rx Outcome (1)	Rx Outcome (2)	Timepoint	28	29	30	31	32	58	93
Gt1a	Gt1a Ref					M	P	Q	L	P	H	Y
Gt1b	Gt1b Ref					L	P	R	L	P	P	Y
Gt1l	P1	SOF/LDV/ RBV	Resp-Rel	Resp-Rel	Pre-Rx	M	P	R	M	P	P	Y
					Post-Rx	M	P	R	M	P	P	Y
	P2	SOF/DCV	Resp-Rel	Resp-Rel	Pre-Rx	M	P	Q	M	P	P	Y
					Post-Rx				NA			
	P3	SOF/LDV/ RBV	Resp-Rel	Resp-Rel	Pre-Rx	M	P	R	M	P	P	Y
					Post-Rx	M	P	R	M	P	P	Y
Gt4a	Gt4a ref					V	P	L	M	P	P	Y
Gt4r	P4	SOF/LDV	Non-Resp	Not re-treated	Pre-Rx	M	P	R	M	P	P	Y
					Post-Rx	M	P	R	M	P	P	Y
	P5	SOF/LDV/ RBV	Resp-Rel	Not re-treated	Pre-Rx	M	P	R	L	P	P	Y
					Post-Rx	M	P	R	L	P	P	H

NA – sequence not available; Rx outcome (1) and (2) – initial (1) and retreatment (2) outcomes.

FIGURE LEGEND

Figure 1. Emergence and loss of RAS S282T in NS5B after therapy in patient P4. Boxes show the percentage reads of S282 and T282 from NGS data at pre- and post-treatment.

Figure 1