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Letter

Baseline drug-resistance mutations are detectable in HCV genes NS3 and NS5A but not NS5B in acute and chronic HIV-coinfected patients

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Running head: HCV resistance-associated polymorphisms

Dear Editor,

In 2012, Plaza *et al* reported the prevalence of natural polymorphisms in HCV NS5A gene associated with resistance to Daclatasvir (DCV) in 78 HIV-HCV-co-infected patients and 635 HCV mono-infected derived NS5A sequences deposited in Los Alamos HCV database [1]. They did not observe NS5A resistance-associated variants (RAVs) in HCV-1a and HCV-3 NS5A sequences, whereas, major RAVs (Y93H) were detected in 7% and 13% in NS5A sequences from co-infected patients infected with HCV-1b and HCV-4 respectively, with a similar frequency of NS5A RAVs observed in HCV mono-infected patients for these HCV genotypes [1]. Additionally, the L31M NS5A variant was observed in 7% of HCV gt 1b patients, irrespective of co-infection status, and occurred in >93% of HCV gt4 mono and co-infected patients [1]. Interestingly, the presence of naturally occurring drug resistance variants in acutely HCV-infected, treatment-naïve HIV patients have been detected by population and deep sequencing in HCV NS3 in a large proportion of subjects [2]. The significance of the threshold at which these RAVs are detectable and whether these will impact on response to therapy with NS3 and NS5A inhibitors in clinical practice is not fully clear. In acute HCV amongst those who are HIV-infected, the role for new HCV direct acting antiviral drugs (DAAs) has not been established. Treatment with pegylated-interferon and ribavirin (pIFN-RBV) early in HCV infection is often successful for most genotypes (gts) [3]. Telaprevir, a first generation PI, has been used in a small study of acute HCV infection in HIV, and results suggest SVRs may be improved using 'triple therapy' with shortened treatment durations [4]. It is likely that in the near future DAA based therapy will be standard of care for both acute and chronic HCV infections, with PIs forming part of this armamentarium [5,6] alongside other DAAs, including NS5B polymerase inhibitors [7] and HCV NS5A

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inhibitors [8]. Ultimately, an interferon-free future is heralded, where drug regimens will consist of combinational DAAs, targeting different HCV gene products [9]. It is therefore important to establish the frequency of RAVs in all patients infected with HCV.

In this study, we investigated the prevalence of RAVs by population sequencing from three groups of HIV/HCV co-infected patients: (1) acute HCV infections (n=25), (2) chronic treatment-naïve patients (n=20) and, (3) chronic treatment-experienced (pIFN-RBV) patients who did not achieve an SVR (n=34) and compared with the prevalence of RAVs in 85 chronic HCV mono-infected patients. Genomic regions (sites of known RAVs) were amplified from HCV RNA using RT-PCR followed by a nested PCR. Typically, amino acids 1-181 of HCV NS3 protease (gt1 only), amino acids 1-213 of domain I of NS5A (for gt1a, 1b, 2, 3 and 4) and amino acids 219-347 of NS5B (pan-genotypic) were included. Purified PCR amplicons were sequenced using ABI PRISM 3730 genetic analyser and consensus sequences aligned against HCV reference sequences.

Baseline RAVs were detected in all 3 cohorts of co-infected patients in NS3 and NS5A but baseline S282T, associated with resistance to Sofosbuvir, was not detected in any of our cohorts (Table 1), possibly attributable to the low fitness of this mutation [10]. The Q80K polymorphism was the predominant NS3 variant for gt1a conferring resistance to 1st and 2nd generation PIs [11], and increased in frequency in acutes vs chronics and from chronic naïve to chronic treatment failure: 5.3% vs 9.1% vs 11.1% respectively. Conversely, the Q30H NS5A variant was only detected in a single acute patient, conferring 1477-fold Daclatasvir (DCV) resistance [12] and variants at codon 30 did not differ markedly in HCV gt1a between the chronic and acute cohorts. The NS5A Y93H variant was present at 33.3% compared with 7% reported by Plaza *et al* [1] in our chronic naïve gt1b co-infected population, and was not detected in our HCV gt1b treatment failure cohort. In contrast to Plaza *et al* [1] we did not observe Y93H in any of our HCV gt4 infected cohorts. However, similar to Plaza *et al*, we observed in the majority of HCV gt 1b and gt4 NS5 sequences, the M28L and L30R variants respectively, conferring minimal resistance effects to DCV. Unlike Plaza *et al*, the L31M NS5A variant was not observed in our HCVgt4a cohort but was detected in single HCV gt1a treatment failure, reportedly conferring 341-fold DCV resistance effects [12]. Due to high viral load and increased rates of primary RAVs reported for HIV/HCV co-infected patients vs HCV mono-infected patients [13,14] we decided to compare the prevalence of DAA resistant variants between these cohorts. We observed a significant increase in NS3 variants in HCV gt1a mono-infected patients compared with acute and/or chronic HCV gt1a co-infected patients ($p<0.0001$ Fisher's Exact test), including the R155K variant, conferring resistance to all licensed PIs [11,15]. We also observed an increase in NS5A variants in the mono-infected gt1a cohort, though this was not statistically significant. Additionally, the L31M NS5A variant was detected in 22.2% HCVgt2 mono-infected patients, conferring 140 fold DCV resistance, whilst the prevalence of NS3 and NS5A variants was minimal for HCV gt3, with NS5A A30K variant observed in a single mono-infected patient.

In conclusion, natural polymorphisms conferring low to moderate NS3 and NS5A DAA resistance effects were detected across genotypes 1-4 in both HCV mono-infected and acute and chronic HIV/HCV co-infected patients, using population sequencing, hence probably under-estimating

the presence of minority variants. The significance of this finding will only come to light as we start to put the use of new DAAs into clinical practice.

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AM, DW, MM, AG, and SB conceived the study design. AM was involved in the generation of the data and CS performed statistical analyses on the data generated. All co- authors substantially contributed to editing and formatting the manuscript for submission.

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Disclosure statement:

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Table 1: Prevalence of resistant associated polymorphisms in HCV NS3 and NS5A according to genotype and cohort.

	NS3 polymorphisms	**NS5A polymorphisms**	**NS5B (S282T)**
Acute			
GT1a (n=19)	1 (5.3%)(1 **Q80K)**	2 (10.5%) (1 **Q30H,** 1 H58P)	0
GT3 (n=2)	N/A	0	0
GT4 (n=4)	N/A	4 (100%)(4 L30R*)	0
Chronic treatment-naïve			
GT1a (n=11)	1 (9.1%)(**Q80KQ**)	1 (9.1%)(**Q30H**)	0
GT1b (n=3)	2 (66.7%)(2 I132V†)	1 (33.3%)(**Y93H**)	0
GT4 (n=6)	N/A	6 (100%)(2 L30R*, 4 L30R* T58P)	0
Chronic treatment-experienced (no SVR)			
GT1a (n=27)	3 (11.1%)**(Q80K**)	2 (7.4%)(1 **Q30QR**,1 **L31M**)	0
GT1b (n=1)	1 (100%)(I132IV†)	0	0
GT3 (n=3)	N/A	1 (33.3%)(P58S)	0
GT4 (n=3)	N/A	3 (100%)(2 L30R*, 1 L30R* +T58P)	0
Mono-infected			
GT1a (n=23)	11 (47.8%)(6 **Q80K**,1 **Q80R**,1 T54S, 1**T54ST+Q80K**, 1 V36L+**Q80K,** 1 **R155K**)	5 (34.8%) (1 H58P, 1 H58HP,1 **Q30R**, 1**Q30HQ** + **Y93HY**, 1 Q30LQ)	0
GT1b (n=14)	10 (71.4%)(9 I132IV†, 1V36L+I132V)	2 (14.3%)(1 **Y93HY,**1 **Y93H**)	0
GT2 (n=18)	N/A	12 (66.7%) (4 **L31M**, 7 S58P, 1 K30R)	0
GT3 (n=17)	N/A	1 (5.9%)(1 **A30K**)	0
GT4 (n=13)	N/A	7 (53.8%) (5 L30R*, 2 L30R* + T58P)	0

RAVs conferring moderate to high fold DAA resistance are shown in bold type. Polymorphisms detected and number of individuals harbouring certain polymorphisms are shown in brackets. Common GT1b NS3 polymorphism (†); common GT4 NS5A polymorphism (*) conferring low level resistance effects. N/A, not applicable.