8 weeks of sofosbuvir/ledipasvir is effective in DAA-naive non-cirrhotic HCV genotype 4 infected patients (HEPNED-001 study)

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Supplement S1: extended version of the methods

Design & subjects

This study was designed as a single arm prospective open label multicenter study in HIV and/or hepatitis C treatment centers in the Netherlands (n=9) and Belgium (n=1). Eligible participants were HIV positive or negative adults (≥18 years), chronically infected with genotype 4 HCV with a screening HCV RNA load <10 million IU/mL. A chronic hepatitis C infection was defined according to the EASL guideline as the presence of both anti-HCV antibodies and HCV RNA for more than 6 months [1]. Patients with an eGFR < 30 mL/min or a history of DAA treatment failure for the current episode of HCV infection were excluded. Only patients with a liver biopsy with a METAVIR score lower than AxF4 or a liver stiffness measurement (Fibroscan®) <12.5 kPa were eligible[2]. Biopsy or shear wave elastography results were allowed to be 24 months old. However, in case of METAVIR score F3 or shear wave elastography result >9,5 kPa [2], results could not be older than 12 months. All concomitant co-medication (e.g. cART) was reviewed for drug-drug interactions with the Hepatitis Drug Interactions tool of the University of Liverpool [3] and co-medication was changed if needed before DAA initiation.

Treatment & assessments

All subjects received 8 weeks of SOF/LDP 90/400 mg QD. HCV RNA loads during therapy were analyzed according to local hospital policy, but at least at baseline and week 20 (SVR12). Because SOF/LDP is already EMA-approved, there was no mandatory reporting of minor side effects during this study, but serious adverse events were registered.

Primary outcome

The primary efficacy outcome was the sustained virological response 12 weeks after the end of the 8 week therapy (SVR12) in the on-treatment (OT) study population. SVR12 was defined as an HCV RNA

below the limit of detection 12 weeks or later after the end of therapy. The OT population was defined as all patients that had completed the 8-week course and of which a HCV RNA was measured at ≥12 weeks after the end of therapy.

Treatment failures

HCV relapse was defined as reoccurrence of the HCV virus with which the patient was infected at the start of therapy after treatment discontinuation and after the documentation of a previously undetectable HCV RNA during therapy. However, as reinfection is frequently observed in HIV+ MSM [4] and, in 2017, approximately 35% of all acute HCV infections in Dutch and Belgian HIV+ MSM were of the genotype 4 [5], it is important to differentiate reinfection from relapse because an HCV reinfection should not be considered therapy failure. Therefore, in patients with a presumed HCV relapse, a genotype analysis with a reverse hybridizing assay (the Versant® HCV Genotype 2.0 System (LiPA)) was performed to differentiate relapse with a new HCV genotype from reinfection. If genotype 4 was again present at the time of the presumed relapse, a phylogenetic analysis was done using a fragment of the envelope E2 gene which includes the hypervariable region 1, to differentiate relapse from a genotype 4 reinfection according to the methods described by Thomas et al. [6]. Patients with a documented HCV reinfection 12 weeks after the end of therapy were not considered as treatment failures in the analysis.

Secondary outcomes

Secondary outcomes included SVR12 in the intention-to-treat (ITT) population defined as all patients that initiated study drugs, SVR12 in the HIV positive compared to the HIV-negative population and SVR12 in the study population with baseline viral loads < 6 million IU/ml HCV RNA.

Sample size

Although the study was a non-randomized single arm study and therefore not a formal non-inferiority randomized clinical trial, we estimated the appropriate sample size for the study by calculating the sample size under the assumption that the cure rate with 8 weeks of SOF/LDP would be 95% and therefore identical to what was observed after 12 weeks of SOF/LDP for chronic HCV genotype 4 in the NIAID SYNERGY[7] and the 1119 study[8] . Our hypothesis is that we can shorten therapy duration to 8 weeks without a loss of effectivity. Therefore we anticipate that the SVR after 8 weeks of therapy is a fixed 95%. We based our 95% estimate on the available results on the treatment of genotype 4 with 12 weeks of sofosbuvir/ledipasvir at the time the protocol was written in 2016; the NIAID SYNERGY study[7]. In both studies combined, an SVR was observed in 61 of the 65 patients (94%) but to be on the conservative site in our sample size calculation we used a fixed 95% SVR as comparator. We use a non-inferiority margin of 10%, which means that the lower 95% C.I. of the difference between the proportion of patients with an SVR in the intervention group and the fixed SVR of 95% should not exceed 10% (e.g. if the SVR result is 95% the difference between proportions is 0% and the 95% CI of this 0% should not exceed 10%). For the study to have 90% power to show non-inferiority under our study hypothesis, and using an alfa error of 5% a sample size of 41 is needed. (Settings are therefore 1-beta of 0,9, alfa 5%, true proportion 0,95, null hypothesis proportion 0,95 and delta 0.1). [9].

Note: *Although we intended to include 41 patients, as a result of the rapid treatment uptake of DAAs in HIV-infected MSM in the Netherlands and Belgium[10], the inclusion of additional patients was not possible because after the screening of 63 and the treatment of 40 HCV genotype 4 patients (of whom 30 were HIV co-infected), no eligible patients were left in any of the participating centers.*

Interim analysis

A single interim safety analysis was planned and performed after 10 patients had reached the SVR12 evaluation endpoint. The stopping rule in the protocol said that the study would be discontinued if <8 of the first 10 patients had an SVR12 because the upper limit of the 95% C.I. of an SVR12 of 7/10 is 89% and with current DAA therapies we considered a SVR12 <90% as unacceptably low.

Statistical analysis

Data was analyzed using IBM SPSS statistics® v21. Baseline characteristics between HIV-negative and HIV-positive patients were compared with Fisher's exact test for categorical variables and 2-sided Mann-Whitney U test for continuous variables. A 2-sided $p<0.05$ was regarded as significant. For the primary as well as the secondary endpoints, the proportion of patients with SVR12 was calculated with a 2-sided C.I. using the exact Clopper-Pearson confidence intervals.

Ethics statement

The protocol was approved by all local medical ethics committees and registered in the Dutch Trial Register 'Nederlands Trial Register' (Trial ID NTR5729). All subjects signed informed consent.

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Supplement S2: flow diagram

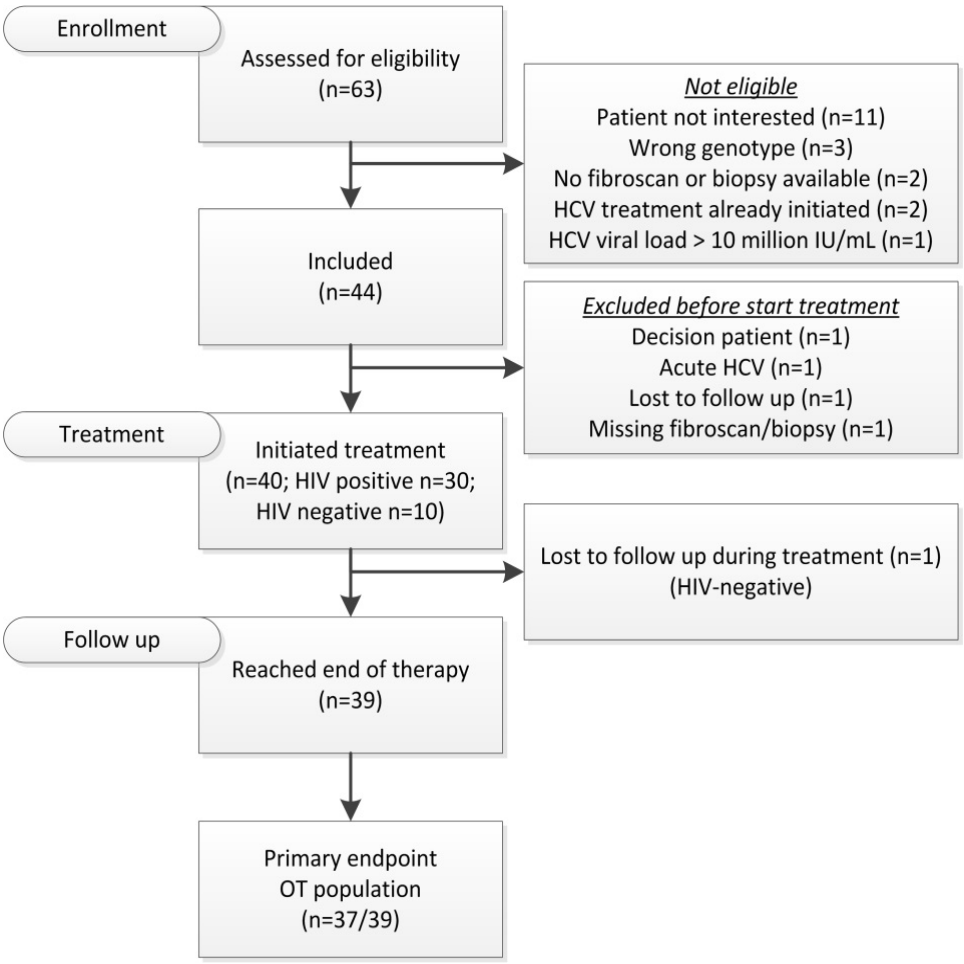

Fig. S1. Flow diagram.

Supplement S3: Neighbor-Joining Tree

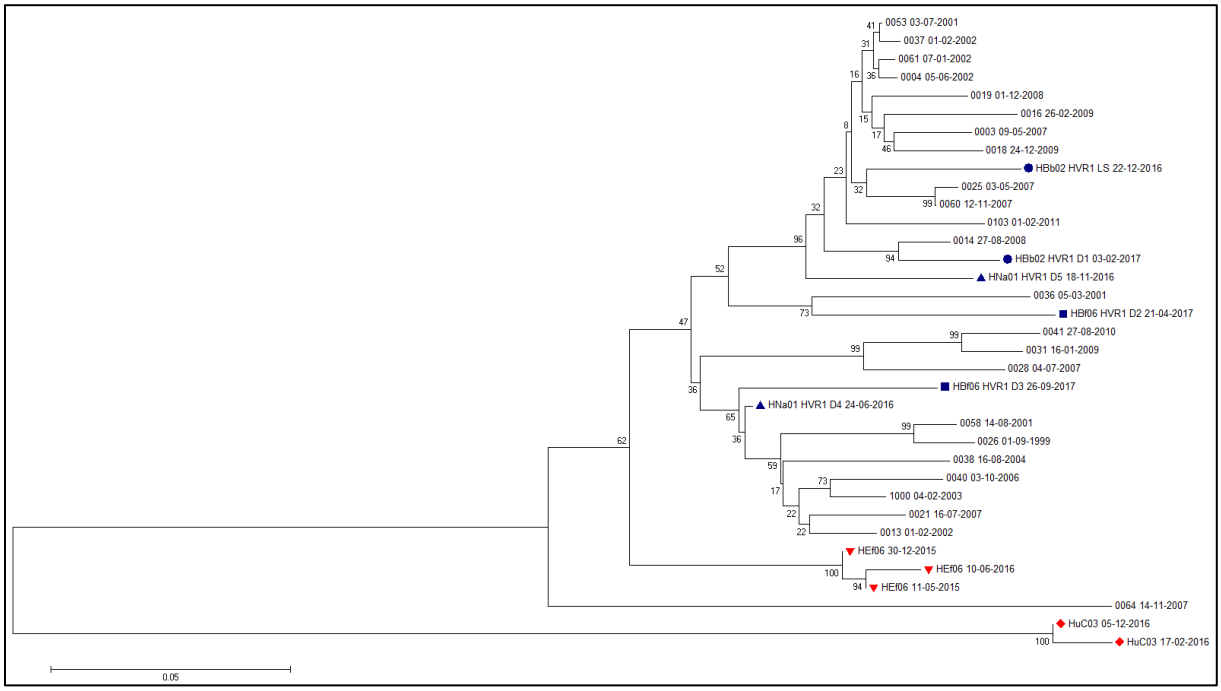

Fig. S2. Evolutionary relationships of patients infected with HCV genotype 4.

Phylogenetic tree of genotype 4 sequences, including relapsers and patients with a reinfection with the same genotype before and after treatment. Relapsers are presented by filled symbols in red and reinfections are presented by filled symbols in blue. Each patient is presented by a unique symbol. The date in the labels indicate sampling dates.

Method to make the tree (copied from MEGA6): The evolutionary history was inferred using the Neighbor-Joining method [1]. The optimal tree with the sum of branch length = 1.30658013 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (10000 replicates) are shown next to the branches [2]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method [3] and are in the units of the number of base substitutions per site. The analysis involved 35 nucleotide

sequences. All ambiguous positions were removed for each sequence pair. There were a total of 427 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 [4].

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