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Inferior cure rate in pilot study of four-week glecaprevir/pibrentasvir treatment with or without ribavirin of chronic hepatitis C

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List of abbreviations

GLE/PIB glecaprevir/pibrentasvir
RAS resistance-associated substitutions
LSM liver stiffness measurement
SVR sustained virological response
HCV hepatitis C virus
DAA direct acting antivirals
PWUD people who use drugs
LDV/SOF ledipasvir/sofosbuvir
NS3P NS3 protease
SVR12 12 weeks sustained virological response
OPEN Open Patient data Explorative Network
e-CRF electronic case report form
SOF/VEL sofosbuvir/velpatasvir
SOF/VEL/VOX sofosbuvir/velpatasvir/voxilaprevir
Conflict of interest.

PBC and AØ have received research grants from AbbVie, Gilead and MSD, not related to this study and have received travel and conference support from AbbVie, Gilead and MSD. LWM, UF, MP and JB have no conflict of interest.

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Acknowledgement

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Abstract

Background & Aims

Shortening the treatment duration for chronic hepatitis C may increase feasibility and reduce cost of cure. The aims of this study were to compare four weeks of glecaprevir/pibrentasvir (GLE/PIB) treatment with and without ribavirin for patients with chronic hepatitis C and favorable baseline characteristics, and to monitor the development of resistance-associated substitutions (RAS) and re-treatment outcomes if treatment failed.

Methods:

We performed an open-label single center randomized controlled trial, in which patients with chronic hepatitis C were randomized 1:1 to GLE/PIB +/- ribavirin, stratified by genotype 3. The main inclusion criteria were treatment naive patients, aged 18-49 with all genotypes accepted, and absence of liver fibrosis, determined by liver stiffness measurement (LSM) less than 8 kPa. Viral genome sequences were determined by deep sequencing at baseline and at the time of relapse.

Results

A total of 32 patients started treatment. Sustained virological response at week 12 (SVR12) was 59% (10/17) for GLE/PIB without ribavirin and 73% (11/15) for GLE/PIB with ribavirin. Drug target specific NS5A RAS were detected at baseline for 45% (5/11) of patients with treatment failure and for 14% (3/21) of patients who achieved SVR12. Ten failure patients were retreated 12 weeks with sofosbuvir-based regimens; all have been cured.

Conclusions

In this pilot study of four-week treatment with GLE/PIB with and without ribavirin we found that baseline RAS were more frequent in patients with virological failure. Development of RAS did occur after short treatment but did not result in retreatment failure with a different regimen.

Word count abstract: 250

EudraCT no: 2017-005179-21

Keywords: Hepatitis C virus, Glecaprevir, Pibrentasvir, Ribavirin, Drug Resistance, Retreatment

Lay Summary:
The majority of patients with chronic hepatitis C and no severe liver disease can be cured by direct acting antivirals with shorter treatment duration than recommended by international guidelines, but the cure rate is disappointing compared to standard of care.

Baseline resistance mutations was associated with a lower SVR12 in ultrashort treatment.

Development of resistance do occur after treatment failure but do not impede retreatment.
INTRODUCTION

The introduction of direct acting antivirals (DAA) targeting essential functions of the hepatitis C virus (HCV) has dramatically improved the treatment outcome for chronic hepatitis C. With this great advance the World Health Organization in 2016 defined the 2030 elimination goal for viral hepatitis C, which includes that 80% of eligible patients should be treated by year 2030. To fulfill this ambitious global goal, worldwide increase in test capacity and access to affordable antiviral treatment for hepatitis C is mandatory. One way to achieve greater treatment coverage is to develop shorter treatment schedules with the same high cure rate as current standard of care. This would reduce costs and may increase feasibility of cure in vulnerable patient groups, such as people who use drugs (PWUD).

So far, there has been only few clinical trials of chronic hepatitis C with treatment duration of four weeks or less, and most of them with disappointing results. These studies have been characterized by different treatment regimens and included different patient groups. Our research group has previously shown that four weeks treatment with ledipasvir/sofosbuvir (LDV/SOF) in combination with weight-based ribavirin gave a cure rate of 92% with no effect of adding interferon in young and easy to treat patients. However, another study, with an older study population and higher baseline viral load, showed week 12 sustained virological response (SVR12) of 68% for 6 weeks of treatment with LDV/SOF + ribavirin.

A major concern in relation to short-term treatment is the development of treatment related resistance-associated substitutions (RAS) in the NS3 protease (NS3P), NS5B polymerase and especially the NS5A protein. Clinical studies have shown high frequency of NS5A RAS after failure to treatment regimens with NS5A targeting DAA with a persistence of NS5A RAS at least 1-2 years after treatment failure. The number of retreatment studies and the impact of treatment emergent RAS after treatment failure of short duration treatment is limited. Studies have so far shown treatment emergent NS5A and NS3P RAS at virological relapse with a frequency between 40-60% and 0-11% respectively. However, this seem not to have any effect on the outcome of re-treatment as SVR12 after retreatment has been reported to be between 91-100%.

Knowledge of RAS development after short duration treatment has become increasingly important as DAA therapy is distributed more widely and includes vulnerable patient groups with possibly unstable living conditions and many competing priorities such as homelessness, people in prison and PWUD. Here decreased compliance and premature cessation of treatment may be significant problems that clinical trials might not capture.

Prior studies of short-term treatment were with sofosbuvir containing regimens. Further, they included NS5A inhibitors with a lower barrier to resistance than pibrentasvir (PIB). Thus, the aim for this randomized controlled trial was to compare 4 weeks of treatment with glecaprevir/pibrentasvir (GLE/PIB) with and
without ribavirin for patients with chronic hepatitis C with no significant liver fibrosis. The primary objective was to compare the percentage of patients achieving SVR 12 in the two groups. Adverse events, safety parameters and hepatitis C RNA viral load were monitored at baseline, during and after treatment. We also investigated RAS at baseline and for patients with treatment-failure at the time of relapse. Finally, we determined the outcome of 12 weeks of retreatment with velpatasvir/sofosbuvir based regimens.

**MATERIALS AND METHODS**

**Patients**

Treatment naive patients aged 18-49 with chronic hepatitis C, defined with two positive HCV test, more than 6 months apart (second test must be a positive HCV RNA result), were included. All HCV genotypes were accepted. Patients had absence of liver fibrosis, defined as a liver stiffness measurement (LSM) measured by Fibroscan of less than 8kPa. In addition, we excluded patients who had any signs of cirrhosis, defined as clinical signs, International Normalized Ratio (INR) >1.3 or platelets <130*10^9/L. Patients with comorbidity of chronic hepatitis B, HIV or autoimmune hepatitis were excluded from the study. Due to possible ribavirin exposure in the trial, we excluded patients with hemoglobin level less than 11.3 g/dl, pregnancy, breastfeeding and refusal to use contraceptives.

**Study design**

Randomized, open label, investigator initiated clinical trial (EudraCT no: 2017-005179-21). The study was conducted at the Department of Infectious Diseases at Odense University Hospital in Denmark. The 4RIBC study was designed in two phases where phase two would depend on the outcome of phase one. The aim for the first phase was to evaluate the feasibility of four weeks treatment with GLE/PIB with or without ribavirin. The plan of enrollment were 40 patients. This paper reports phase one. Randomization was done after screening and patients were randomized 1:1 stratified by genotype 3/non 3 to GLE/PIB or GLE/PIB with ribavirin for 4 weeks with a cap of 50% of any genotype in each arm. Randomization was handled by open patient explorative network (OPEN), an independent research support facility at Odense University Hospital and University of Southern Denmark and the stratification and block size was set up by a data manager and blinded to the investigators. Blood for viral genome sequencing was sampled at baseline and at week 4 and 12 after the end of treatment. Drugs were dosed in weekly dosing boxes with 2 weeks interval. Ribavirin was dosed as 15 mg/kg b.i.d with an upper limit of 1400 mg.

**Data collection**

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All clinical data were recorded in a REDCap database that also served as electronic case report form (e-CRF) (hosted by OPEN at the Region of Southern Denmark)\(^\text{15}\), see supplemental 1. Time of infection was defined as first year of injection for people who disclosed having acquired the infection by intravenous drug use. For patients who had never injected drugs or who had an unknown route of transmission, the time of infection was defined as the year of the hepatitis C diagnosis.

Safety was evaluated at each visit. For details on safety, see supplemental 2. During treatment all study participant was inquired by both project nurse and investigator about compliance and was asked to return empty dosage boxes in relation to medication intake monitoring.

**HCV RNA**

Plasma samples were collected at each study visit and HCV RNA viral load was determined by the COBAS HCV assay, run at the 6800/8800 systems (Roche Molecular System, Inc.). All analyses were performed according to the manufacturer’s instructions. The lower detection limit of the COBAS HCV assay was 15 IU/ml.

**Viral genome sequencing and data analysis**

The HCV genome sequence was determined from virus recovered from plasma samples. An in-house developed Trizol protocol\(^\text{16}\) was used to extract the RNA from the plasma. The RNA was subsequently prepped for total RNA-seq using the NEBNext Ultra II directional RNA library kit as described\(^\text{17}\) and sequenced on an Illumina NextSeq platform. Data was analyzed by *de novo* assembly and subsequent mapping as recently presented\(^\text{16,18}\). In order to identify RAS of the HCV NS3, NS5A and NS5B genome regions resistance analysis was performed using HCV-GLUE offline software with a 5% sensitivity threshold\(^\text{19}\). Sequencing was performed for all patients at baseline and at time of virological relapse in those with treatment failure.

**Virological relapse**

All patients with measurable HCV RNA (HCV RNA > 15 IU/ml) after post treatment week 12 were defined as treatment relapse and offered re-treatment with sofosbuvir/velpatasvir (SOF/VEL) or sofosbuvir/velpatasvir/voxtiaprevir (SOF/VEL/VOX) for 12 weeks according to the presence of RAS. Patients were retreated in a standard of care setting and SVR12 was recorded according to protocol.

**Statistical analysis**
Descriptive statistics was reported as proportion for categorical variables with 95% confidence intervals (CI) and medians with interquartile ranges (IQR) for continuous variables. This exploratory study planned enrollment of 40 patients over 52 weeks (20 patients in each treatment arm) and was not designed to evaluate statistical hypotheses. An observed SVR12 of 80% in the experimental arm would have a CI at (0.56-0.94). Interim analysis was performed after the first 20 patients had reached SVR12.

STATA version 15 (StataCorp LP, Texas) was used for data processing and analyses.

Ethical

All participants signed informed consent and participants could withdraw their consent at any time. This study was approved by Danish Health and Medicines Authorities, The Regional Committees on Health Research Ethics for Southern Denmark ID S-20180013 and the Danish Data Protection agency (j. no 18/21965) and monitored by the Good Clinical Practice Institute at the University of Southern Denmark in compliance with the Good Clinical Practice Guidelines and the Declaration of Helsinki.

All data were handled in accordance with The General Data Protection Regulation (GDPR), the Danish Act on Data Protection, the Danish Act on Research Ethics Review of Health Research Projects and the Danish Health Act. The study adheres to the CONSERT guidelines for randomized clinical trials.

The corresponding author had access to all data and the integrity of the study participants were respected during data analysis.

RESULTS

From May 2018 to August 2018, 33 patients were screened and followed according to protocol until October 2019. One patient withdrew informed consent before randomization. Overall, 32 patients were randomized, and all randomized patients were evaluated for primary outcome (SVR12) of the trial. In total 15 patients were randomized to treatment with GLE/PIB plus ribavirin and 17 patients received GLE/PIB (Figure 1).

Baseline characteristic

Baseline characteristics of the study population are shown in table 1. Most treated patients were male with current or prior drug abuse, but only a minority had used intravenous drugs within the last year. The most common genotypes were 1 and 3, with the most frequent being subtype 1a and 3a. One patient with genotype 1 could not be subtyped by normal procedure. Baseline demographic characteristics were generally well balanced between treatment arms, but baseline viral loads were notable higher in the group.
without ribavirin. The median infection duration with hepatitis C, for both treatment groups were more than 20 years.

Virological Response
In the GLE/PIB treatment arm 59% (10/17) (95% CI 0.33 – 0.82) achieved SVR12 compared to 73% (11/15) (95% CI 0.45-0.92) in the GLE/PIB + ribavirin treatment arm. Except one patient with virological failure, all patients had HCV RNA below lower limit of quantification by the end of treatment. At post treatment week 4, six patients had measurable virus in the blood, of which one achieved SVR12. Six study participants had virological relapse between post treatment week 4 and 12 (Figure 2).

All patients, except one lost to follow up, who obtained SVR 12 also achieved SVR 48.

Safety and compliance
Most adverse events were mild, and no patients stopped treatment or had any dose adjustment due to adverse reactions. No severe events or adverse events due to laboratory abnormalities were reported. For detailed description of adverse events, see supplemental 3.

The group of patients who received ribavirin had a mean reduction in hemoglobin level of 2.24 g/dl range (-0.32-5.32) from treatment start to end of treatment. The decrease in hemoglobin level for the ribavirin group was transient and four weeks after cessation of treatment the mean hemoglobin level was 14.50 g/dl and almost at the same level as at the start of treatment where the mean level was 14.66 g/dl (figure 3).

A high adherence to study drugs was observed; only three doses were missed, in three individual patients (compliance 99.8%).

Virological Relapse
No significant association between SVR and baseline variables were identified. In total 11 patients experienced virological relapse and their median age was 40 and nine (81.8%) were men. Viral genome sequencing did not indicate that the treatment relapse was due to re-infection and all patients were therefore characterized with virological relapse. For patients who did not achieve SVR12, two (18.2%) had genotype 3 (one 3a and one 3b) and nine (81.8%) had genotype non 3, four patients with genotype 1a, two with 1b and 2b and one patient with an unknown subtype of genotype 1. Baseline viral load in the ribavirin treatment arm was lower for patients who achieved SVR12, median 5.8 log_{10} IU/ml (IQR 5.6-3.4) versus the group who did not achieve SVR12, median 6.8 log_{10} IU/ml (IQR 6.4-7.0). In the ribavirin free treatment group, the median baseline viral load was 6.6 log_{10} IU/ml for the group who experienced virological failure versus 6.5 log_{10} IU/ml for the group achieving SVR.
Infection duration with hepatitis C was comparable for the group that achieved SVR12 relative to the group that had virological relapse. In total nine patients were IL28b genotype CC of which one patient did not obtain SVR 12. This patient also had double baseline NS5A RAS (see below).

**Resistance-associated substitutions**

Baseline RAS were detected for 23% (5/21) of patients with SVR12 and 54.5% (6/11) of patients with virological relapse. The five patients who achieved SVR12 had one baseline RAS with three having one mutation in NS5A. Among the six patients with treatment failure and baseline RAS, all had more than one mutation, with 83% (5/6) having at least one RAS in NS5A and three patients with two or more RAS in NS5A at baseline. In 60% (3/5) of the patients with treatment failure and baseline NS5A RAS, the virus acquired additional RASs in NS5A during treatment. RAS development in NS3P was seen for three patients with treatment failure. One patient developed RAS in both NS3P and NS5A (table 2). RAS development was only observed for those patients who already had baseline RAS.

**Retreatment**

In total 10 of the 11 chronic hepatitis C patients with treatment-failure have completed retreatment (standard of care) and achieved SVR12. Eight patients have been retreated with (SOF/VEL) for 12 weeks. Two patients have been retreated with (SOF/VEL/VOX) according to their pretreatment RAS profile: one patient with genotype 3b had double baseline NS5A RASs (30K, 31M) and the other patient with an undesccribed subtype of genotype 1 (suspected West African origin) had triple baseline NS5A RASs (28M, 30Q, and 31M).

**DISCUSSION**

This randomized clinical trial is among the first to treat chronic hepatitis C with GLE/PIB for only four weeks, with or without ribavirin, and the first short duration study without a sofosbuvir-containing regimen. Including the NS5A-inhibitor PIB in short-term treatments might be beneficial due to its high barrier to resistance. Overall, 11 patients experienced treatment failure of which six patients had detectable baseline RAS. Development of RAS during treatment did occur, but none led to failure in 12 weeks retreatment with sofosbuvir-containing regimens. Our results suggest that the majority of patients with chronic hepatitis C and favorable baseline characteristics can be cured with DAA therapy of shorter duration than is currently recommended by international guidelines.

Conduction of this study have some ethical considerations. None of the study participants were eligible for standard of care treatment, at the time of study inclusion, due to fibrosis treatment restrictions (F2 or LSM.
> 10 kPa) in Denmark (until November 1, 2018). Furthermore, because of the study criteria, no one suffered from severe liver disease and based on previous retreatment studies after short treatment the likelihood of cure after retreatment was high. Outreach clinics and well-developed registers in Denmark make it possible to maintain close contact with patients, even in a study population that may have unstable living conditions. All elements of hepatitis C treatment and retreatment is free of cost to the patients in the Danish health system.

A major concern for suboptimal treatment regimens is the development of RAS, associated with treatment failures, and the risk of diminished effective retreatment. In this study development of RAS in NS5A were only detected in 27% (3/11) of the patients with virological relapse, whereas the development of NS5A RAS has been seen with much higher frequency in other studies. Development of NS3P RAS was detected but only one patient with treatment failure developed a mutation at residue 168, which is a known resistance position for GLE, while all other NS3P resistance substitutions must be considered minor and possibly not contributing much to resistance and treatment failure.

Treatment-emerging substitutions in NS5A, in a pooled analysis for both treatment experienced and treatment naïve patients were detected in 82% of patients after treatment failure with 8-12 weeks treatment with GLE/PIB. A reason for the observed lower proportion of treatment emerging substitutions in the present study may be the fact that the original patient virus may not be completely eradicated under short duration treatment and thus rebound without emerging RAS after end of treatment. During the first phase of the biphasic viral load decline during hepatitis C treatment, therapy with direct-acting antivirals efficiently inhibit replication of the original patient virus, which result in fast viral load decline. However, as described above it is not certain that the original virus population was eradicated during short duration treatment and thereby led to treatment failure. Furthermore, the distribution of the quasispecies is important in the first phase of virus decline, as the decrease of variants with reduced susceptibility or partial resistance is slower and exhibit a longer first phase. If treatment duration is not sufficient for complete eradication, the variants will rebound again after DAA withdrawal. This is in accordance with the findings in this study where baseline NS5A RAS were detected in 45% (5/11) of the patients with virological relapse compared to 14% (3/21) among SVR patients. This suggest that NS5A RAS could be a concern for short-term treatment, but testing for baseline RAS will not be a realistic option in a clinical setting. The most important baseline RAS associated with treatment failures are located in NS5A domain I. These also affected RNA titer decline and quasispecies composition after end of treatment.

The strategy for retreatment in this study was not according to protocol and patients were treated by standard of care. We believe that retreatment after ultrashort treatment is a different issue than retreatment after 8-12 weeks DAA and therefore we did not follow standard guidelines according to...
retreatment. We were uncertain that using GLE/PIB for an 8-week regimen would be sufficient for patients who had failed 4 weeks of the same drugs and prolonging GLE/PIB to 12 weeks was not an option in standard of care. In addition, a sofosbuvir regimen of 12 weeks was desirable after failure to a NS5A regime and therefore SOF/VEL was chosen as retreatment. However, the combination of (SOF/VEL/VOX) was used for two patients with double or triple baseline NS5A RASs. The clinical impact of RAS and retreatment is still not completely clear, but retreatment can be optimized with personalized retreatment regime after RAS testing. However, clinical studies showed high probability of successful retreatment regardless of RAS at retreatment baseline. The most crucial pre-treatment parameter associated with retreatment outcome has primarily been the presence of cirrhosis, and this patient group was not included in our study.

The hypothesis of this study was that by adding ribavirin the SVR12 proportion of patients treated for four weeks with DAA would be higher. The ribavirin arm had numerically higher SVR12 (73%), but with >95% cure in standard of care, our result is still disappointing. Until now, only a few trials with a treatment duration of 6 weeks or less have used ribavirin. Everson et al found no differences between SOF/VEL with and without ribavirin given for 8 weeks in genotype 1 and 2. A cohort study showed that ribavirin increase the likelihood of SVR12 among patients with genotype 3, treatment failure on DAA and decompensated cirrhosis. In continuation to this, another study has shown the benefit of ribavirin to shorten the duration from 24 to 12 weeks in previously non-responder genotype 1 patients with compensated cirrhosis. In relation to treatment with duration of less than 8 weeks, the role of adding ribavirin has not been demonstrated convincingly. We have shown that in cell culture, ribavirin sensitivity is dependent of specific regions of the HCV genome but the clinical correlate of this finding remains to be shown.

Lower cure rate for genotype 3 than 1 have been reported for DAA including GLE/PIB for 8-12 weeks of treatment. Contrary to expectations, this study did not find that genotype 3 was a predictor of virological relapse. The two patients with genotype 3 and treatment relapse in the present study did have baseline RAS as a potential factor for virological relapse. Apart from one study, which also did not find genotype 3 as a predictor of virological relapse, most ultrashort trials have omitted the inclusion of genotype 3. Hepatitis C virus have a high level of genetic heterogeneity throughout the genome where the genome of different genotypes differ by about 30%. The impact of the different genotypes and subtypes on the treatment response is crucial which was also reflected in this study where a patient with an unknown genotype 1 subtype failed treatment. From a clinical point of view short treatment can only be used if a pangenotypic treatment regimen with SVR >90% based on few and easily obtained baseline variables can be identified, especially in resource-limited settings.

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Half of the patients with baseline RAS were cured, and half of the patients who failed did not have or developed treatment associated RAS. Furthermore, one patient with positive HCV RNA 4 weeks post treatment obtained SVR. This suggests the importance of the host immune response for virus elimination. The immune system was not addressed in this study, but we speculate whether an immune-stimulant, less toxic than interferon-alpha should be considered for future short treatment trials.

The study population in this study had hepatitis C for a mean duration of 20 years. A recent pilot study showed that GLE/PIB was highly effective when given for 6 weeks in people with acute and recent hepatitis C infection.

**Strengths and limitations**

The major strength of this study is the low rate of lost to follow up confirming that there was no late treatment relapse after post treatment week 12. Another strength is that the study population represents the majority of infected hepatitis C patients. The most important limitations are the small sample size and the single center setting. We aimed to include 40 patients but because of financial issues, we could only include 32 patients. However, the reduced number of included patients changed the CI from (95% CI 0.56-0.94) to (95% CI 0.54-0.96) and we therefore do not believe that the reduced number of included patients in this pilot study compared to that originally planned, will have any impact for the final outcome. Additionally, it is unlikely that the small sample size affects the low detection of baseline RAS and high rate of SVR12 after retreatment in our study.

**CONCLUSION**

We observed a low treatment response to GLE/PIB with and without ribavirin after four-week treatment. This pilot study shows that baseline RAS were more frequently observed in patients with treatment failure, than patients who achieved SVR12, after ultrashort treatment for chronic hepatitis C. Furthermore, this study shows development of RAS during ultrashort treatment. However, these mutations did not impede cure after longer-term retreatment with SOF-based regimens.

**Author contributions**

AØ and PBC designed the study. AØ, PBC and LWM were involved in patient screening, recruitment and data collection. UF, MSP and JB performed and analyzed viral resistance. LWM conducted the data analyses. All authors contributed to the interpretation of the data. LWM drafted the manuscript with input from all authors. All authors have reviewed and approved the final manuscript.

**Data Availability**

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Due to Danish rules on data availability, we are unable to make an anonymized dataset public. These rules are based on the Data Protection Act, imposed by The Danish Data Protection Agency. An English translation of the Data Protection Act can be found on the official website for The Danish Data Protection Agency (https://www.datatilsynet.dk/english/legislation/). For further information, the corresponding author can be contacted.

**References**


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Table 1. Baseline demographic and clinical characteristics of study patients.

<table>
<thead>
<tr>
<th>Study population, n (%)</th>
<th>Glecaprevir/Pibrentasvir (n=17)</th>
<th>Glecaprevir/Pibrentasvir + Ribavirin (n=15)</th>
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<tr>
<td>Age (years) median (range)</td>
<td>41 (33-48)</td>
<td>43 (28-49)</td>
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<tr>
<td>Male</td>
<td>14 (82.4)</td>
<td>9 (60.0)</td>
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<tr>
<td>BMI, median (IQR)</td>
<td>27 (24 – 31)</td>
<td>27 (24-29)</td>
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<tr>
<td>Current or past alcohol overuse</td>
<td>10 (58.8)</td>
<td>8 (53.3)</td>
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<tr>
<td>Current or past intravenous drug use</td>
<td>12 (70.6)</td>
<td>12 (80.0)</td>
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<tr>
<td>Injection within the last year</td>
<td>2 (11.8)</td>
<td>3 (20.0)</td>
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<td>HCV Genotype 1/2/3/4</td>
<td>8/2/7/0</td>
<td>6/2/6/1</td>
</tr>
<tr>
<td>HCV RNA (log_{10} IU/ml), median (IQR)</td>
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<td>6.0 (5.6-6.6)</td>
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<td>IL28b genotype CC/non CC</td>
<td>5 (29.4) / 12 (70.6)</td>
<td>4 (26.7) / 11 (73.3)</td>
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<td>LSM in kPa, median (range)</td>
<td>5.3 (3.1-7.8)</td>
<td>5.4 (4.3 -7.8)</td>
</tr>
<tr>
<td>Opiate Substitution Therapy</td>
<td>5 (29.4)</td>
<td>4 (26.7)</td>
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<tr>
<td>Year since infected, median (IQR)</td>
<td>21 (15-26)</td>
<td>23 (19-27)</td>
</tr>
</tbody>
</table>

Abbreviations: Interquartile range (IQR), Body Mass Index (BMI), Hepatitis C (HCV), Liver stiffness measurement (LSM)

Table 2. All detectable Resistance Associated Substitutions at baseline and time of virological relapse. Significant changes marked in bold. Lack of statement of the percentage correlates with a detection level of 100%.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Genotype</th>
<th>Treatment</th>
<th>Baseline</th>
<th>Relapse</th>
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<table>
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<tr>
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<tbody>
<tr>
<td><strong>Virological Cure</strong></td>
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<tr>
<td>Patient 1</td>
<td>1a</td>
<td>GLE/PIB + ribavirin</td>
<td>Q80K</td>
<td></td>
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<tr>
<td>Patient 2</td>
<td>3a</td>
<td>GLE/PIB</td>
<td>A166S</td>
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<td></td>
<td></td>
<td></td>
<td>(59%)</td>
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<tr>
<td>Patient 3</td>
<td>1a</td>
<td>GLE/PIB + ribavirin</td>
<td>M28V</td>
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<tr>
<td>Patient 4</td>
<td>4d</td>
<td>GLE/PIB + ribavirin</td>
<td>58S</td>
<td></td>
</tr>
<tr>
<td>Patient 5</td>
<td>3a</td>
<td>GLE/PIB</td>
<td>30K</td>
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<tr>
<td><strong>Virological Relapse</strong></td>
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<tr>
<td><strong>Patients with NS5A RAS development at relapse</strong></td>
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<tr>
<td>Patient 6</td>
<td>2b</td>
<td>GLE/PIB</td>
<td>30K</td>
<td>L28F (54%), 30K, 31M (23%)</td>
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<tr>
<td>Patient 7</td>
<td>3b</td>
<td>GLE/PIB</td>
<td>30K,31M, 80R,168L, 30K,31M,93H</td>
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<tr>
<td>Patient 8</td>
<td>1a</td>
<td>GLE/PIB</td>
<td>M28T (0.6%) †, R81K (9%)</td>
<td>M28T (14%), R81K (61%)</td>
</tr>
<tr>
<td><strong>Patients with NS3 RAS development at relapse</strong></td>
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<tr>
<td>Patient 9</td>
<td>1*</td>
<td>GLE/PIB</td>
<td>56F, 170I, V71I (6%), 28M,30Q,31M, 56F, 170I, V71I (100%), 28M,30Q,31M</td>
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<tr>
<td>Patient 10</td>
<td>1b</td>
<td>GLE/PIB + ribavirin, Y56F, 170I, V71I (15%), Y56F, 170I, V71I (80%)</td>
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<tr>
<td><strong>No RAS development at relapse</strong></td>
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<tr>
<td>Patient 11</td>
<td>3a</td>
<td>GLE/PIB + ribavirin</td>
<td>30S</td>
<td>30S</td>
</tr>
</tbody>
</table>

| † is included although detected below 5% at baseline as it increases to above 5% at virological relapse. Patient 7 has development in both targets and thereby fits both categories.
Figure 1. Flow diagram of patient inclusion and randomization.

Abbreviation; (GLE/PIB) glecaprevir/pibrentasvir

† could not be subtyped with normal procedure

Figure 2:
Proportion of patients with viral load below LLOQ from EOT to post treatment week 12

Abbreviations: LLOQ: Lower limit of quantitation, EOT; End of Treatment, SVR4; Sustained Virological Response week 4, SVR12; Sustained Virological Response week 12.

† 2 missing values. Both achieved SVR12.

Figure 3
Mean Hemoglobin level from treatment start week 0 to SVR12 (week 16) for the two treatment groups.

Week 4 for the group treated with GLE/PIB + ribavirin is calculated for 14 patients.
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