



## Long-term safety and efficacy of microRNA-targeted therapy in chronic hepatitis C patients



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### ABSTRACT

**Background and aims:** MicroRNA-122 (miR-122) is an important host factor for hepatitis C virus (HCV) and promotes HCV RNA accumulation. Decreased intra-hepatic levels of miR-122 were observed in patients with hepatocellular carcinoma, suggesting a potential role of miR-122 in the development of HCC. Miravirsin targets miR-122 and resulted in a dose dependent and prolonged decrease of HCV RNA levels in chronic hepatitis C patients. The aim of this study was to establish the sustained virological response rate to peginterferon (P) and ribavirin (R) following miravirsin dosing and to assess long-term safety in patients treated with miravirsin.

**Methods:** In this multicenter, retrospective follow-up study we included 36 treatment naïve patients with chronic hepatitis C genotype 1 who received five weekly subcutaneous injections with miravirsin or placebo over a 29-day period in a phase 2a study. Patients were offered PR therapy 3 weeks (3 mg/kg group) or 6 weeks (5 or 7 mg/kg group) after completion of miravirsin or placebo dosing.

**Results:** PR therapy was started in 14/36 patients of whom 12 had received miravirsin. SVR was achieved in 7/12 patients previously dosed with miravirsin. All patients dosed with 7 mg/kg miravirsin who were subsequently treated with PR achieved SVR. One patient had a prolonged undetectable HCV RNA period from week 14 to week 29 after baseline without subsequent antiviral therapy and relapsed thereafter. None of the patients treated with anti-miR-122 developed HCC or other liver-related complications.

**Conclusion:** No long-term safety issues were observed among 27 miravirsin-treated patients. Targeting miR-122 may be an effective and safe treatment strategy for HCV infection and should be investigated in larger clinical trials.

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**Abbreviations:** ALT, alanine aminotransferase; APRI, AST to platelet ratio index; AST, aspartate aminotransferase; DAA, direct-acting antiviral; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IL28B, interleukin-28B; ISG, interferon-stimulated gene; miRNA, microRNA; miR-122, microRNA-122; P, peginterferon; R, ribavirin; SVR, sustained virological response; RVR, rapid viral response.

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### 1. Introduction

Hepatitis C virus (HCV) is a single-stranded RNA virus and represents a major causative agent of chronic liver disease. Worldwide, 170 million people have a chronic HCV infection and are at risk to develop cirrhosis, leading to clinical complications such as hepatocellular carcinoma (HCC) (Hajarizadeh et al., 2013; Lauer and Walker, 2001). The aim of chronic hepatitis C treatment

is to achieve a sustained virological response (SVR), which is associated with reduced occurrence of liver failure and HCC, and with prolonged overall survival (Backus et al., 2011; Cardoso et al., 2010; van der Meer et al., 2012). Many highly potential direct-acting antiviral (DAA) agents are being assessed in clinical trials and various combinations of DAA's result in high SVR rates. Some DAAs target viral proteins, such as NS3/4A protease and NS5A/B replication inhibitors, whereas others target host factors that are essential for HCV replication, such as cyclophilin A or microRNA-122 (miR-122) (Flisiak et al., 2012; Janssen et al., 2013).

MicroRNAs (miRNAs) are small (19–24 nucleotides), non-coding, RNA molecules that are involved in various cellular processes by post-transcriptional suppression of gene expression (Ambros, 2004; Bartel, 2004). MiR-122, a highly abundant miRNA expressed in the liver (Lagos-Quintana et al., 2002), regulates lipid metabolism and acts as a tumor suppressor (Coulouarn et al., 2009; Esau et al., 2006; Krützfeldt et al., 2005). MiR-122 is also involved in HCV replication by binding to two highly conserved seed sites in the 5' UTR of the HCV genome and promotes HCV RNA accumulation by stabilizing the viral genome and stimulating its translation (Jopling et al., 2005; Lanford et al., 2010). Furthermore, the miR-122-HCV complex protects the HCV genome from degradation and prevents induction of an innate immune response against HCV (Jopling et al., 2005; Machlin et al., 2011). This discovery led to the development of the first successful miRNA-based therapeutic strategy wherein an anti-miR silences miR-122. In chimpanzees infected with HCV, silencing of miR-122 led to potent and prolonged inhibition of HCV replication without viral resistance (Lanford et al., 2010). Recently, the results of the first study in which an anti-miR was administered to HCV infected patients was presented (Janssen et al., 2013). In this phase 2a study, chronic HCV genotype 1 infected patients received five weekly injections of miravirsin, a locked nucleic acid-modified phosphorothioate oligonucleotide targeting miR-122. This resulted in a prolonged and dose-dependent decrease in HCV RNA, alanine aminotransferase (ALT) and cholesterol levels (Janssen et al., 2013). Patients were followed for an additional 14 weeks after the last dose of miravirsin and effects on HCV RNA and ALT could still be observed at the end of the study. The prolonged antiviral effect could be explained by the fact that miravirsin has a long tissue half-life (approximately 30 days) which suggests that the biological effect of miravirsin can last for weeks. As earlier studies revealed that miR-122 has a tumor suppressive role and that mice lacking the gene encoding for miR-122 were at high risk to develop hepatosteatosis and HCC (Hsu et al., 2012; Tsai et al., 2012), it is of great importance to evaluate the long-term safety among the patients treated with this first anti-miR therapy. The primary objective of this study was to assess the long-term safety and clinical efficacy of miR-122 targeted therapy among patients with chronic HCV genotype 1 infection. The secondary objective was to determine the virological response among those patients who subsequently received peginterferon (P) and ribavirin (R) therapy.

## 2. Materials and methods

### 2.1. Study population and design

This follow-up study was a retrospective analysis which assessed the long-term safety and clinical outcome of patients treated with different doses of miravirsin, with or without a subsequent course of PR therapy. All 36 HCV genotype 1 infected, treatment naïve patients who previously participated in a multicenter, randomized, placebo-controlled, phase 2a study to assess the safety and efficacy of miravirsin were included (Janssen et al., 2013). In this study, patients were randomized in a 3:1 ratio

to receive either miravirsin (in doses of 3 mg, 5 mg or 7 mg/kg) or placebo. Miravirsin was administered subcutaneously in five weekly doses over a 29-day period. After the administration period, patients returned for follow-up visits for a period of 14 weeks. Patients were allowed to start PR therapy at the discretion of the investigator 3 weeks (patients dosed with 3 mg/kg) or 6 weeks (patients dosed with 5 or 7 mg/kg) after completion of miravirsin or placebo dosing. Patients were treated with pegylated interferon alfa-2a (dose 180 µg/0.5 ml) and weight-based doses of ribavirin (1000 mg for ≤75 kg and 1200 mg for >75 kg). Treatment response was subdivided in virological breakthrough, virological relapse, non-response or SVR. Virological breakthrough refers to the reappearance of HCV RNA before treatment is completed. Virological relapse was defined as a decrease in HCV RNA below the limit of detection during treatment, but detectable HCV RNA after treatment was stopped. Non-response was defined as <2 log decline of HCV RNA at week 12 or HCV RNA positive HCV RNA at week 24 during treatment. SVR was defined as undetectable HCV RNA 24 weeks after treatment was stopped. A rapid viral response (RVR) was defined as undetectable HCV RNA at week 4 during treatment. End points regarding safety were liver failure (such as ascites, jaundice, variceal bleeding or hepatic encephalopathy), liver transplantation, HCC, hospitalization or death.

### 2.2. Data collection

We collected prolonged follow-up data to assess the long-term efficacy and safety. The obtained data included clinical safety data, local laboratory results, virological responses to PR therapy, side effects and stage/grade of liver disease (fibroscan or liver biopsy). The aspartate aminotransferase to platelet ratio index (APRI) score was calculated by the formula:  $(AST/reference\ AST)/(platelets \times 100)$ .

### 2.3. Ethics

The study was approved by the Medical Ethics Review Committee of the Academic Medical Center Amsterdam and was carried out in compliance with the protocol, the principles laid down in the Declaration of Helsinki, in accordance with the ICH Harmonised Tripartite Guideline for Good Clinical Practice and the local national laws governing the conduct of clinical research studies.

### 2.4. Statistical analysis

To compare the baseline characteristics and outcome measures of the study groups we used the Student's *t*-, one-way ANOVA, Kruskal–Wallis, and  $\chi^2$  tests. A *p*-value of <0.05 was considered statistically significant. All analyses were performed with the use of SPSS, version 20.

## 3. Results

### 3.1. Patients characteristics

This study included 36 patients of whom 27 had received various doses of miravirsin and nine received placebo. Baseline characteristics were similar among the four study groups (Table 1). PR therapy was initiated in 14 (39%) patients.

### 3.2. Virological response

Five subcutaneous injections with miravirsin resulted in a prolonged and dose-dependent decrease in HCV RNA levels (Janssen et al., 2013). The mean of the maximum reduction in HCV RNA

**Table 1**Baseline characteristics of all patients ( $n = 36$ ).

|                                   | Miravirsen          |                     |                     | Placebo<br>( $n = 9$ ) |
|-----------------------------------|---------------------|---------------------|---------------------|------------------------|
|                                   | 3 mg/kg ( $n = 9$ ) | 5 mg/kg ( $n = 9$ ) | 7 mg/kg ( $n = 9$ ) |                        |
| Median age (range) – year         | 35 (26–66)          | 46 (33–65)          | 48 (31–61)          | 56 (42–66)             |
| Male sex – no. (%)                | 5 (56)              | 8 (89)              | 6 (67)              | 3 (33)                 |
| Race – no. (%)                    |                     |                     |                     |                        |
| White                             | 9 (100)             | 9 (89)              | 7 (78)              | 7 (78)                 |
| Black                             | 0                   | 1 (11)              | 1 (11)              | 2 (22)                 |
| Asian                             | 0                   | 0                   | 1 (11)              | 0                      |
| HCV RNA – log <sub>10</sub> IU/ml | 6.0 ± 0.7           | 6.3 ± 0.3           | 5.9 ± 0.6           | 6.3 ± 0.4              |
| IL28B CC genotype – no. (%)       | 2 (22)              | 4 (44)              | 4 (44)              | 2 (22)                 |
| Subtype of HCV – no. (%)          |                     |                     |                     |                        |
| 1a                                | 5 (56)              | 7 (78)              | 5 (56)              | 6 (67)                 |
| 1b                                | 2 (22)              | 1 (11)              | 3 (33)              | 2 (22)                 |
| 1a/1b                             | 2 (22)              | 0                   | 1 (11)              | 1 (11)                 |
| 1a/3a                             | 0                   | 1 (11)              | 0                   | 0                      |
| Alanine aminotransferase – IU/L   | 74.3 ± 38.7         | 69.1 ± 21.4         | 81.3 ± 71.8         | 92.7 ± 36.9            |
| APRI score <sup>a</sup>           | 0.36 ± 0.13         | 0.34 ± 0.07         | 0.44 ± 0.30         | 0.93 ± 0.76            |
| Stage of fibrosis <sup>b</sup>    |                     |                     |                     |                        |
| F0–F1                             | 3 (33)              | 6 (67)              | 7 (78)              | 4 (44)                 |
| F2–F3                             | 2 (22)              | 2 (22)              | 1 (11)              | 2 (22)                 |
| F4                                | 0                   | 0                   | 0                   | 1 (11)                 |
| Unknown                           | 4 (44)              | 1 (11)              | 1 (11)              | 2 (22)                 |

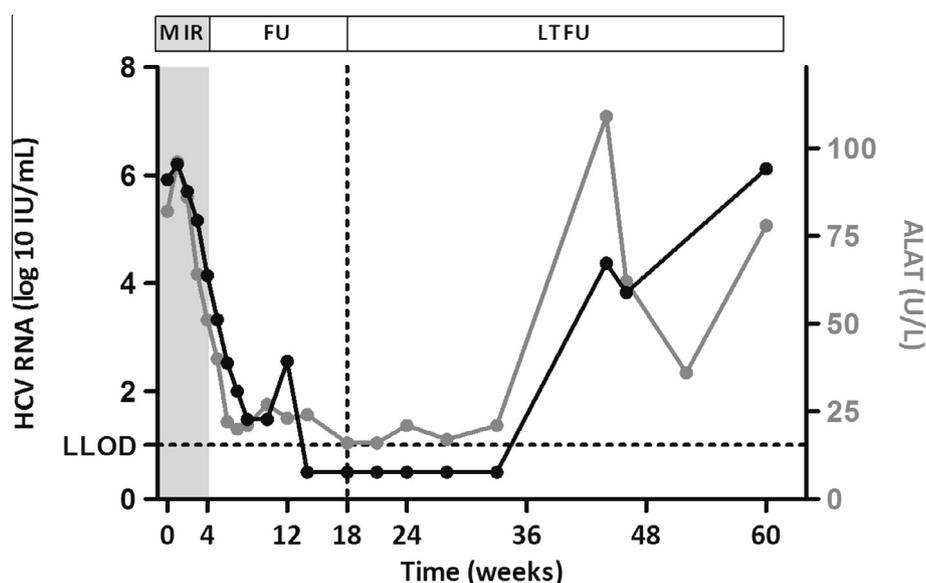
Data are given as median (minimum–maximum), as mean ± SD or as number (percentage).

<sup>a</sup> APRI score was calculated by the formula: ((AST/reference AST)/platelets \* 100).

<sup>b</sup> Stage of fibrosis was determined by liver biopsy or liver elastography.

levels (log<sub>10</sub> IU/mL) from baseline was 1.2 ( $p = 0.01$ ) for patients receiving 3 mg/kg, 2.9 ( $p = 0.003$ ) for those receiving 5 mg/kg, and 3.0 ( $p = 0.002$ ) for those receiving 7 mg/kg, compared with a decline of 0.4 in the placebo arm (Janssen et al., 2013). Undetectable HCV RNA was achieved in one patient in the 5-mg group and in four patients in the 7-mg group. Levels of virus rebounded in most patients who were not treated with PR therapy. One patient, a 43 year old female with fibrosis stage F0–F1 and HCV genotype 1b infection who was dosed with miravirsen 7 mg/kg, became HCV RNA negative at study week 14 and remained this for a period of at least 15 weeks without the initiation of PR

therapy (Fig. 1). This patient was followed up frequently and experienced a virological relapse 44 weeks after miravirsen dosing, at which time the HCV RNA level (log<sub>10</sub> IU/mL) was 4.37 and the ALT level (IU/L) was 109. Two weeks after the virological relapse, the HCV RNA level decreased to 3.83 with a simultaneous decrease in ALT level to 62. However, three months later, the viral load and ALT were back at the pre-treatment levels, with a HCV RNA level of 6.12 as compared to 5.92 at baseline and an ALT level of 78 compared to 82 at baseline. Population sequencing showed no nucleotide changes in the 5'UTR or amino acid differences in NS3, NS5A and NS5B regions.



**Fig. 1.** HCV RNA and ALT levels for an individual patient with a prolonged antiviral effect of anti-miR therapy. This figure shows a prolonged antiviral effect of anti-miR-122 therapy in an individual patient. The lower limit of detection (LLOD) is 12 IU/mL (or 1.08 log<sub>10</sub> IU/mL). This patient was treated with the highest dose of miravirsen (7 mg/kg) and became HCV RNA negative (<LLOD) 14 weeks after the first dose of miravirsen. At week 18 the regular follow-up ended, and extended follow-up shows that this patient remained HCV RNA negative up to 29 weeks after miravirsen dosing. However, 44 weeks after the first dose of miravirsen this patient had a virological relapse, with a simultaneous increase in ALT levels.

### 3.3. Patients treated with PR therapy

PR therapy was started in 14/36 patients of whom 2 received placebo, 5 received 3 mg/kg, 4 received 5 mg/kg and 3 received 7 mg/kg miravirsin (Table 2). The dose of ribavirin was reduced in two patients during treatment due to anaemia and gingival bleeding. SVR was achieved in 7/12 (58%) of the patients previously treated with different doses of miravirsin. All patients ( $n = 3$ ) who received the highest dose of miravirsin (7 mg/kg) and were treated with PR achieved RVR and SVR. Of these patients, 2/3 had undetectable HCV RNA at the start of PR therapy (Fig. 2). The median treatment duration of patients who achieved SVR was 24 weeks (IQR 14–48 weeks), compared to 47 weeks (IQR 24–48 weeks) in patients without SVR ( $p = 0.01$ ). Mean HCV RNA levels ( $\log_{10}$  IU/mL) at the start of PR therapy were significantly lower for patients achieving SVR compared to patients who did not achieve SVR, respectively 3.1 versus 5.2 ( $p = 0.029$ ). The interleukin-28B (IL28B) genotype distribution of patients achieving SVR was CC ( $n = 1$ ), CT ( $n = 4$ ) and TT ( $n = 2$ ). Therapy failed in five patients which was due to non-response ( $n = 2$ ), virological relapse ( $n = 2$ ), and virological breakthrough after therapy cessation due to hospitalization for a pneumonia ( $n = 1$ ) (Table 2). The IL28B genotype distribution of patients who failed PR therapy was CT ( $n = 4$ ) and TT ( $n = 1$ ). Two serious adverse events occurred during PR therapy. One patient was hospitalized due to bronchopneumonia and one patient was observed overnight in the hospital due to loss of consciousness that occurred after a fall. Both events were considered unrelated to miravirsin dosing.

### 3.4. Long-term safety

Patients were followed up to 35 months after the start of miravirsin therapy, with a median duration of 24 months (IQR 14–28 months). None of the patients were diagnosed with HCC, or with any other cirrhosis-related complication. There were no clinically relevant events or hospitalizations during follow-up, other than the serious adverse events that occurred during PR therapy. None of the patients died. Mean ALT levels (IU/L) at follow-up were lower compared to baseline in patients who achieved SVR, respectively 50 at baseline versus 24 at end-of-follow-up ( $p = 0.03$ ). Mean ALT levels were comparable between baseline and end-of-follow-up among patients who did not achieve SVR with PR therapy or did not start PR therapy, respectively 78 versus 67 ( $p = 0.45$ ) and 101 versus 100 ( $p = 0.97$ ). Median APRI score of patients treated

with miravirsin was comparable between baseline and follow-up, respectively 0.34 versus 0.32 ( $p = 0.97$ ). There was no significant difference between baseline and end-of-follow-up median APRI score in patients who achieved SVR, respectively 0.32 versus 0.15 ( $p = 0.11$ ), or in patients who did not achieve SVR, respectively 0.44 versus 0.48 ( $p = 0.57$ ).

## 4. Discussion

Here we present the results of the first study to assess long-term safety of miR-targeted therapy in humans. Up to 35 months following therapy no long-term safety problems were observed among the 27 chronic hepatitis C patients that were treated with miravirsin. None of the patients treated with anti-miR-122 developed HCC or cirrhosis related morbidity such as ascites or variceal bleeding. In addition, antiviral therapy with PR following miravirsin resulted in SVR in 58% of HCV genotype 1, treatment-naïve patients.

MiR-122 is believed to have a tumor suppressive role and has been related to the development of HCC. In vitro studies showed that miR-122 levels were reduced in human HCC cells compared to normal hepatocytes, and that restoration of miR-122 in HCC cells reversed their malignant phenotype and tumorigenic properties (Bai et al., 2009; Coulouarn et al., 2009). Short-term inhibition of miR-122 using antisense oligonucleotides for 5 weeks was well tolerated in adult mice, and these mice did not develop HCC (Esau et al., 2006). In an obesity mouse model induced by a high fat diet, miR-122 inhibition led to a reduction of steatosis (Esau et al., 2006). In contrast, mice lacking the gene encoding for miR-122 developed microsteatosis and inflammation of the liver that progressed to steatohepatitis and HCC later on in life (Hsu et al., 2012; Tsai et al., 2012). It was postulated that hepatocarcinogenesis was initiated by activation of several oncogenic pathways and the production of pro-tumorigenic cytokines (Hsu et al., 2012). However, the biological and clinical effect of transient inhibition of miR-122 and the subsequent long-term risk for HCC development in humans is still unknown and should be carefully studied in future studies with miR-122 inhibiting agents.

It was demonstrated that elevated serum miR-122 level is a sensitive marker for inflammatory activity in the liver and strongly correlates with serum ALT activity (Bihrer et al., 2011; van der Meer et al., 2013). Furthermore, several studies showed that the expression of miR-122 was related to the progression of liver fibrosis and that serum and hepatic miR-122 levels decreased

**Table 2**  
Treatment outcome of patients treated with PR ( $n = 14$ ).

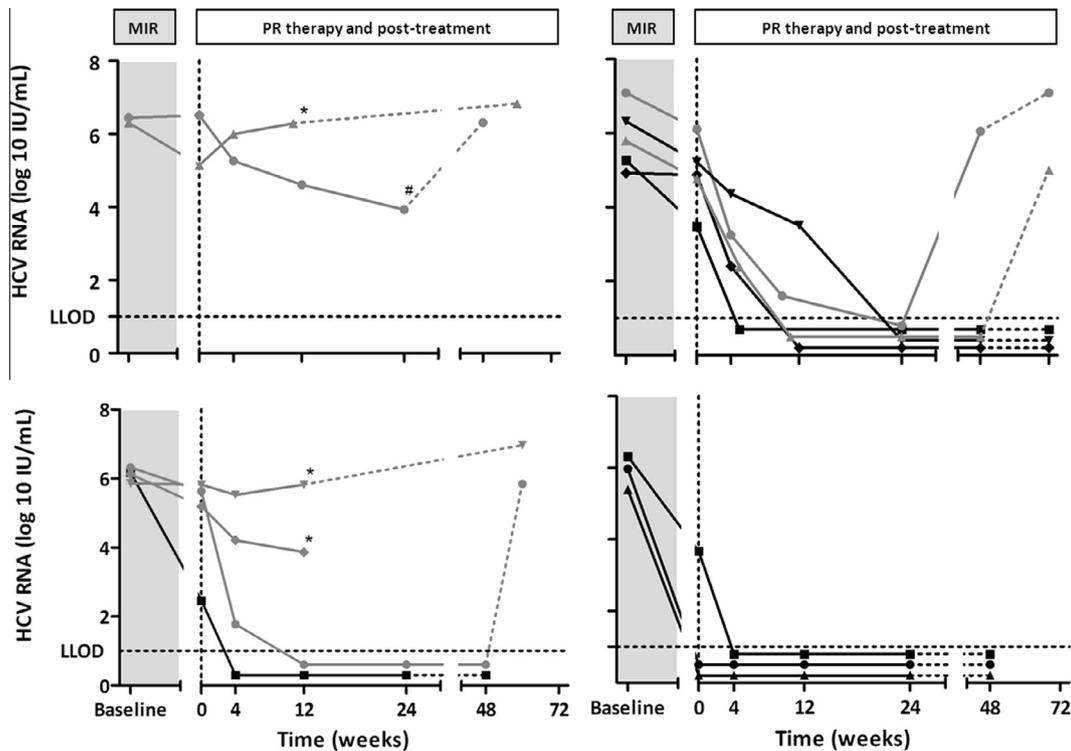
|                                            | Miravirsin          |                     |                     | Placebo<br>( $n = 2$ ) |
|--------------------------------------------|---------------------|---------------------|---------------------|------------------------|
|                                            | 3 mg/kg ( $n = 5$ ) | 5 mg/kg ( $n = 4$ ) | 7 mg/kg ( $n = 3$ ) |                        |
| Median time until start PR – weeks (range) | 4 (3–10)            | 6 (6–15)            | 7 (7–16)            | 3 (3–3)                |
| Median duration of PR – weeks (range)      | 48 (47–48)          | 20 (13–47)          | 24 (23–24)          | 17 (11–22)             |
| HCV RNA start PR – $\log_{10}$ IU/ml       | 4.2 $\pm$ 1.9       | 4.8 $\pm$ 1.6       | 2.8 $\pm$ 1.2       | 6.3 $\pm$ 0.3          |
| IL-28B genotype – no (%)                   |                     |                     |                     |                        |
| CC                                         | 0                   | 0                   | 1 (33)              | 0                      |
| CT                                         | 3 (60)              | 3 (75)              | 2 (67)              | 1 (50)                 |
| TT                                         | 2 (40)              | 1 (25)              | 0                   | 1 (50)                 |
| SAE's – no. (%) <sup>a</sup>               | 1 (20)              | 0                   | 1 (33)              | 0                      |
| RVR – no. (%)                              | 1 (20)              | 0 <sup>b</sup>      | 3 (100)             | 0                      |
| SVR 24 – no. (%)                           | 3 (60)              | 1 (25)              | 3 (100)             | 0                      |
| Non-responder – no. (%)                    | 0                   | 2 (50)              | 0                   | 2 (100) <sup>c</sup>   |
| Virological breakthrough – no. (%)         | 1 (20)              | 0                   | 0                   | 0                      |
| Virological relapse – no. (%)              | 1 (20)              | 1 (25)              | 0                   | 0                      |

Data are given as median (minimum–maximum), as numbers (percentage) or as mean  $\pm$  SD. SAE: serious adverse event; RVR: rapid viral response; SVR 24: sustained virological response 24 weeks after treatment was stopped.

<sup>a</sup> Hospitalization due to bronchopneumonia and trauma capitis, both during PR therapy.

<sup>b</sup> Unknown in 3/4 patients.

<sup>c</sup> PR therapy was stopped due to bad tolerability and insufficient viral response.



**Fig. 2.** HCV RNA levels for individual patients treated with PR, according to study group. Before the start of PR therapy patients were dosed with miravirsen 3, 5, 7 mg/kg or placebo. The difference between HCV RNA levels at baseline and start of PR therapy due is simplified and illustrated in the gray shading. PR therapy is started at week 0 and the treatment duration differs between the individual patients. The solid line represent the HCV RNA measurements during PR therapy, and the dashed line the HCV RNA measurements post-treatment. Black lines represent all patients who achieved a sustained virological response ( $n = 7$ ), and gray lines represent patients who failed on PR therapy ( $n = 7$ ). The lower limit of detection (LLOD) is 12 IU/mL (or 1.08 log<sub>10</sub> IU/mL). \*PR therapy was stopped due to non-response (<2 log decline at week 12); #PR was stopped due to non-response (positive HCV RNA at week 24 on treatment).

significantly if the stage of liver fibrosis progressed (Marquez et al., 2010; Trebicka et al., 2013). In this study we compared baseline and end-of-follow-up fibrosis stage of patients treated with miravirsen using the APRI score. We demonstrated that patients treated with miravirsen showed no difference in APRI score between baseline and end-of-follow-up. This finding suggests that there is no increase in fibrosis in patients treated with anti-miR-122 therapy.

It was suggested that both miR-122 expression and lambda-3-interferon gene (IFNL3) polymorphisms could predict treatment response to PR therapy in chronic hepatitis C patients (Ge et al., 2009; Sarasin-Filipowicz et al., 2009). Patients with the IFNL3 CC genotype have a more rapid early HCV viral decline and achieve higher SVR rates compared to patients with genotype CT/TT (Thompson et al., 2010). Furthermore, several studies demonstrated that low pre-treatment levels of hepatic and serum miR-122 were associated with a poor virological response to PR therapy (Murakami et al., 2010; Sarasin-Filipowicz et al., 2009; Su et al., 2013), however another study did not confirm this finding (Waidmann et al., 2012). Recently, a strong association between the expression of miR-122 and IFNL3 polymorphisms, which is independent of the response to treatment, was demonstrated (Estrabaud et al., 2014). This finding suggests that miR-122 may play a role in the early viral decline that is dependent on IFNL3 and the innate immune response (Estrabaud et al., 2014). Furthermore, it is established that patients with a pre-activated interferon system, which thus express hundreds of ISGs at high levels before treatment, are poor responders to interferon-based therapies (Chen et al., 2005). It was demonstrated that a reduced hepatic miR-122 level was inversely correlated with a high ISG expression in non-responders (Ge et al., 2009; Heim, 2013; Sarasin-Filipowicz et al., 2009). Furthermore, miR-122 blockade in HCV infected chimpanzees, which resulted in the inhibition of viral replication,

induced a simultaneous down-regulation of ISGs in the liver of the chimpanzees (Lanford et al., 2010), and thus reverted the activation of the endogenous interferon system. In this study, one-third of the patients previously dosed with miravirsen started PR therapy. We demonstrated that patients treated with miravirsen had similar treatment responses to PR as expected in treatment-naïve chronic HCV, genotype 1 patients. In fact, all patients who were treated with the highest dose of miravirsen followed by PR therapy achieved RVR and subsequent SVR with a short treatment course of 24 weeks. This favorable treatment response might be explained by the low baseline HCV RNA levels when PR therapy was initiated, however a possible relationship with normalization of ISG levels, permitting the endogenous interferon pathway to respond to therapy, should also be considered.

Compared to direct acting antivirals, which have a half-life of several hours, miravirsen has a long tissue half-life and prolonged antiviral activity. Miravirsen is rapidly cleared out of plasma, approximately within 1 h, and taken up into tissues. The highest concentration of miravirsen is accomplished in liver and kidney tissue. However, the terminal elimination half-life of miravirsen is approximately 30 days. The slow elimination from the liver contributes to the sustained activity of miravirsen and could explain the prolonged effects of treatment. It was shown that miravirsen does not only target mature miR-122, but also suppresses the biogenesis of miR-122 at the primary- and precursor-miRNA levels in vitro (Gebert et al., 2014), which could contribute to this prolonged antiviral effect as well. In this context, the patient who remained HCV RNA negative for more than 7 months after the last dose of miravirsen is illustrative. The possibility of infection with a new virus or development of viral resistance was excluded by population sequencing. Sequence analyses showed no nucleotide changes in the 5'UTR nor amino acid differences in NS3, 5A and 5B regions.

A limitation of this study is the small number of patients, which is due to the fact that this study was the first to administer an anti-miR to humans. Furthermore, there was only one patient with fibrosis stage F4 included in the study, which made it difficult to evaluate the clinical effect of miR-122 inhibition in relation to cirrhosis. Another limitation of this study was that the extended follow-up was not part of the prospective study design, which led to a variation in follow-up duration. Nevertheless, the clinical efficacy on the long-term remains of great importance regarding the potential risk of HCC development. In fact, the theoretical risk to induce HCC by miR-122 suppression is the main reason why the Food and Drug Administration now requests a total follow-up duration of five years for patients treated with anti-miR-122 therapy. Since the initial follow-up period of these patients was 18 weeks, this study provides important additional clinical and safety information of the first patients treated with anti-miR therapy.

The therapeutic field for HCV is changing quickly with the ongoing development and recent registration of several DAAs. This study was the first to evaluate the long-term safety and efficacy data of chronic hepatitis C patients treated with an anti-miR-122. Currently a regimen of 12 weeks monotherapy with miravirsin is being evaluated in clinical trials. The potential and safety of miR-122 inhibition as a therapeutic target for HCV eradication needs to be further examined. Nevertheless, the rapid progression in miRNA research and its possible clinical implications in several human diseases may lead to comparable clinical trials in the future, making these first long-term safety experiences with miravirsin treatment of relevance. In conclusion, no long-term safety problems were observed in a limited number of miravirsin-treated patients and targeting of miR-122 may be an effective treatment strategy for HCV infected patients.

## Supplementary information

This study was initiated by the Academic Medical Center, Amsterdam in the Netherlands. Other participating hospitals were Erasmus Medical Center in the Netherlands, J.W. Goethe University Hospital in Germany, University of Texas Health Science Centre in the USA, Fundacion de Investigacion in Porto Rico, University Hospital Bratislava in Slovakia and Medical University of Warsaw in Poland in collaboration with PRA International and Santaris Pharma.

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