Accepted Manuscript

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

Stefan Zeuzem, Masashi Mizokami, Stephen Pianko, Alessandra Mangia, Kwang-Hyub Han, Ross Martin, Evguenia Svarovskaia, Hadas Dvory-Sobol, Brian Doehle, Charlotte Hedskog, Chohee Yun, Diana M. Brainard, Steven Knox, John G. McHutchison, Michael D. Miller, Hongmei Mo, Wan-Long Chuang, Ira Jacobson, Gregory J. Dore, Mark Sulkowski
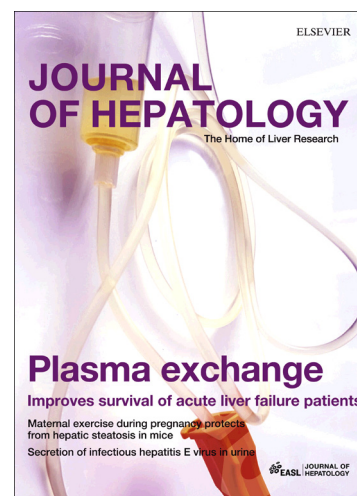
Please cite this article as: Zeuzem, S., Mizokami, M., Pianko, S., Mangia, A., Han, K-H., Martin, R., Svarovskaia, E., Dvory-Sobol, H., Doehle, B., Hedskog, C., Yun, C., Brainard, D.M., Knox, S., McHutchison, J.G., Miller, M.D., Mo, H., Chuang, W-L., Jacobson, I., Dore, G.J., Sulkowski, M., NS5A Resistance-Associated Substitutions in Patients with Genotype 1 Hepatitis C Virus: Prevalence and Effect on Treatment Outcome, *Journal of Hepatology* (2017), doi: http://dx.doi.org/10.1016/j.jhep.2017.01.007

NS5A Resistance-Associated Substitutions in Patients with Genotype 1 Hepatitis C Virus:

Prevalence and Effect on Treatment Outcome

Stefan Zeuzem[1], Masashi Mizokami[2], Stephen Pianko[3], Alessandra Mangia[4], Kwang-Hyub Han[5],

Ross Martin[6], Evguenia Svarovskaia[6], Hadas Dvory-Sobol[6], Brian Doehle[6], Charlotte Hedskog[6],

Chohee Yun[6], Diana M. Brainard[6], Steven Knox[6], John G. McHutchison[6], Michael D. Miller[7],

Hongmei Mo[6], Wan-Long Chuang[8], Ira Jacobson[9], Gregory J. Dore[10], Mark Sulkowski[11]

[1]Goethe-Universität, Frankfurt am Main, Germany

[2]National Center for Global Health and Medicine, Chiba, Japan

[3]Monash Health and Monash University, Melbourne, Australia

[4]IRCCS Hospital 'Casa Sollievo della Sofferenza', San Giovanni Rotondo, Italy

[5]Yonsei University College of Medicine, Seoul, Korea

[6]Gilead Sciences, Inc., Foster City, California, United States

[7]University of Dundee, Dundee, United Kingdom

[8]Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan

[9]Icahn School of Medicine at Mount Sinai, New York, New York, United States

[10]The Kirby Institute, University of New South Wales, Sydney, Australia

[11]Johns Hopkins University School of Medicine, Baltimore, Maryland, United States

Corresponding author: Stefan Zeuzem, Johann Wolfgang Goethe University Medical Center,

Theodor Stern Kai 7, 60590 Frankfurt, Germany, or at zeuzem@em.uni-frankfurt.de

26 **Masashi Mizokami**: Research: Asahi Kasei Pharma Corporation, Mitsubishi Tanabe Pharma

27 Corp, G&G Science Co, SRL Technical Services; Speaker: Abbott, Astellas, Bristol-Myers

28 Squibb, Chugai Pharmaceutical, Daiichi Sankyo, Dainippon Sumitomo, Eisai, G&G,

29 GlaxoSmithKline, Minophagen, Merck Sharp & Dohme, Otsuka Pharmaceutical, Taisho

30 Toyama Pharmaceutical, Takeda Pharmaceutical, The Chemo-Sero-Therapeutic Research

31 Institute, Toray Industries, and Sysmex Corporation.

32 **Stephen Pianko:** Personal Fees: Gilead Sciences, Brisol-Myers Squibb

33 **Alessandra Mangia:** Consultant: BMS, Gilead Sciences, MSD; Advisory Board: BMS, Gilead

34 Sciences, MSD; Research: Janssen, BMS.

35 **Wan-Long Chuang:** Advisory Board: Gilead, Abbvie, BMS, Roche, PharmaEssentia; Speaker:

36 Gilead, BMS, MSD, Roche.

37 **Ira Jacobson:** Consultant: AbbVie, Achillion, Bristol-Myers Squibb, Gilead Sciences, Janssen,

38 and Merck; Resarch: AbbVie, Bristol-Myers Squibb, Gilead Sciences, Janssen, Merck, and

39 Tobira; Speaker: Bristol-Myers Squibb, Gilead Sciences, Janssen, and Merck.

40 **Gregory J. Dore:** Consultant, Advisory Board and Research: AbbVie, BMS, Gilead, Merck

41 **Mark Sulkowski:** Advisory Board: Gilead, AbbVie, Janssen, Merck, Cocrystal, Trek; Research:

42 Gilead, AbbVie, BMS, Janssen, Merck.

43 Employees and stock holders of Gilead Sciences: **Ross Martin, Evguenia Svarovskaia, Hadas**

44 **Dvory-Sobol, Michael D. Miller, Brian Doehle, Charlotte Hedskog, Chohee Yun, Diana M.**

45 **Brainard, Steven Knox, John G. McHutchison, Hongmei Mo.**

46 Nothing to declare: **Kwang-Hyub Han.**

47

48 **Authors' Contributions:**

49 Conception/Design: Stefan Zeuzem, Ross Martin, Evguenia Svarovskaia, Hadas Dvory-Sobol,

50 Brian Doehle, Charlotte Hedskog, Michael D. Miller, Hongmei Mo; Data Acquisition/Analysis:

51 Ross Martin, Evguenia Svarovskaia, Hadas Dvory-Sobol, Brian Doehle, Charlotte Hedskog,

52 Michael D. Miller, Hongmei Mo; Data Interpretation: Stefan Zeuzem, Masashi Mizokami,

53 Stephen Pianko, Alessandra Mangia, Kwang-Hyub Han, Diana M. Brainard, Steven Knox, John

54 G. McHutchison, Wan-Long Chuang, Ira Jacobson, Gregory J. Dore, Mark Sulkowski;

55 Manuscript Drafting/Review: All authors.

56

61

ABSTRACT

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

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

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

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

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

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.

INTRODUCTION

Due to high rates of viral replication and an error prone HCV RNA polymerase, tremendous

variability of HCV has been observed within infected patients (quasispecies) with all single

mutations that do not abolish viral replication thought to be pre-existing (1). As a result, NS5A

RASs are observed at baseline in patients infected with chronic HCV. Deep sequencing enables

detection of HCV substitutions, point deletions, or insertions within the quasispecies down to a

frequency of 1%. However, commercially available assays based on standard population HCV

sequencing or not cross-validated next generation, also called deep sequencing, report variants

with a frequency of ≥15% of the quasispecies.

The prevalence of baseline NS5A RASs has been reported to be 6% to 16% using population

sequencing (cut off 15-25%) or deep sequencing (cut off 1%) (2-4). Interestingly, the prevalence

and type of baseline NS5A RASs may vary by geographic regions. For example, the prevalence

of the NS5A M28V in genotype 1a-infected patients was shown to be higher in the United States

than in Europe, 7% versus 0%, respectively (5). Furthermore, the prevalence of genotype 3

NS5A Y93H varied between 0% and 17% in different geographic regions (6). A comparison of

baseline prevalence of RASs in Japanese and Western patients showed that the prevalence of

Q80L and S122G in NS3, and L28M, R30Q and Y93H in NS5A was significantly higher in

Japanese patients than the Western counterparts (7).

Many currently approved interferon (IFN)-free regimens for the treatment of chronic hepatitis C

(HCV) include an inhibitor of HCV NS5A. To date, there are five NS5A inhibitors approved for

treatment of chronic HCV infection; ledipasvir (LDV), daclatasvir, and velpatasvir (which are all

administered with the NS5B inhibitor sofosbuvir), and ombitasvir (in a fixed-dose combination

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 RAPs, 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 RAPs, 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-naive 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 ($\geq$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-naive and treatment-

244 experienced patients for NS5A RAPs, 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-naive 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 detections

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.

345 **Acknowledgement**

349

350 **REFERENCES**

351 1. Rong L, Dahari H, Ribeiro RM, Perelson AS. Rapid emergence of protease inhibitor

352 resistance in hepatitis C virus. Sci Transl Med 2010;2:30ra32.

353 2. Sarrazin C, Dvory-Sobol H, Svarovskaia ES, et al. Baseline and post-baseline resistance

354 analyses of phase 2/3 studies of ledipasvir/sofosbuvir ± RBV. Hepatology 2014;60:1128A.

355 3. Bartels DJ, Sullivan JC, Zhang EZ, et al. Hepatitis C virus variants with decreased sensitivity

356 to direct-acting antivirals (DAAs) were rarely observed in DAA-naive patients prior to treatment.

357 J Virol 2013;87:1544-1553.

358 4. Suzuki F, Sezaki H, Akuta N, et al. Prevalence of hepatitis C virus variants resistant to NS3

359 protease inhibitors or the NS5A inhibitor (BMS-790052) in hepatitis patients with genotype 1b. J

360 Clin Virol 2012;54:352-354.

361 5. Krishnan P, Tripathi R, Schnell G, et al. Resistance analysis of baseline and treatment-

362 emergent variants in hepatitis C virus genotype 1 in the AVIATOR study with paritaprevir-

363 ritonavir, ombitasvir, and dasabuvir. Antimicrob Agents Chemother 2015;59:5445-54.

364 6. Hernandez D, Zhou N, Ueland J, Monikowski A, McPhee F. Natural prevalence of NS5A

365 polymorphisms in subjects infected with hepatitis C virus genotype 3 and their effects on the

366 antiviral activity of NS5A inhibitors. J Clin Virol 2013;57:13-8.

367 7. Krishnan P, Schnell G, Tripathi R, et al. Analysis of HCV Genotype 1b Resistance Variants in

368 Japanese Patients Treated with Paritaprevir/Ritonavir and Ombitasvir. Antimicrob Agents

369 Chemother 2015;60:1106-13.

370   8. Harvoni (ledipasvir and sofosbuvir) tablets. Summary of product characteristics.

371   http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-

372   _Product_Information/human/003850/WC500177995.pdf

373   9. Epclusa (sofosbuvir and velpatasvir). Summary of product characteristics.

374   http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-

375   _Product_Information/human/004210/WC500211151.pdf

376   10. Daklinza (daclatasvir) tablets. Summary of product characteristics.

377   http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-

378   _Product_Information/human/003768/WC500172848.pdf

379   11. Viekirax (dasabuvir, ombitasvir, paritaprevir, and ritonavir) extended-release tablets,

380   http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-

381   _Product_Information/human/003839/WC500183997.pdf

382   12. Zepatier (elbasvir and grazoprevir) tablets. Summary of product characteristics.

383   http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-

384   _Product_Information/human/004126/WC500211235.pdf

385   13. Nelson DR, Cooper JN, Lalezari JP, et al. All-oral 12-week treatment with daclatasvir plus

386   sofosbuvir in patients with hepatitis C virus genotype 3 infection: ALLY-3 phase III study.

387   Hepatology 2015;61:1127-1135.

388   14. Sarrazin C, Dvory-Sobol H, Svarovskaia ES, et al. Prevalence of resistance-associated

389   substitutions in HCV NS5A, NS5B, or NS3 and outcomes of treatment with ledipasvir and

390   sofosbuvir. Gastroenterology 2016 [epub].

391   15. Lontok E, Harrington P, Howe A, et al. Hepatitis C virus drug resistance–associated

392   substitutions: state of the art summary. Hepatology 2015;62:1623-32.

393   16. Sarrazin C. The importance of resistance to direct antiviral drugs in HCV infection in clinical

394   practice. J Hepatol 2016;64:486-504.

395   17. Krishnan P, Beyer J, Mistry N, et al. In vitro and in vivo antiviral activity and resistance

396   profile of ombitasvir, an inhibitor of hepatitis C virus NS5A. Antimicrob Agents Chemother

397   2015;59:979-987

398   18. Bilello JP, Lallos LB, McCarville JF, et al. In vitro activity and resistance profile of

399   samatasvir, a novel NS5A replication inhibitor of hepatitis C virus. Antimicrob. Agents

400   Chemother 2014;58:4431-42.

401   19. McPhee F, Hernandez D, Zhou N, et al. Virological escape in HCV genotype-1-infected

402   patients receiving daclatasvir plus ribavirin and peginterferon alfa-2a or alfa-2b. Antivir Ther

403   2014;19:479-90.

404   20. Black S, Pak I, Ingravallo P, et al. Resistance analysis of virologic failures in hepatitis C

405   genotype 1 infected patients treated with grazoprevir/ elbasvir +/− ribavirin: the C-WORTHY

406   study. J Hepatol 2015;62(Suppl 2):S677-8.

407   21. Ng T, Pilot-Matias T, Tripathi R, et al. Analysis of HCV genotype 1 variants detected during

408   monotherapy and combination therapy with next generation HCV direct-acting antiviral agents

409   ABT-493 and ABT-530. Hepatology 2015;62:558A.

410   22. Feld JJ, Jacobson IM, Hézode C, et al. Sofosbuvir and velpatasvir for HCV genotype 1, 2, 4,

411   5, and 6 infection. N Engl J Med 2015;373:2599-607.

412   23. Foster GR, Afdhal N, Roberts SK, et al. Sofosbuvir and velpatasvir for HCV genotype 2 and

413   3 infection. N Engl J Med 2015;373:2608-17.

414   24. Curry MP, O'Leary JG, Bzowej N, et al. Sofosbuvir and velpatasvir for HCV in patients with

415   decompensated cirrhosis. N Engl J Med 2015;373:2618-28.

416   25. Wong KA, Worth A, Martin R, et al. Characterization of Hepatitis C Virus Resistance from a

417   Multiple-Dose Clinical Trial of the Novel NS5A Inhibitor GS-5885. Antimicrob Agents

418   Chemother 2013;57:6333-40.

419   26. Cheng G, Peng B, Corsa A, et al. Antiviral activity and resistance profile of the novel HCV

420   NS5A inhibitor GS-5885. J Hepatol 2012;56:S464.

421   27. Ioannou GN, Beste LA, Chang MF, et al. Effectiveness of sofosbuvir, ledipasvir/sofosbuvir,

422   or paritaprevir/ritonavir/ombitasvir and dasabuvir regimens for treatment of patients with

423   hepatitis C in the Veterans Affairs National Health Care System. Gastroenterology 2016 [in

424   press].

425   28. Terrault N, Zeuzem S, Di Bisceglie AM, et al. Treatment outcomes with 8, 12 and 24 week

426   regimens of ledipasvir/sofosbuvir for the treatment of hepatitis C infection: analysis of a

427   multicenter prospective, observational study. Hepatology 2015;62:256A.

428   29. Curry MP, Bacon B, Flamm SL, et al. Effectiveness of 8 or 12 week LDV/SOF in treatment-

429   naïve patients with non-cirrhotic, genotype 1 hepatitis C: real-world experience from the TRIO

430   Network. Hepatology 2015;62:721A.

431   30. Christensen S, Mauss S, Hueppe D, et al. Sofosbuvir and ledipasvir for 8 weeks in patients

432   with hepatitis C virus (HCV) mono-infection and human immunodeficiency virus (HIV)-HCV

433   co-infection with genotype 1 and 4 in clinical practice – Results from the GErman hepatitis C

434   COhort (GECCO) Hepatology 2015;62:740A.

435   31. Buggisch P, Wursthorn K, Stoehr A, et al. Real-world effectiveness of ledipasvir/sofosbuvir

436   8 weeks chronic hepatitis C treatment Hepatology 2015;62:806A.

437   32. Backus LI, Belperio PS, Shahoumian T, et al. Effectiveness of ledipasvir/sofosbuvir in

438   treatment naïve genotype 1 patients treated in routine medical practice. Hepatology

439   2015;62:255A.

440   33. Marshall V, Rife K, Hirsch A, et al. Real life treatment outcomes with 8 week course

441   treatment for hepatitis C without cirrhosis confirmed by transient elastography. Hepatology

442   2015;62:779A.

443   34. Roytman M, Tang AL, Wu C, et al. Real life experience with sofosbuvir and ledipasvir fixed

444   dose regimen in the multiethnic cohort of patients with chronic hepatitis C. Hepatology

445   2015;62:761A.

446   35. Lai JB, Witt DJ. Real-word effectiveness of ledipasvir (LDV)/sofosbuvir (SOF)-based

447   therapy for hepatitis C virus (HCV): preliminary analysis in a large integrated health care

448   system. Hepatology 2015;62:725A.

449   36. Gill K, Fante G, Nafisi S, et al. Sofosbuvir and ledipasvir for the treatment of HCV GT-1,

450   cirrhotics and non-cirrhotics: real-world effectiveness. Hepatology 2015;62:757A.

Figure Legends

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

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

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

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)
IL-28B, 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

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

Geno-type	RAS	N. America	Europe	Oceania	Asia Pacific	Overall
1a	*N*	*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	*N*	*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%

- N. America included USA, Canada and Puerto Rico; Europe included Austria, Belgium, Switzerland, Czech Republic, Germany, Spain, France, United Kingdom, Italy, Netherlands and Poland; Asia Pacific included China, India, Japan, Korea, Russia and Taiwan; Oceania included Australia and New Zealand
- Only variants with prevalence >1% are listed
- No LDV-specific RASs were observed at NS5A positions (26), 32, 38, 58, and 92 with prevalence >1%
- Prevalence of NS5A class RASs that are not LDV RASs are shown in parenthesis
- *Prevalence of Y93H was not included in this table due to low prevalence in genotype 1a. Prevalence of Y93H was 0.6%, 0.9%, and 0.9% in Europe, Oceania, and overall, respectively
†The number of patients in the Asia Pacific region with genotype 1a HCV was too small to be the basis for prevalence estimates.

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)
IL-28B, 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
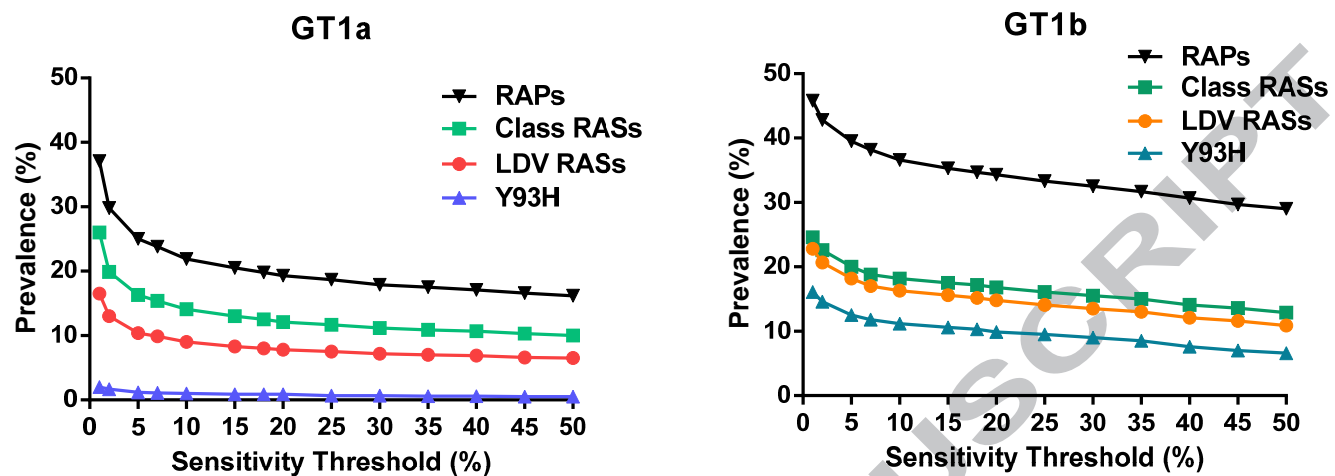
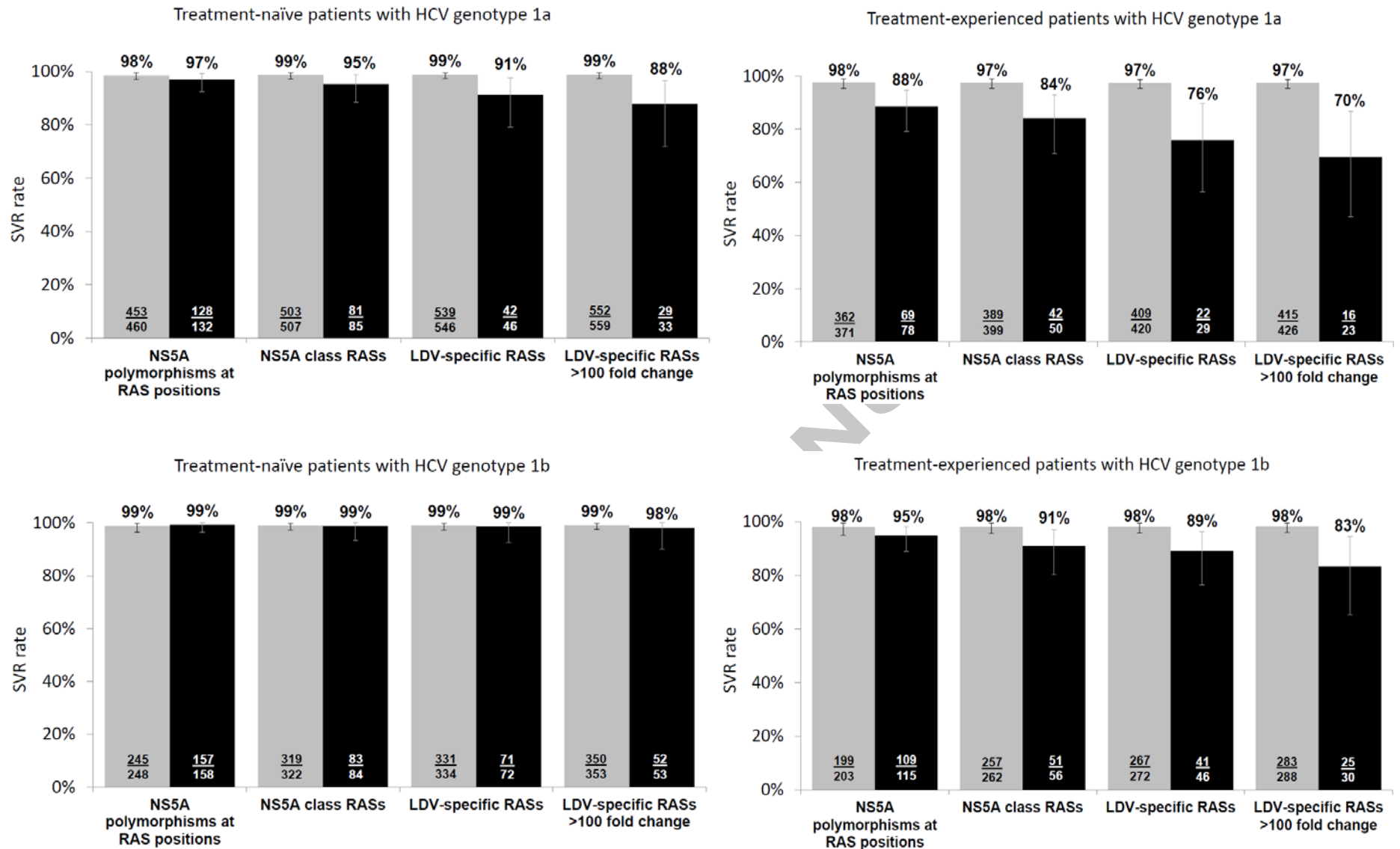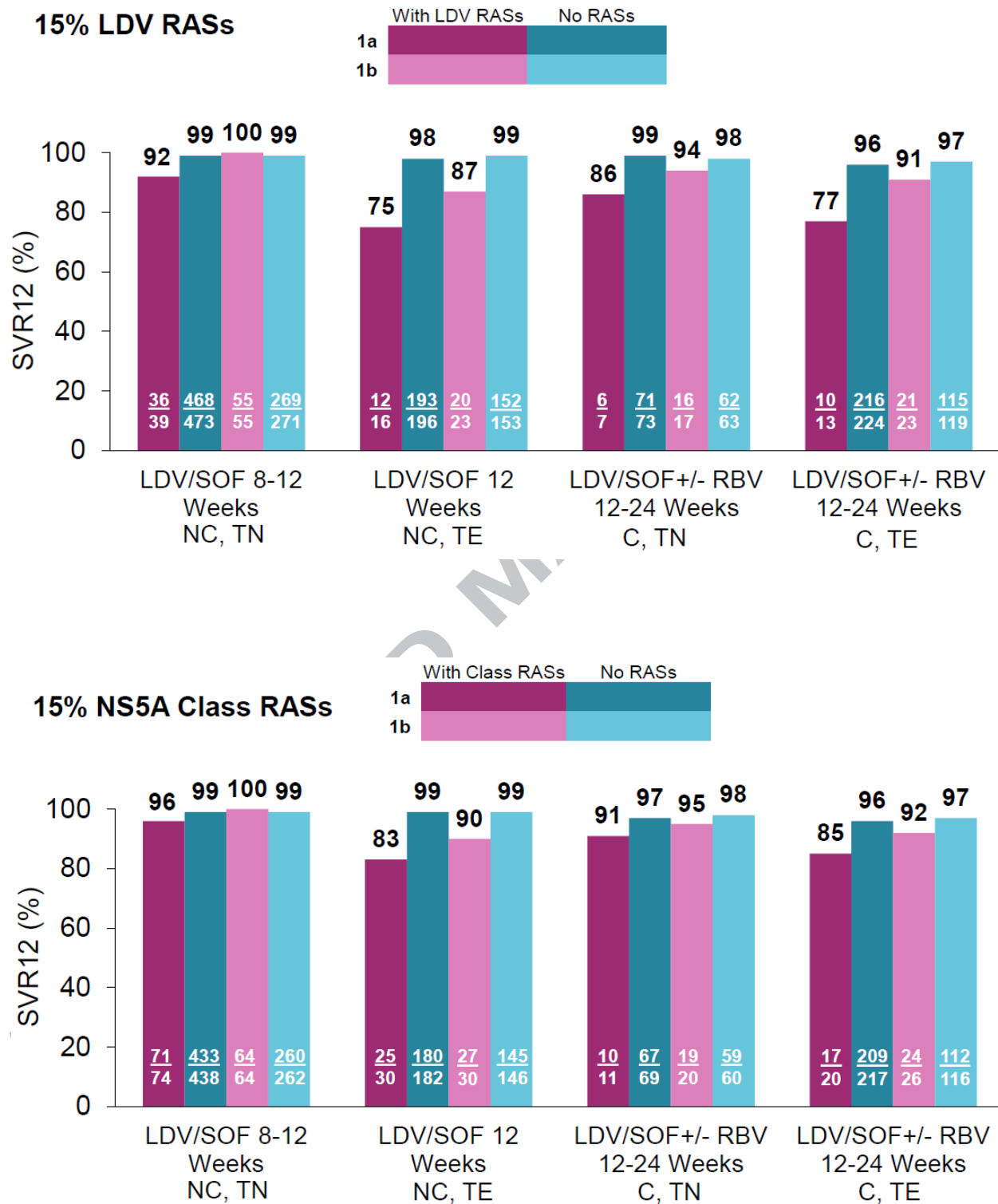**Figure 2. SVR rates in patients with and without NS5A RASs**

Figure 3. SVR12 Rates by Treatment History and Cirrhosis State

Figure 3. SVR12 Rates by Treatment History and Cirrhosis State