

# Hepatitis C Resistance-Associated Substitutions Among People Who Inject Drugs Treated With Direct-Acting Antiviral-Containing Regimens

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**Background.** Resistance-associated substitutions (RASs) to HCV direct-acting antivirals (DAAs) can contribute to virologic failure and limit retreatment options. People who inject drugs (PWID) are at highest risk for transmission of resistant virus. We report on RASs at baseline and after virologic failure in DAA-naïve and protease inhibitor-experienced PWID.

**Methods.** We sequenced the NS3/4A, NS5A, and NS5B regions from 150 PWID with genotype 1 (GT1) viruses; 128 (85.3%) GT1a, 22 (14.7%) GT1b.

**Results.** Among the 139 (92.7%) DAA-naïve PWID, 85 of 139 (61.2%) had baseline RASs—67 of 139 (48.2%) in NS3 (predominantly Q80K/L); 25 of 139 (18.0%) in NS5A; and 8 of 139 (5.8%) in NS5B. Of the 11 protease inhibitor-experienced participants, 9 had baseline NS3 RASs (V36L N = 1, Q80K N = 9) and 4 had baseline NS5A RASs (M28V N = 2, H58P N = 1, A92T N = 1). Among the 11 participants who had posttreatment samples with detectable virus (7 treatment failures, 1 late relapse, 3 reinfections), 1 sofosbuvir/ledipasvir failure had a baseline H58P. Two sofosbuvir/ledipasvir-treated participants developed new NS5A mutations (Q30H, Y93H, L31M/V). Otherwise, no RASs were detected.

**Conclusions.** Our results demonstrate RAS prevalence among DAA-naïve PWID is comparable to that in the general population. Only 2 of 150 (1.3%) in our longitudinal cohort developed treatment-emergent RASs. Concern for transmission of resistant virus may therefore be minimal.

**Keywords.** direct-acting antivirals (DAAs); hepatitis C virus (HCV); people who inject drugs (PWID); resistance-associated substitutions (RASs); transmitted resistance.

People who inject drugs (PWID) are at the heart of the hepatitis C virus (HCV) pandemic. Globally, an estimated 8.2 million (52%) of PWID are HCV antibody positive [1, 2]. In the United States, approximately 53% of PWID are anti-HCV positive, with rates ranging from 38.1% to 68.0% [3], compared with 1.7% in the general American population [4]. Although advancements in HCV treatment have made sustained virologic response (SVR), or HCV cure, attainable in almost every individual who receives direct-acting antiviral (DAA) therapy, few data are available on the prevalence of resistance-associated substitutions (RASs) among PWID and the associated treatment outcomes within this population [5, 6]. Resistance-associated substitutions make

individuals more prone to failing HCV treatment, leading to lower SVR. Moreover, treatment-induced RASs may hinder future retreatment options and lead to transmitted resistance [7–10].

Due to the single-stranded nature of the HCV ribonucleic acid virus (RNA), it is inherently prone to resistance [7, 11]. Resistance can be present before treatment due to natural variation, or it can develop during the course of treatment due to viral replication in the presence of drug pressure associated with DAAs, which may cause DAA regimens to fail [12]. Resistance-associated substitution prevalence varies by the region sequenced and among genotypes. For example, among treatment-naïve individuals in the general population with genotype (GT) 1a or 1b, approximately 45% have NS3 RASs, whereas rates in the NS5A and NS5B regions are typically much lower; approximately 13% and 8%, respectively [13]. Depending on the individual's HCV genotype and the DAA drug class used, RASs that can become predominant during treatment may disappear once drug pressure is removed. For example, the treatment-induced S282T variant in the NS5B region, which is associated with nucleoside inhibitor resistance, has been shown to rapidly decrease after treatment cessation,

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among those being treated with sofosbuvir (SOF) [14–16]. However, treatment-induced variants in the NS5A regions may persist for years [7, 8, 17].

Hepatitis C virus treatment-failure can be attributed to host, viral, or treatment-related factors [7]. Host factors include clinical characteristics, such as the presence of cirrhosis. Viral factors include genotype, baseline viral load, and presence of baseline RASs. Treatment-related factors include previous treatment experience, limited adherence, or treatment interruption leading to a reduced course of therapy [7]. Therefore, RASs are only one element that predicts treatment failure; when combined with other adverse host, viral, or treatment-related factors, treatment failure may be more likely. In addition, certain genotypes/subtypes are more prone to resistance. For example, HCV GT1a, which is the most prevalent worldwide and among PWID, has been found to be more likely to develop resistance [7, 11, 18, 19] due to a lower genetic barrier for RASs [9].

To date, data are limited on RAS prevalence among PWID. Resistance-associated substitutions are theoretically more likely to occur among PWID than in the general population due to concerns about PWID having lower adherence leading to insufficient drug pressure with subsequent emergence of RASs. Although there is little evidence to support this, risky behaviors such as needle sharing among actively injecting PWID may also increase the risk for forward transmission to injection partners [20, 21]. This could contribute to an increase in drug-resistant strains of HCV infection and reinfection within the PWID community and eventually the general population [21]. Therefore, there is a critical need to understand the prevalence of RASs and the interrelationship between DAA adherence and the development of RASs among PWID.

The primary aim of this study was to outline the prevalence of RASs in a cohort of GT1-infected PWID engaged in opioid agonist therapy (OAT). A secondary aim was to assess RAS presence among those with detectable viremia at the end of treatment. We anticipate that these findings will aid in understanding the risk of baseline and transmitted RASs among PWID.

## METHODS

### Participants and Setting

Participants were part of the PREVAIL study, a randomized controlled trial that evaluated the effectiveness of 3 models of HCV care—directly observed therapy, group treatment, and self-administered individual treatment—among 150 PWID on OAT in the Bronx, New York [22]. As part of the trial, clinical characteristics such as the presence of cirrhosis were established at baseline, and participants completed biobehavioral surveys at each research visit that included questions related to their demographics, risk behaviors, HCV-related knowledge, social support, and treatment adherence, as described elsewhere [23]. All participants had HCV GT1. Trial participants were initiated

on HCV treatments according to American Association for the Study of Liver Diseases (AASLD)/Infectious Diseases Society of America (IDSA) guidelines from October 2013 to April 2017: telaprevir, pegylated interferon, and ribavirin (TVR/IFN/RBV); sofosbuvir, pegylated interferon, and ribavirin (SOF/IFN/RBV); sofosbuvir and ribavirin (SOF/RBV); or a combination DAA regimen of sofosbuvir and simeprevir (SOF/SMV) or sofosbuvir/ledipasvir (SOF/LDV).

We developed a biorepository containing samples from the PREVAIL study visits at baseline, treatment week (TW) 4, end of treatment, and during posttreatment weeks (PT) 4, 12, and 24. In the parent study, HCV viral load at PT12 was used to assess for SVR, and the study visit at PT24 was used to assess short-term reinfection rates. We collected blood samples at each visit for RAS assessment by Monogram Biosciences, LabCorp (South San Francisco, CA).

### Sequencing Analysis

We performed consensus sequencing of NS3/4A, NS5A, and NS5B regions on samples submitted for RAS analysis. For each sample, we used GT1a- or GT1b-specific primers to amplify the NS3/4A, NS5A, and NS5B regions by reverse-transcription polymerase chain reaction. We analyzed amplified regions by Sanger-based sequencing or using the Illumina MiSeq platform with a 10% variant reporting threshold (comparable to the sensitivity obtained in the Sanger-based assays). We reported substitutions relative to the H77 (GT1a) and Con1 (GT1b) reference sequences that represented naturally occurring polymorphisms or treatment-emergent variants associated with reductions in SVR rates, variants that emerge during DAA treatment or relapse, and/or that may confer reductions in susceptibility based on *in vitro* data. Resistance-associated substitution prevalence in the PREVAIL samples was compared with the prevalence in Monogram Biosciences' database of HCV samples submitted for routine RAS testing between August 2011 and March 2017 for NS3, between May 2015 and March 2016 for NS5A and NS5B, and published literature. Our primary outcome was the presence of RASs among PREVAIL participants at baseline. Our secondary outcome was presence of RASs among those who failed DAA treatment.

Before conducting this substudy, we had also performed HCV phylogenetic analysis in which we applied next-generation sequencing to the hypervariable region 1 (HVR1) of the HCV genome, as described elsewhere [24]. We used findings from our phylogenetic analysis to support findings in this study, such as in instances of suspected reinfection or relapse.

### Treatment Outcomes

We obtained results of HCV RNA tests through medical chart review or from blood draws. We defined SVR as an HCV RNA level below the limit of quantification at PT12, using COBAS TaqMan real-time reverse transcriptase polymerase chain

reaction assay (Roche Diagnostics, Indianapolis, IN). We defined treatment failure as having a detectable HCV viral load 12 weeks after HCV treatment completion, late relapse as a detectable HCV viral load later than 12 weeks after HCV treatment completion, and reinfection as a detectable viral load after treatment completion with confirmation of a distinct viral strain using phylogenetic analysis, as described elsewhere [24].

### Adherence

Medication adherence was measured using electronic Med-ic blister packs (Information Mediary Corp., Ottawa, Canada), which have a 99.6% event accuracy (time of dose removal correctly recorded within  $\pm 2$  minutes) [25]. Adherence was defined as a continuous outcome, calculated as the percentage of expected blister-pack medication dispensed during 2-week intervals [23].

### Patient Consent Statement

All participants provided written informed consent, and the study was conducted in accordance with Good Clinical Practice and the ethical principles that originated in the Declaration of Helsinki. The study was approved by the institutional review board of Albert Einstein College of Medicine.

## RESULTS

Of the 150 PWID enrolled in the study, 128 (85.3%) were infected with HCV GT1a and 22 (14.7%) were infected with HCV GT1b. Most ( $N = 139$ , 92.7%) were DAA-naive. The remaining 11 participants (7.3%) were DAA-experienced with a protease inhibitor (PI). Sixteen (11%) were cirrhotic (Table 1).

In terms of treatment regimens, 104 participants were treated with SOF/LDV, 32 participants were treated with SOF/RBV  $\pm$  IFN, 11 participants were treated with SMV/SOF, and 3 participants were treated with TVR/RBV/IFN (Table 1). Overall, 97% of study participants completed treatment and 94% achieved SVR [22]. Among all participants, 9 (6%) failed treatment (2 of whom died while on HCV treatment due to unrelated causes) and 1 had late relapse (viremic at PT24). Three other participants were reinfected (2 at the PT24 timepoint, 1 occurred 17 months after treatment) [26]. Eleven participants had follow-up viral load samples with detectable virus during or after treatment completion (7 treatment failures, 1 late relapse, 3 reinfections), depicted in Table 3.

### Baseline

Among all 150 participants, 96 participants (64.0%) had at least 1 RAS present at baseline, with half (50.7%) of all participants presenting with RASs present in the NS3/4A region (Table 2). The Q80K/L polymorphism was the most prevalent variant, appearing in 55 (36.7%) of all participants. NS5A and NS5B RASs were less prevalent among participants—19.3% and 5.3%, respectively.

**Table 1. PREVAIL Study Participant Characteristics**

Genotype		
1a	128	85.3%
1b	22	14.7%
Prior Treatment With DAAs		
DAA-naive	139	92.7%
DAA-experienced (protease inhibitor-experienced)	11	7.3%
Treatment		
TVR/RBV/IFN	3	2.0%
SMV/SOF	11	7.3%
SOF/RBV $\pm$ IFN	32	21.3%
SOF/LDV	104	69.3%
Treatment Outcome		
Successfully completed	137	91.3%
Late relapse	1	0.7%
Treatment failure	7	4.7%
Died during treatment	2	1.3%
Reinfection	3	2.0%
Treatment Failure Regimens		
SOF/RBV	3	42.9%
SOF/LDV	4	57.1%
Cirrhosis	16	11%
Baseline RAS		
Yes (n = 96)	96	64.0%
NS3/4A	76	79.2%
NS5A	29	30.2%
NS5B	8	8.4%
No	54	36.0%

Abbreviations: DAA, direct-acting antiviral; SOF/RBV  $\pm$  IFN, sofosbuvir, ribavirin, with or without pegylated interferon; RAS, resistance-associated substitution; SOF/LDV, sofosbuvir/ledipasvir; SOF/RBV, sofosbuvir and ribavirin; SOF/SMV, sofosbuvir and simeprevir; TVR/IFN/RBV, telaprevir, pegylated interferon, and ribavirin.

A total of 139 participants were DAA-naive, and over half (61.2%) of these individuals had RASs at baseline (Table 2). All of those classified as PI-experienced had RASs present at baseline. The majority of baseline RASs were in the NS3/4A region for both the DAA-naive and PI-experienced individuals—67 of 139 (48.2%) and 9 of 11 (81.8%), respectively. The Q80K/L polymorphism was the most common variant found in 46 (33.15%) of the DAA-naive participants at baseline. Resistance-associated substitutions were also present in the NS5A region and the NS5B region among DAA-naive participants at baseline—25 (18.0%) and 8 (5.8%), respectively. Among the PI-experienced participants, 9 of 11 (81.8%) had virus with NS3/4A RASs, 4 of 11 (36.4%) had NS5A RASs, and none had NS5B RASs.

When examined by genotype, a greater proportion of participants with HCV GT1a had RASs present at baseline; 86 of 128 (67.2%) in GT1a versus 10 of 22 (45.5%) in GT1b (Table 2). Among the 128 participants with HCV GT1a, 69 (53.9%) had RASs in the NS3/4A region compared with 7 (31.8%) of the 22 GT1b participants. NS5A RASs were less prevalent among those with GT1a vs GT1b; 23 of 128 (18.0%) versus 6 of 22 (27.3%). NS5B RASs were present at baseline for 8 of 128 (6.3%)

**Table 2. Resistance-Associated Substitution Prevalence at Baseline Among PREVAIL Study Participants**

	DAA-Naive (n = 139)		DAA-Experienced <sup>a</sup> (n = 11)		GT1a (N = 128)		GT1b (n = 22)		Total (n = 150)	
	No.	%	No.	%	No.	%	No.	%	No.	%
No. of participants with any RASs overall	85	61.2%	11	100.0%	86	67.2%	10	45.5%	96	64.0%
No. of participants with any RASs in NS3/4A	67	48.2%	9	81.8%	69	53.9%	7	31.8%	76	50.7%
S122G/N/T	14	10.1%	0	0.0%	9	7.0%	5	22.7%	14	9.3%
Q80K/L	46	33.1%	9	81.8%	55	43.0%	0	0.0%	55	36.7%
V55A/I	6	4.3%	0	0.0%	5	3.9%	1	4.5%	6	4.0%
T54S	3	2.2%	0	0.0%	3	2.3%	0	0.0%	3	2.0%
V36M/L	3	2.2%	1	9.1%	4	3.1%	0	0.0%	4	2.7%
I132V	1	0.7%	0	0.0%	1	0.8%	0	0.0%	1	0.7%
Y56F	3	2.2%	0	0.0%	1	0.8%	2	0.0%	3	2.0%
Total RASs in NS3/4A	76	-	10	-	78	-	8	-	86	-
No. of participants with any RASs in NS5A	25	18.0%	4	36.4%	23	18.0%	6	27.3%	29	19.3%
Y93H/S	5	3.6%	0	0.0%	1	0.8%	4	18.2%	5	3.3%
H58H/P	6	4.3%	1	9.1%	7	5.5%	0	0.0%	7	4.7%
H54Y	1	0.7%	0	0.0%	1	0.8%	0	0.0%	1	0.7%
L31M	2	1.4%	0	0.0%	1	0.8%	1	4.5%	2	1.3%
M28V	8	5.8%	2	18.2%	10	7.8%	0	0.0%	10	6.7%
K24K/R	3	2.2%	0	0.0%	3	2.3%	0	0.0%	3	2.0%
A92T	0	0.0%	1	9.1%	0	0.0%	1	4.5%	1	0.7%
Total RASs in NS5A	25	-	4	-	23	-	6	-	29	-
No. of participants with any RASs in NS5B	8	5.8%	0	0.0%	8	6.3%	0	0.0%	8	5.3%
S556G/R	5	3.6%	0	0.0%	5	3.9%	0	0.0%	5	3.3%
S473S/T	1	0.7%	0	0.0%	1	0.8%	0	0.0%	1	0.7%
G307G/R	2	1.4%	0	0.0%	2	1.6%	0	0.0%	2	1.3%
Total RASs in NS5B	8	-	0	-	8	-	0	-	8	-

Abbreviations: DAA, direct-acting antiviral; GT, genotype; RAS, resistance-associated substitution.

<sup>a</sup>DAA-experienced included protease inhibitors only.

of GT1a participants, but these were not present among any of the GT1b participants.

## Treatment Outcomes

### Treatment Failures

Of the 7 treatment failures, 4 were treated with SOF/LDV and 3 were treated with SOF/RBV. Resistance-associated substitution presence at baseline and persistence throughout the study period varied among these individuals (Table 3). For example, participant AL2020 had no baseline RASs, but he/she had detectable virus at PT4 despite having an undetectable viral load upon treatment completion and an average daily adherence of 82.2%. At PT4, Q30H and Y93H RASs were present in the NS5A region along with other variants in this region. Phylogenetic analysis revealed that a minority viral sequence that was present at baseline at very low frequency became predominant at PT4 through PT24, as described previously [24].

The 3 other SOF/LDV treatment failures had baseline RASs, 1 in the NS5A region and 2 in the NS3/4A region. All had undetectable virus during treatment, but they had poor adherence ranging from 31% to 58% with detectable viral loads from the end of treatment through PT24. AL1046 had virus persistent with NS5A RASs (H58P) at baseline and throughout the posttreatment period, and they acquired an additional

NS5A RAS (L31M/V) posttreatment. Participant AL1035 had Q80K present at baseline, which persisted throughout the posttreatment period. Participant AL1080 had a baseline Q80K. Although a follow-up specimen was not available for RAS analysis, this participant had an average daily adherence of 38.0% and a detectable viral load at TW12 with viremia persisting throughout the posttreatment period. Phylogenetic analysis revealed similar sequences during the baseline and posttreatment periods [24], all of which support a treatment failure in this participant.

In the SOF/RBV-treated participant, AL2009's HCV RNA levels were never fully suppressed. This participant had a Q80K polymorphism at all available timepoints from baseline to PT4 and denied injection drug use 30 days before the baseline and end of treatment visits. In addition, at the end of treatment a L159L/F RAS emerged in the NS5B region but was no longer present in the posttreatment timepoint. Two of the other participants on SOF/RBV, AL1021 and AL1024, had baseline NS3/4A RASs and discontinued treatment early. For AL1021, these RASs persisted posttreatment. For AL1024, R155R/K was detected at baseline, TW4, PT4, and PT12, but not at EOT or PT24. This may reflect fluctuating levels of the R155K RAS above and below the assay reporting threshold, as described elsewhere [27].

**Table 3. Resistance-Associated Substitution Distribution Among PREVAIL Participants With Virologic Relapse**

Study ID	Genotype	Regimen	Cirrhosis	Average Daily Adherence	Treatment Outcome	Baseline		Treatment Week 4		End of Treatment		Week 4 Posttreatment		Week 12 Posttreatment		Week 24 Posttreatment	
						NS3/4A	NS5A	NS5B	NS3/4A	NS5A	NS5B	NS3/4A	NS5A	NS3/4A	NS5A	NS5B	NS3/4A
AL1021	1b	SOF/RBV	No	78.73	Treatment failure	S122G		S122G		S122G		S122G		S122G		S122G	
AL1024	1a	SOF/RBV/PEG	No	-	Treatment failure	R155R/K		R155R/K		R155R/K		R155R/K		R155R/K		R155R/K	
AL1035	1a	SOF/LDV	Yes	43	Treatment failure	Q80K		Q80K		Q80K		Q80K		Q80K		Q80K	
AL1046	1a	SOF/LDV	No	31.4	Treatment failure	H58P		H58P		L31M/V, H58P		L31M/V, H58P		L31M, H58P		L31M, H58P	
AL2009	1a	SOF/RBV	No	45.17	Treatment failure	Q80K		Q80K		L159L/F		Q80K		Q80K		Q80K	
AL2020	1a	SOF/LDV	No	82.17	Treatment failure												
AL1080	1a	SOF/LDV	No	38	Treatment failure	Q80K		Q80K		Q80K		Q80K		Q80K		Q80K	
AL1019	1a	SOF/RBV	Yes	53.5	Late relapse												
AL1020	1a	SOF/RBV	No	88.77	Reinfection		G307R										
AL1075	1a	SOF/LDV	Yes	58.42	Reinfection	Q80K/L		Q80K/L		Q80K/L		Q80K/L		Q80K/L		Q80K/L	
AL2019	1a	SIM/SOF	Yes	57.09	Reinfection	Q80K		Q80K		Q80K		Q80K		Q80K		Q80K	

Abbreviations: ID, identification; PEG, pegylated; SIM, simeprevir; SOF/LDV, sofosbuvir/ledipasvir; SOF/RBV, sofosbuvir, ribavirin.

### Reinfections

Three participants experienced reinfections. All 3 were infected with HCV GT1a and reported ongoing injection drug use [26]. Both AL1075 and AL2019 had baseline virus with a Q80K and daily adherence of 58.4% and 57.1%, respectively. The Q80K/L variant was the only RAS present for AL1075 at baseline. Although RAS data were not available for this participant beyond baseline, phylogenetic analysis revealed they were reinfected with phylogenetically distinct strains by PT24 [24]. Participant AL2019, treated with SIM/SOF, had a Q80K present at baseline and no detectable virus during the treatment period; however, at PT24, the Q80K was absent, and this participant had detectable virus again with several variants that were not present at baseline. This, along with phylogenetic analysis, which revealed 3 distinct strains after treatment completion, is consistent with reinfection [24].

Participant AL1020 had a G307R NS5B variant present at baseline, although not clinically significant for the SOF/RBV treatment regimen. Despite missing RAS data for the posttreatment period, this individual presented as transiently viremic at 17 months posttreatment and therefore is suspected to have experienced a reinfection.

### Late Relapse

In addition to the 10 participants discussed above, another was treated with SOF/RBV with viral rebound at PT24 and classified as a late relapse. At baseline, this participant, AL1019, did not present with any RASs but was cirrhotic. Although on SOF/RBV, they achieved an undetectable viral load by the end of treatment, but they experienced virologic relapse at PT24. At TW4, the participant's virus had similar nonsignificant variants to the BL virus, and a variant profile consistent with the prior virus re-emerged at PT24 (data not shown).

## DISCUSSION

To our knowledge, this is the first study to (1) outline baseline and postbaseline RASs among PWID and (2) define associations between adherence and the development of resistance within several timepoints on DAA therapy. We identified 85 (61.2%) DAA-naive PWID participants who had RASs present at baseline. Among these individuals, RASs were most common in the NS3/4 region, particularly for the Q80K/L polymorphism, which was found in 33.1% of all DAA-naive participants. Overall, RAS prevalence among treatment-naive PWID in our cohort is largely comparable to that expected in DAA-naive individuals in the general population [28, 29]. For example, NS3 RASs were present at baseline for 45% of treatment-naive participants with GT1a and 1b in the Wang et al [29] cohort, with 13% and 8% having RASs present in the NS5A and NS5B regions, respectively, at baseline. Only 2 of 150 (1.3%) in our longitudinal cohort developed treatment-emergent RASs. Therefore, transmission of resistant strains

may be minimal, particularly given the high SVR rate in this PWID cohort.

Consistent with existing studies, the Q80K variant was more common among our participants with GT1a compared to those with GT1b, and with previous PI treatment experience compared to those without. Of the 128 participants in PREVAIL with HCV GT1a, 55 (43.0%) had a Q80K/L variation present at baseline. Past studies have found Q80K polymorphism to be present in 34% to 48% of individuals with GT1a [9, 13, 29–35]. For example, 44% of GT1a participants in the Wang et al [29] cohort had the Q80K variant. In Monogram Bioscience's database, which includes both treatment-naive and -experienced individuals, 42.0% of GT1a samples had a Q80K polymorphism [36]. The latter is consistent with findings from other US-based studies where greater than 40% of individuals with HCV GT1a are found to have a Q80K variant [29, 36, 37].

Resistance-associated substitutions in the NS5A region are more commonly found in individuals with HCV GT1b than those with GT1a, and these differences are often exacerbated during treatment with LDV/SOF [8, 28, 38]. Consistent with other literature, we found a higher frequency of RASs in this region among participants with HCV GT1b compared with GT1a. Of the 128 people with GT1a virus, 23 (18.0%) had RASs in the NS5A region compared with 6 (27.3%) of the 22 participants with GT1b. As demonstrated by Wyles and Luetkemeyer [38], NS5A RASs emerge in treatment failures with NS5A inhibitor-containing regimens. Only 2 participants in our study had treatment-emergent RASs, and these were located in the NS5A region. For one participant, an L31M/V emerged and persisted posttreatment. Another participant had a Q30H and Y93H that emerged and persisted as well. These RASs were not detected in either of these participants' baseline consensus sequencing; however, supplemental phylogenetic analysis confirmed that the infection observed during follow-up for the second participant likely resulted from selection of the minority variant that was present at baseline but was present at a quantity too low to be detected through consensus sequencing [24]. Presence of this minority variant could explain this participant's treatment failure despite a daily adherence rate of 82.2%.

The most common NS5B variants in our sample were S556G/R and G307G/R with prevalence rates of 3.6% and 1.3%, respectively. Similar to our findings, of 500 routine clinical samples submitted to Monogram Biosciences for NS5B resistance analysis, the most prevalent RASs observed were S556G/N (2.8%) and G307R (4.2%) [39]; therefore, the prevalence of these RASs appeared to be similar in our cohort of PWID. In terms of treatment outcomes, the only NS5B RAS observed was in AL2009 who developed an L159L/F at the end of treatment timepoint. This RAS developed in the setting of poor average daily adherence (45%) and disappeared posttreatment suggesting selection of L159L/F, possibly due to inadequate drug pressure. Similar phenomena have been reported elsewhere,

and it is not uncommon for individuals being treated with SOF/RBV who have the L159F mutation to have lower SVR rates [40, 41].

This study has limitations. First, although these findings are consistent with RAS prevalence in the general population, these may underestimate the current and future prevalence of RAS among PWID. This study was conducted from October 2013 to May 2016 when newly available DAA therapy regimens became available. Therefore, sufficient exposure to DAA-containing regimens to reach a meaningful degree of circulating resistance transmitted within the PWID communities was unlikely during this study timeframe. Second, given the characteristics of the sample population, these findings are not generalizable to all PWID with HCV. This study only characterized the prevalence of RASs among participants from the parent study who are individuals with HCV GT1a and GT1b. In addition, all individuals were enrolled in OAT programs; therefore, these findings are only generalizable to PWID. Third, RAS testing was not available at each timepoint for all participants; however, we were able to use supplementary phylogenetic analysis to understand treatment patterns where RAS data were missing. Finally, RASs were determined using population-based Sanger sequencing or using the Illumina MiSeq platform with a threshold applied to match the sensitivity of the population-based sequence assay because clinical studies indicated that the detection of variants at lower thresholds may not be clinically relevant. Although this is the form of sequencing that is commercially available, more information could be gained from next-generation sequence analysis using a lower threshold to determine whether minority variants harboring RASs were present in individuals with treatment failure and whether this correlated to adherence. This technique may also be required to determine whether changes in variants after treatment is due to reinfection or the emergence of existing minority variants.

## CONCLUSIONS

In conclusion, our findings suggest that the PWID in this study did not harbor a significantly greater prevalence of RASs compared with expected population prevalence. Moreover, few participants failed treatment and developed RASs leading to low concern for transmission of resistant strains. Given that the few participants who did fail treatment were not adequately adherent, support should be provided to PWID facing barriers to adequate adherence; and because PWID are at the greatest theoretical risk for transmitting resistant HCV strains among whom RASs develop, harm reduction should be provided to those with ongoing risk behaviors. Now that there has been greater DAA coverage, future studies should assess the presence of RASs among PWID, particularly those not receiving OAT. If enrichment of RASs is demonstrated, there may be implications for initial treatment strategies among PWID despite the availability of newer pangenotypic

regimens with higher barriers to resistance. Since PWID may be itinerant and seek care at multiple healthcare facilities, it is important to screen for previous treatment failures with DAA-containing regimens that may require second-line regimens and possibly RAS testing, especially in the setting of failed retreatment. Moreover, it may be prudent to screen PWID who report injecting with another individual who has failed treatment since this could result in transmitted resistance. Such targeted strategies may be more efficient in selecting PWID to test for baseline RASs and initiation of second-line regimens to improve treatment outcomes and prevent further DAA resistance. Consideration should also be made for better HCV surveillance systems to monitor for emerging resistant strains to support local and global HCV elimination efforts.

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