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NS5A Resistance-Associated Substitutions in Patients with Genotype 1 Hepatitis C Virus: Prevalence and Effect on Treatment Outcome

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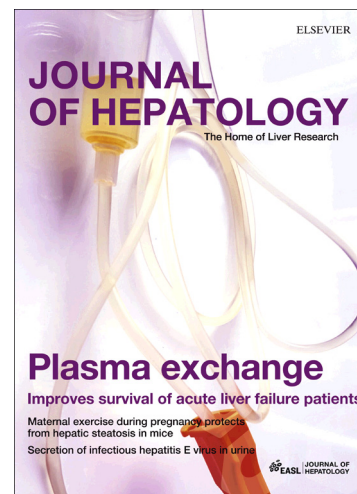
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1 **NS5A Resistance-Associated Substitutions in Patients with Genotype 1 Hepatitis C Virus:**
2 **Prevalence and Effect on Treatment Outcome**

3
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22
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47

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56

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61

62 **ABSTRACT**

63 **Background:** The efficacy of NS5A inhibitors for the treatment of patients chronically infected
64 with hepatitis C virus (HCV) can be affected by the presence of NS5A resistance-associated
65 substitutions (RASs). We analyzed data from 35 phase 1, 2, and 3 studies in 22 countries to
66 determine the pretreatment prevalence of various NS5A RASs, and their effect on outcomes of
67 treatment with ledipasvir-sofosbuvir in patients with genotype 1 HCV.

68 **Methods:** NS5A gene deep sequencing analysis was performed on samples from 5,397 patients
69 in Gilead clinical trials. The effect of baseline RASs on sustained virologic response (SVR) rates
70 was assessed in the 1,765 patients treated with regimens containing ledipasvir-sofosbuvir.

71 **Results:** Using a 15% cut-off, pretreatment NS5A and ledipasvir-specific RASs were detected in
72 13% and 8% of genotype 1a patients, respectively, and in 18% and 16% of patients with
73 genotype 1b. Among genotype 1a treatment-naïve patients, SVR rates were 91% (42/46) vs 99%
74 (539/546) with and without ledipasvir-specific RASs, respectively. Among treatment-
75 experienced genotype 1a patients, SVR rates were 76% (22/29) vs 97% (409/420) with and
76 without ledipasvir-specific RASs, respectively. Among treatment-naïve genotype 1b patients,
77 SVR rates were 99% for both those with and without LDV-specific RASs (71/72 vs 331/334) ,
78 and among treatment-experienced genotype 1b patients, SVR rates were 89% (41/46) vs 98%
79 (267/272) for those with and without ledipasvir-specific RASs, respectively.

80 **Conclusions:** Pretreatment ledipasvir-specific RASs that were present in 8%-16% of patients
81 have an impact on treatment outcome in some patient groups in particular treatment-experienced
82 patients with genotype 1a HCV.

83 • **Keywords:** NS5A RAS, HCV genotype 1, ledipasvir-sofosbuvir

84

85 **LAY SUMMARY**

86 The efficacy of treatments using NS5A inhibitors for patients with chronic Hepatitis C virus
87 (HCV) infection can be affected by the presence of NS5A resistance-associated substitutions
88 (RASs). We reviewed results from 35 clinical trials where patients with genotype 1 HCV
89 infection received treatments that included ledipasvir-sofosbuvir to determine how prevalent
90 NS5A RASs are in patients at baseline, and found that ledipasvir-specific RASs were present in
91 8-16% of patients prior to treatment and had a negative impact on treatment outcome in subset of
92 patient groups in particular treatment-experienced patients with genotype 1a HCV.

93 INTRODUCTION

94 Due to high rates of viral replication and an error prone HCV RNA polymerase, tremendous
95 variability of HCV has been observed within infected patients (quasispecies) with all single
96 mutations that do not abolish viral replication thought to be pre-existing (1). As a result, NS5A
97 RASs are observed at baseline in patients infected with chronic HCV. Deep sequencing enables
98 detection of HCV substitutions, point deletions, or insertions within the quasispecies down to a
99 frequency of 1%. However, commercially available assays based on standard population HCV
100 sequencing or not cross-validated next generation, also called deep sequencing, report variants
101 with a frequency of $\geq 15\%$ of the quasispecies.

102 The prevalence of baseline NS5A RASs has been reported to be 6% to 16% using population
103 sequencing (cut off 15-25%) or deep sequencing (cut off 1%) (2-4). Interestingly, the prevalence
104 and type of baseline NS5A RASs may vary by geographic regions. For example, the prevalence
105 of the NS5A M28V in genotype 1a-infected patients was shown to be higher in the United States
106 than in Europe, 7% versus 0%, respectively (5). Furthermore, the prevalence of genotype 3
107 NS5A Y93H varied between 0% and 17% in different geographic regions (6). A comparison of
108 baseline prevalence of RASs in Japanese and Western patients showed that the prevalence of
109 Q80L and S122G in NS3, and L28M, R30Q and Y93H in NS5A was significantly higher in
110 Japanese patients than the Western counterparts (7).

111 Many currently approved interferon (IFN)-free regimens for the treatment of chronic hepatitis C
112 (HCV) include an inhibitor of HCV NS5A. To date, there are five NS5A inhibitors approved for
113 treatment of chronic HCV infection; ledipasvir (LDV), daclatasvir, and velpatasvir (which are all
114 administered with the NS5B inhibitor sofosbuvir), and ombitasvir (in a fixed-dose combination
115 with the protease inhibitor paritaprevir, the nonnucleoside NS5B polymerase inhibitor dasabuvir,

116 and ritonavir, a potent inhibitor of CYP3A4 enzymes), and elbasvir (in a fixed-dose combination
117 with the protease inhibitor grazoprevir) (8-12). The presence of baseline NS5A RASs may
118 impact treatment outcome of some NS5A inhibitor containing HCV regimens due to the intrinsic
119 qualities of the NS5A inhibitor, drug pharmacology, or effects of the other compounds within the
120 treatment regimen. However, depending on how NS5A RASs are defined and included in
121 resistance analysis, as well as what level of variant detection is utilized, different results may be
122 obtained. To date, three definitions of NS5A variants that are associated with resistance have
123 been used most commonly; polymorphisms at RAS positions (RAPs), class RASs, and drug-
124 specific RASs. Polymorphisms at RAS positions are defined as any change from reference
125 sequence for a specific genotype at positions associated with NS5A inhibitor resistance. NS5A
126 class RASs are substitutions that have been shown to emerge on treatment or confer a significant
127 reduction in susceptibility in vitro (e.g., >2.5 fold change in EC_{50}) to any approved or
128 investigational NS5A inhibitor. Drug-specific RASs refer to substitutions that have been shown
129 to emerge on the specific drug treatment or confer significantly reduced susceptibility in vitro to
130 the specific NS5A inhibitor. In addition, drug-specific RASs can be categorized into groups with
131 different levels of reduced susceptibility to the drug.

132 To enable comparisons of resistance analyses between clinical trials, standardization of RAS
133 definitions and sensitivity cut offs is needed. In several studies, population sequences were used
134 for resistance analysis (cut off of variant detection 15%-25%) and NS5A polymorphisms at RAS
135 positions were defined as RAPs. In these studies, the presence of baseline NS5A polymorphisms
136 at RAS positions had shown no significant impact on treatment outcome (5, 12). Further study is
137 needed to understand the role of RASs present at frequencies below 15% and whether

138 substitutions without an in vitro susceptibility change to the NS5A inhibitor may dilute a clinical
139 signal by RASs that do confer reduced susceptibility to a specific NS5A inhibitor.

140 Here, we characterized the prevalence of baseline NS5A RASs in 5,397 NS5A inhibitor-naïve
141 patients infected with genotype 1a or 1b HCV according to geographic regions. Moreover, we
142 assessed the effect of baseline NS5A RASs, defined as NS5A RASs, NS5A class RASs or LDV-
143 specific RASs using 1% and 15% sensitivity of substitution detection cut offs, on treatment
144 outcome among 1,765 patients treated with currently recommended regimens containing
145 ledipasvir-sofosbuvir. A previous analysis using a portion of the same dataset has recently been
146 published (13). That analysis concerned the prevalence and effect on treatment of NS3, NS5A,
147 and NS5B RASs, and included data on patients who had been treated with regimens/durations
148 that have not been incorporated into label recommendations or treatment guidelines. The current
149 study covers only NS5A RASs and includes data only from patients who received guideline-
150 recommended regimens.

151

152 MATERIAL AND METHODS

153 Sequencing Analysis

154 Deep sequencing of baseline plasma samples was performed in 5,397 patients from 22 countries
155 across the HCV Gilead clinical development program from 2010 to 2015. The list of clinical
156 trials and identification numbers are included in the supplement materials (supplemental Table
157 1). The HCV NS5A coding regions were amplified by DDL Diagnostic Laboratory (Rijswijk,
158 Netherlands) using proprietary amplification primers and standard reverse transcription
159 polymerase chain reaction (RT-PCR) technology, if a plasma sample was available and baseline
160 HCV RNA was >1000 IU/mL. Deep sequencing using MiSeq platform (Illumina, Inc., San

161 Diego, CA) was performed by WuXi AppTec (Shanghai, China) or DDL Diagnostic Laboratory
162 (Rijswijk, Netherlands). Deep sequencing data was split into one file per sample using only
163 100% matched barcodes to bin the reads. Sequence analysis was performed using internally
164 developed software in a stepwise fashion. Briefly, raw reads from the FASTQ files were trimmed
165 and filtered based on quality scores and read length. Trimming was carried out on reads when
166 quality score decreased below 15, and reads shorter than 50 nucleotides were removed. Deep
167 sequencing data was aligned using MOSAIK v1.1.0017. All aligned reads were then translated
168 in-frame and changes from a reference sequence were determined. Assay sensitivity and assay
169 background cutoffs were evaluated based on plasmid and RNA controls. There are no
170 standardized HCV deep sequencing assays available as commercialized kits, therefore cross-
171 validation of deep sequencing data from DDL and WuXi was performed on a subset of control
172 samples.

173 **Ethics Statement**

174 All studies were conducted in accordance with the Declaration of Helsinki, Good Clinical
175 Practice guidelines, and local regulatory requirements. All patients provided written informed
176 consent.

177 **Definition of NS5A Polymorphisms at RAS Positions (RAPs) and Resistance-Associated 178 Substitutions (RASs)**

179 NS5A RAPs were defined as any change from genotype 1a or 1b reference strains (1a-H77 or
180 1b-Con1) at NS5A positions associated with NS5A drug resistance. NS5A class RASs were
181 summarized by the HCV Drug Resistance Advisory Group group (14), and/or recently observed
182 in clinical trials with ledipasvir, velpatasvir, daclatasvir, pibrentasvir, and elbasvir (15-23),
183 specifically variants at NS5A positions 24, 28, 30, 31, 32, 38, 58, 92, 93 that confer >2.5-fold

184 reduced susceptibility to any NS5A inhibitor. Ledipasvir-specific RASs were classified as
185 variants at NS5A positions 24, 28, 30, 31, 32, 38, 58, 92, 93 that confer >2.5-100 or >100-fold
186 reduced susceptibility to ledipasvir in vitro or were selected in clinical trials in patients treated
187 with ledipasvir-containing regimens (2, 24, 25) (Table 1).

188 **Assessment of Sustained Virologic Response (SVR) in patients with and without** 189 **pretreatment NS5A Inhibitor RASs**

190 SVR12 rates were assessed only in the 1,765 patients who were treated with currently
191 recommended regimens containing ledipasvir-sofosbuvir (according to AASLD/IDSA and EASL
192 guidelines) in 15 phase 2 and phase 3 Gilead-sponsored clinical trials (supplemental table 2).
193 Only patients who were not previously exposed to NS5A inhibitors were included in these
194 analyses. Patients were excluded from these analyses if they did not achieve SVR due to non-
195 virologic failure (e.g., lost to follow up). The results were analyzed according to the 1% and 15%
196 detection cut-offs of NS5A RASs, class RASs or ledipasvir-specific RASs.

197

198 **RESULTS**

199 **Patient Baseline Characteristics**

200 Demographic and baseline clinical characteristics of the 5,393 NS5A inhibitor-naïve patients
201 included in the NS5A baseline prevalence RAS analysis are provided in Table 2. The majority of
202 patients were treatment naïve (56%) and male (64%), with HCV genotype 1a (65%) and non-CC
203 interleukin (IL) 28B alleles (73%). Approximately one third (32%) of patients had cirrhosis.

204 **Prevalence and Type of Pretreatment RASs across Geographic Regions**

205 Baseline prevalence of NS5A polymorphisms at RAS positions, NS5A class RASs, ledipasvir
206 RASs and the specific Y93H NS5A variant was evaluated in genotype 1a (n=3501) and 1b

207 (n=1887) patients using 1% through 50% sensitivity cut-offs (Figure 1). Higher prevalence of all
208 categories for NS5A RASs was observed at 1% sensitivity cut-offs and sharply declined with
209 reduction in sensitivity of variant detection to 15%. No significant changes in NS5A RASs
210 prevalence was observed with further reductions in assay sensitivity from 15% to 50%.

211 The prevalence of NS5A polymorphisms at RAS positions was significantly higher as compared
212 to NS5A class RASs in both genotype 1a and 1b across all sensitivity cut-offs. The prevalence
213 of NS5A class RASs was about 5% higher than that of LDV RASs in genotype 1a. This
214 difference was mostly represented by prevalence of the M28V NS5A class RAS that is not an
215 LDV RAS. There was little difference between NS5A class and LDV RASs in genotype 1b.
216 Prevalence of Y93H was higher in genotype 1b as compared to genotype 1a across all assay cut
217 offs. Based on the observation of a sharp decline in prevalence from 1% to 15%, further analyses
218 were performed with both 1% and 15% cut-offs.

219 Overall at the 15% assay cut off, pretreatment NS5A class RASs were detected in 13.0% of
220 genotype 1a patients (Table 3). The prevalence of NS5A class RASs overall in patients with
221 genotype 1a HCV did not differ significantly across most of the geographic regions with the
222 frequency ranging from 12.1% to 15.6%, but the prevalence of ledipasvir RASs was significantly
223 higher among patients in Oceania, than among those from other regions combined (12.7% vs
224 7.9%, $p=0.005$). The overall prevalence of baseline ledipasvir RASs in genotype 1a patients was
225 8.3% with some numeric differences between different regions, the highest in Oceania (12.7%)
226 and the lowest in Europe (7.7%). Specific RASs were detected at a similar frequency in genotype
227 1a patients across geographic regions, including K24R, M28V/T, Q30H/R, L31M and Y93H.

228 The overall prevalence of baseline NS5A class RASs was slightly higher (17.6%) in patients
229 infected with genotype 1b than in those infected with genotype 1a (Table 3). The frequency of
230 detection of NS5A class RASs ranged from 16.1% to 20.4% in genotype 1b patients with only
231 minor numeric differences across geographic regions. The prevalence of baseline LDV RASs
232 among genotype 1b patients was also similar across different regions (15.2-16.4%). Y93H was
233 detected at a much higher frequency (10.6%) than other RASs, including L28M and L31I/M/V
234 among genotype 1b patients, but differences in the prevalence of each RAS between regions
235 were small. In both subtypes, the prevalence of multiple (≥ 2) RASs was low; ranging from 0 to
236 3.8% in genotype 1a, and all less than 1.5% in genotype 1b.

237 **Assessment of the Effect of Baseline RASs on Treatment Outcome with Ledipasvir-** 238 **Sofosbuvir by RAS Categories and Sensitivity Cut offs**

239 To evaluate the effect of baseline RASs on treatment outcome, SVR12 rates were assessed in
240 1,765 patients from 15 ledipasvir-sofosbuvir clinical trials who were treated with currently
241 recommended regimens according to the 2015 AASLD/IDSA and EASL guidelines. The
242 baseline characteristics of this population are given in Table 4. A systematic comparison of the
243 effect on SVR12 rates was performed in genotype 1a and 1b treatment-naïve and treatment-
244 experienced patients for NS5A RASs, class RASs, and LDV RASs, and LDV RASs with >100-
245 fold change, using a 15% sequencing assay cut off (Figure 2).

246 In treatment-naïve patients with genotype 1b HCV infection, the presence of baseline NS5A
247 polymorphisms at RAS positions or NS5A class RASs did not impact the treatment outcome
248 with ledipasvir-sofosbuvir regimens with SVR12 rates of 98%-99% in every group. The SVR12
249 rate in genotype 1a patients with baseline LDV RASs was 94% and 91% (1% and 15% cut offs,

250 respectively) compared to 99% in patients without LDV RASs (Table 5). The presence of
251 baseline LDV RASs in genotype 1b patients had no impact on SVR12 rates.

252 In treatment-experienced patients with genotype 1a or 1b HCV infection, LDV RASs had the
253 most notable impact on SVR12 rates (76%-80% vs 97%-98% and 89%-91% vs. 98% in
254 genotype 1a and 1b, respectively). Even though similar results were obtained when 1% and 15%
255 sensitivity assay cut offs were used, SVR12 rates were slightly lower when 15% assay cut off
256 was used.

257 Taken together, the comparison of the different categories of NS5A RASs and assay cut offs,
258 LDV RASs detected with a 15% assay cut off was identified as the most discriminating for
259 ledipasvir-sofosbuvir regimen baseline analyses and this cut off was used to perform further
260 subgroup evaluations.

261 **Effect of Baseline Ledipasvir RASs on Treatment Outcome by Patient Population**

262 SVR12 rates by treatment history and cirrhosis status according to baseline ledipasvir RASs
263 using a 15% assay cutoff was performed for HCV genotype 1a and genotype 1b (Figure 3)
264 infected patients. The SVR12 rate in treatment-naïve non-cirrhotic patients was not substantially
265 impacted by the presence of ledipasvir RASs at baseline (92% SVR). Numerically lower SVR12
266 rates (86%) were observed in treatment-naïve cirrhotic genotype 1a patients with baseline
267 ledipasvir RASs but only 7 patients limits the interpretability of this finding. Of genotype 1a
268 patients, prior exposure to HCV treatment appeared to impact the SVR12 rates in both non-
269 cirrhotic and cirrhotic groups (75% and 77% respectively) in the presence of baseline ledipasvir
270 RASs, but the number of patients in these groups was also small (<20). Of genotype 1b patients,
271 the SVR12 rates remained >90% across the groups regardless of treatment history and presence

272 of cirrhosis with or without baseline ledipasvir RASs, except for the treatment-experienced non-
273 cirrhotic group which showed an SVR rate of 87%, but it only included 23 patients. The number
274 and prevalence of patients with multiple RASs were small ($N < 30$, $< 1\%$) for both ledipasvir-
275 specific and NS5A class RASs in genotype 1a and 1b. The overall SVR rates were 64% (9/14) in
276 genotype 1a and 100% (2/2) in genotype 1b patients with multiple ledipasvir RASs
277 (supplemental Table 3). Of those with multiple NS5A class RASs, the SVR rates were 74%
278 (17/23) and 83% (5/6) among genotype 1a and 1b, respectively (supplemental Table 3).
279 Among patients with cirrhosis, there were too few patients with baseline RASs to further assess
280 the impact of treatment duration and/or the addition of ribavirin on treatment outcome
281 (supplemental Table 4).

282 **DISCUSSION**

283 Current NS5A inhibitors show overlapping but distinct resistance profiles with RASs described
284 at the NS5A amino acid positions 24, 28, 30, 32, 31, 38, 58, 92, and 93. There are advantages
285 and disadvantages with each of the three main approaches to defining NS5A RASs. The
286 advantage of using the NS5A RAPs definition is that it provides a uniform list of variants for all
287 NS5A inhibitors. It does not require extensive phenotypic testing of all variants with several
288 NS5A inhibitors and provides inclusive assessment of variants that developed in patients treated
289 with NS5A inhibitors. However, for baseline analyses that investigate the role of pre-existing
290 variants on treatment outcome, substitutions that are fully susceptible to a specific NS5A
291 inhibitor dilute the investigated effect. To characterize NS5A class RASs, i.e. those that show
292 reduced susceptibility to one or more NS5A inhibitors in vitro, standardized phenotypic testing is
293 needed for each NS5A inhibitor. Even though the NS5A class RAS definition would exclude
294 variants that are known to be sensitive to NS5A inhibitors and thus provide a more sensitive

295 analysis of the effect of baseline RASs on SVR, some attenuation of the signal may still be
296 observed due to different resistance profiles among the NS5A inhibitors. With further
297 optimization of NS5A inhibitors to improve resistance profiles, the list of NS5A variants and
298 positions that confer reduced susceptibility to the next generation drugs is shortening. Using
299 drug-specific RASs is the most scientifically rigorous way to perform efficacy and baseline
300 resistance analyses. However, extensive standardized phenotypic testing is needed to accurately
301 define drug-specific RASs. Additionally, novel resistance substitutions that develop rarely in
302 vivo may be missed during resistance monitoring and it may be difficult to compare results to
303 those from other studies since drug-specific RASs will be different between various NS5A
304 inhibitors. Another disadvantage of using drug-specific RASs is that this definition fails to
305 capture relevant information regarding the response in patients with resistance to other NS5A
306 inhibitors.

307 The results presented here show that analysis of ledipasvir drug-specific RASs shows more
308 impact on ledipasvir-sofosbuvir treatment outcomes overall as compared to the analysis of RAPs
309 or class RASs, as would be predicted based on these RASs having demonstrated reductions in
310 susceptibility to ledipasvir. However the presence of drug-specific RASs may affect SVR12 rates
311 to a greater or lesser extent depending on the specific pharmacology of an inhibitor and the drug
312 combination regimen being utilized for treatment. For example, previous analyses of ledipasvir-
313 sofosbuvir clinical trials have shown that only ledipasvir-specific RASs contributing >100-fold
314 reduction in susceptibility result in lower SVR rates with ledipasvir-sofosbuvir regimens (2).

315 As multiple options for HCV treatment containing NS5A inhibitors have become available and
316 more broadly applicable, understanding the prevalence of baseline NS5A RASs in specific
317 regions has become more important. In this comprehensive analysis using >5,000 patient

318 samples from 21 countries in 4 continents, it is shown that the prevalence of both NS5A class
319 and ledipasvir RASs does not differ significantly across regions for both genotype 1a and 1b.
320 Numerically lower prevalence of NS5A RASs is observed for genotype 1a in Asia Pacific, but
321 there were small numbers of genotype 1a patients included from this region (n=27) for
322 epidemiological reasons. The prevalence of specific NS5A class and ledipasvir RASs is also
323 similar across regions for both genotypes 1a and 1b. For genotype 1b, the prevalence of Y93H
324 was the highest in Asia Pacific whereas the prevalence of L31M/I/V was the lowest in this
325 region. It must be noted, however, that large regions of the world—including much of Asia, and
326 all of Africa, South America and the Caribbean—are not represented in this analysis.

327 The rates of SVR among patients without pretreatment ledipasvir RASs at all detection
328 thresholds were high regardless of subtype and treatment history, ranging from 97% to 99%. The
329 greatest impact of ledipasvir RASs on SVR was among treatment-experienced patients with
330 genotype 1a HCV, who had an SVR rate of 76% (at the 15% cut-off). This difference was
331 approximately the same at all detection thresholds. Among treatment-naïve patients with
332 genotype 1b HCV, pretreatment ledipasvir RASs appeared to have little to no impact on SVR,
333 with rates ranging from 98-99% for all detection thresholds. Treatment-naïve patients with
334 genotype 1a HCV and treatment-experienced patients with genotype 1b HCV fell somewhere in
335 between, with differences of 4% to 10% between those with and without ledipasvir RASs.

336 The clinical interpretation of these findings remains challenging. The decision to perform pre-
337 treatment RAS testing may be made based on the magnitude of the effect of these RASs on
338 treatment outcome. The effect of NS5A or ledipasvir-specific RASs on treatment outcome was
339 greatest in treatment-experienced patients and/or those with cirrhosis, groups that are at highest
340 risk of disease progression. An argument in favor of pre-treatment RAS testing could thus be

341 made, with the decision to possibly extend treatment duration and/or add ribavirin for those with
342 ledipasvir-specific RASs. However, it should be noted that the number of patients within these
343 subgroups was small (≤ 23 patients) and these data may not be generalizable to the broader
344 population.

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349

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451 **Figure Legends**

452

453 **Figure 1. Prevalence of RASs According to Sensitivity Threshold.** The figures show the
454 prevalence of polymorphisms at RAS positions (RAPs), NS5A class RASs, ledipasvir-specific
455 RASs, and the Y93H RAS by sensitivity threshold. Figure 1A shows prevalence in patients with
456 genotype 1a HCV. Figure 1B shows prevalence in patients with genotype 1b HCV.

457

458 **Figure 2. SVR rates in patients with and without NS5A RASs.** The figures show the rates of
459 SVR12 by presence at baseline of NS5A polymorphisms at RAS positions (RAPs), NS5A class
460 RASs, and ledipasvir-specific RASs, and ledipasvir-specific RASs that confer >100-fold change
461 at a 15% sensitivity threshold.

462

463 **Figure 3. SVR12 rates by treatment history and cirrhosis status.** The figures show the rates
464 of SVR12 by regimen, treatment history (naïve vs previously treated), and cirrhosis status
465 (present vs absent). NC = non-cirrhotic, C = cirrhotic, TE = treatment-experienced, and TN =
466 treatment-naïve.

467

468 **Table 1. List of NS5A Class RASs and Ledipasvir RASs**

469

GT	Reference AA NS5A Position	NS5A Class RASs	LDV RASs	
		Substitutions that confer >2.5 fold change in EC ₅₀ to any NS5A inhibitor	Substitutions that confer >2.5-100 fold change in EC ₅₀ to ledipasvir (FC)	Substitutions that confer >100 fold change in EC ₅₀ to ledipasvir (FC)
1a	K24	G/N/R	G /(43), N (28), /R (4)	--
	M28	A/G/T/V	T (61)	A/G (>1000)
	Q30	C/E/G/H/I/K/L/R/S/T/Y	L /(4), T (4)	E/G/H/K /(>1000), R (632)
	L31	F/I/M/V	F (60)	I /(370), M (554), /V (683)
	P32	L	--	L (348)
	S38	F	F (54)	--
	H58	D/L	--	D (>1000)
	A92	K/T	T (15)	K (>1000)
	Y93	C/F/H/L/N/R/S/T/W	F (7)	C/H/N/S (>1000)
1b	Q24	-	-	--
	(L28)	M		--
	R30	-	-	--
	L31	F/I/M/V	F /(8), I /(29), M /(3), V (43)	--
	P32	L	L (8)	--
	S38	-	-	--
	P58	D	--	D (238)
	A92	K	--	K (>1000)
Y93	C/H/N/S	C (5)	H /(>1000), N /(110), S (142)	

470 FC = Fold Change

471

472

473 **Table 2. Patient Demographics and Baseline Characteristics**

	N. America (n= 3437)	Europe (n= 972)	Oceania (n= 387)	Asia Pacific (n= 597)	Total (n= 5393)
Median age, years (range)	56 (18-81)	54 (18-80)	56 (22-74)	57 (20-80)	56 (18-81)
Male, n (%)	2322 (68)	610 (63)	272 (70)	268 (45)	3472 (64)
Race, n (%)					
White	2571 (80)	945 (97)	329 (85)	27 (5)	4052 (75)
Black	579 (17)	14 (1)	0	0	593 (11)
Asian	44 (1)	10 (1)	24 (6)	570 (96)	648 (12)
Other	34 (1)	3 (<1)	14 (4)	0	51 (<1)
Median BMI, kg/m ² (range)	27 (17-66)	25 (17-57)	27 (18-57)	24 (16-42)	27 (16-66)
Genotype, n (%)					
1a	2635 (77)	531 (55)	314 (81)	27 (5)	3507 (65)
1b	802 (23)	441 (45)	73 (19)	570 (95)	1886 (35)
Median HCV RNA, log ₁₀ IU/mL (range)	6.5 (1.4-8.0)	6.4 (3.2-8.0)	6.4 (1.9-7.7)	6.7 (3.7-7.6)	6.5 (1.4-8.0)
Prior HCV treatment, n (%)					
Treatment-naïve	1961 (57)	559 (58)	184 (48)	332 (56)	3036 (56)
Non-responder	756 (22)	217 (22)	105 (27)	86 (14)	1164 (22)
Relapse/breakthrough	659 (19)	180 (19)	97 (25)	144 (24)	1080 (20)
Other	61 (2)	16 (2)	1 (<1)	35 (6)	113 (2)
<i>IL-28B</i> , n (%)*					
CC	790 (23)	215 (22)	130 (34)	324 (54)	1459 (27)
CT	1922 (56)	568 (59)	197 (51)	247 (41)	2934 (55)
TT	697 (20)	187 (19)	59 (15)	26 (4)	969 (18)
Cirrhosis	1002 (29)	410 (42)	184 (48)	127 (21)	1723 (32)
Median ALT (range), U/L	60 (9-578)	61 (7-420)	66 (12-494)	50 (11-619)	60 (7-619)

474 *IL28B genotype was determined by sequencing of the rs12979860 single-nucleotide polymorphism.

475

476

477

478 **Table 3. Prevalence of NS5A RASs in Patients Naïve to Treatment with NS5A Inhibitors by**
 479 **Region (15% cut off)**

480

Geno-type	RAS	N. America	Europe	Oceania	Asia Pacific	Overall
1a	<i>N</i>	2635	531	314	27 [†]	3507
	K24R	None	1.5%	1.6%	ND	1.1%
	M28T	None	1.1%	2.5%	ND	1.1%
	M28V	5.9%	4.7%	4.1%	ND	5.4%
	Q30H	1.8%	None	2.2%	ND	1.7%
	Q30R	None	1.7%	2.2%	ND	1.1%
	L31M	None	2.2%	4.1%	ND	2.3%
	Y93H	1.0%	None	None	ND	None
	Any LDV RASs	7.9%	7.7%	12.7%	ND	8.3%
	Any NS5A RASs	12.9%	12.1%	15.6%	ND	13.0%
1b	<i>N</i>	802	441	73	570	1886
	L28M	None	1.6%	None	5.4%	2.4%
	L31M	5.9%	4.8%	2.3%	2.1%	4.3%
	L31I	None	None	5.5%	None	None
	Y93H	9.4%	10.2%	9.6%	12.8%	10.6%
	Any LDV RASs	15.5%	15.2%	16.4%	16.0%	15.6%
	Any NS5A RASs	16.1%	16.8%	16.4%	15.6%	17.6%

481 - N. America included USA, Canada and Puerto Rico; Europe included Austria, Belgium, Switzerland, Czech
 482 Republic, Germany, Spain, France, United Kingdom, Italy, Netherlands and Poland; Asia Pacific included China,
 483 India, Japan, Korea, Russia and Taiwan; Oceania included Australia and New Zealand

484 - Only variants with prevalence >1% are listed

485 - No LDV-specific RASs were observed at NS5A positions (26), 32, 38, 58, and 92 with prevalence >1%

486 -Prevalence of NS5A class RASs that are not LDV RASs are shown in parenthesis

487 *Prevalence of Y93H was not included in this table due to low prevalence in genotype 1a. Prevalence of Y93H was
 488 0.6%, 0.9%, and 0.9% in Europe, Oceania, and overall, respectively

489 †The number of patients in the Asia Pacific region with genotype 1a HCV was too small to be the basis for
 490 prevalence estimates.

491

492

493 **Table 4. Patient Demographics and Baseline Characteristics**

	N. America (n= 1103)	Europe (n= 264)	Oceania (n= 67)	Asia Pacific (n= 331)	Total (n= 1765)
Mean age, years (range)	53 (22, 78)	55 (18, 77)	55 (40, 72)	57 (20, 80)	54 (18, 80)
Male, n (%)	795 (72)	165 (63)	50 (75)	140 (42)	1150 (65)
Race, n (%)					
White	817 (74)	258 (98)	50 (75)	0	1125 (64)
Black	251 (23)	5 (2)	0	0	256 (15)
Asian	15 (1)	1 (<1)	6 (9)	331 (100)	353 (20)
Other	20 (2)	0	11 (16)	0	31 (1)
Mean BMI, kg/m ² (range)	28 (18, 66)	25 (18, 40)	29 (18, 50)	24 (17, 38)	27 (17, 66)
Genotype, n (%)					
1a	829 (75)	139 (53)	51 (76)	17 (5)	1036 (59)
1b	271 (25)	124 (47)	16 (24)	313 (95)	724 (41)
1 (no confirmed subtype)	3 (<1)	1 (<1)	0	1 (<1)	5 (<1)
Mean HCV RNA, log ₁₀ IU/mL (range)	6.4 (1.4, 7.8)	6.4 (3.7, 7.5)	6.3 (4.9, 7.7)	6.6 (3.7, 7.6)	6.4 (1.4, 7.8)
Treatment-naïve	682 (62)	135 (51)	28 (42)	178 (54)	1023 (58)
Treatment-experienced	421 (38)	129 (49)	39 (58)	153 (46)	742 (42)
<i>IL-28B</i> , n (%)*					
CC	239 (22)	41 (16)	24 (36)	203 (61)	507 (29)
CT	626 (57)	166 (63)	27 (41)	119 (36)	938 (53)
TT	238 (22)	57 (22)	15 (23)	9 (3)	319 (18)
Cirrhosis	263 (24)	175 (66)	45 (67)	56 (17)	539 (31)
Mean ALT (range), U/L	75 (9, 557)	82 (13, 344)	100 (27, 494)	66 (11, 619)	75 (9, 619)

494 *IL28B genotype was determined by sequencing of the rs12979860 single-nucleotide polymorphism.

495

496

497 **Table 5. SVR12 Rates in Patients with and without LDV RASs Using Various Sensitivity**
 498 **Thresholds**
 499

Genotype	Cut-off	Treatment-naïve		Treatment-experienced	
		With LDV RASs	No LDV RASs	With LDV RASs	No LDV RASs
Patients with genotype 1a HCV	1%	94% (84/89)	99% (497/503)	80% (44/55)	98% (387/394)
	2%	93% (68/73)	99% (513/519)	78% (35/45)	98% (396/404)
	5%	92% (57/62)	99% (524/530)	77% (27/35)	98% (404/414)
	7%	92% (55/60)	99% (526/532)	77% (27/35)	98% (404/414)
	10%	90% (46/51)	99% (535/541)	76% (22/29)	97% (409/420)
	15%	91% (42/46)	99% (539/546)	76% (22/29)	97% (409/420)
	25%	93% (38/41)	99% (543/551)	77% (20/26)	97% (411/423)
	50%	94% (34/36)	98% (547/556)	76% (19/25)	97% (412/424)
Patients with genotype 1b HCV	1%	99% (102/103)	99% (300/303)	91% (63/69)	98% (245/249)
	2%	99% (97/98)	99% (305/308)	90% (57/63)	98% (251/255)
	5%	99% (85/86)	99% (317/320)	88% (46/52)	98% (262/266)
	7%	99% (78/79)	99% (324/327)	88% (42/48)	99% (266/270)
	10%	99% (75/76)	99% (327/330)	87% (41/47)	99% (267/271)
	15%	99% (71/72)	99% (331/334)	89% (41/46)	98% (267/272)
	25%	98% (62/63)	99% (340/343)	88% (36/41)	98% (272/277)
	50%	98% (48/49)	99% (354/357)	85% (28/33)	98% (280/285)

500
 501
 502

Figure 1. Prevalence of RASs According to Sensitivity Threshold

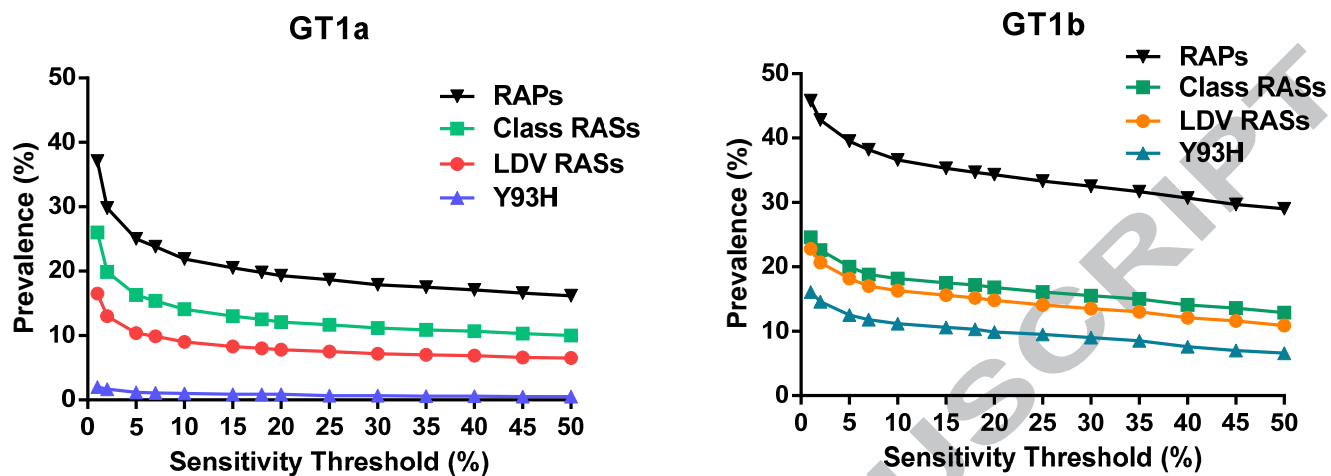


Figure 2. SVR rates in patients with and without NS5A RASs

■ No ■ Yes

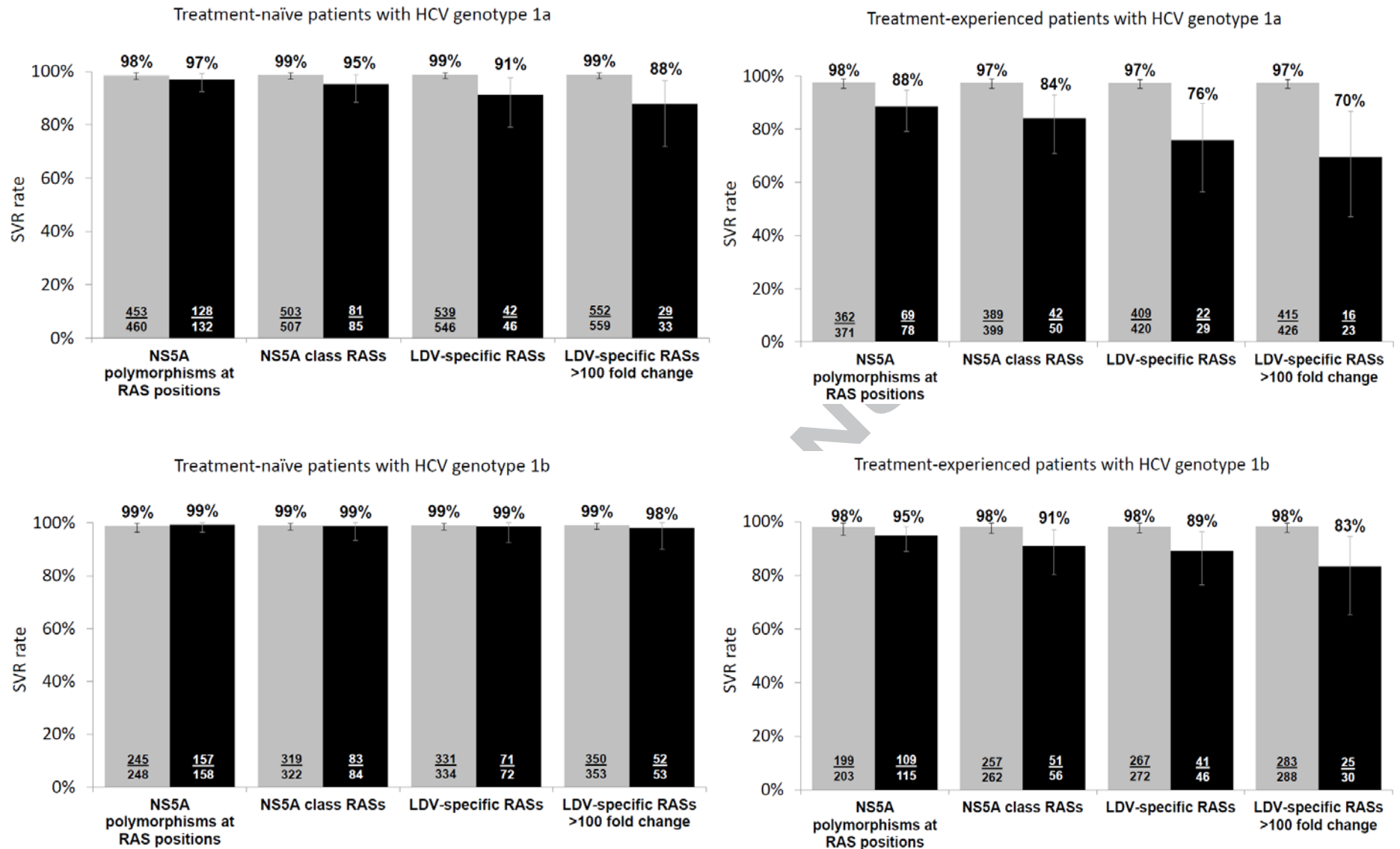


Figure 3. SVR12 Rates by Treatment History and Cirrhosis State

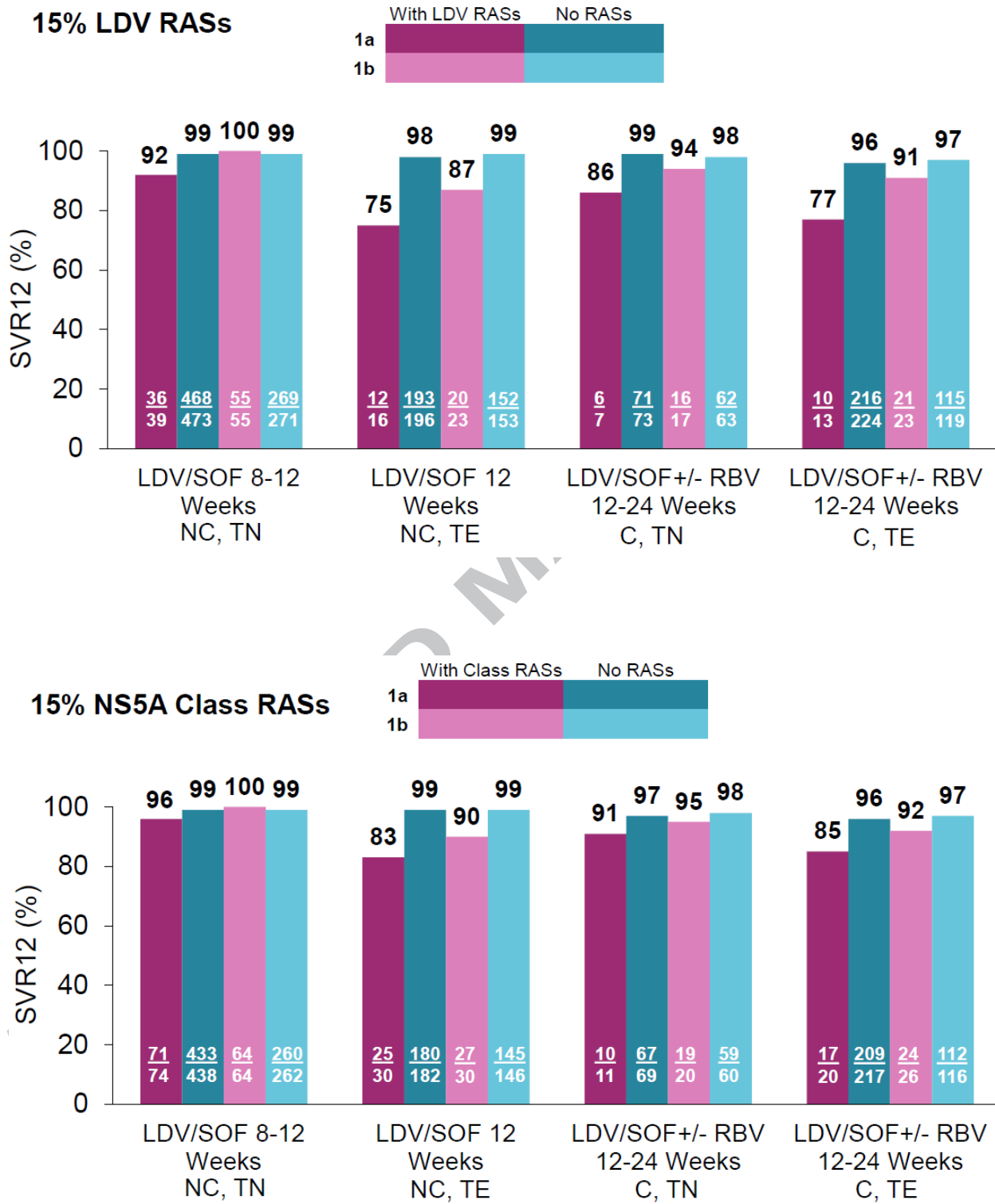


Figure 3. SVR12 Rates by Treatment History and Cirrhosis State

