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ORIGINAL ARTICLE

Efficacy of an escalating dose regimen of pegylated interferon α-2a plus ribavirin in the early phase of HCV reinfection after liver transplantation

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Summary

We evaluated the safety and efficacy of an escalating dose regimen of pegylated interferon α-2a (PEG-IFN$_{\alpha\text{-}2a}$) and ribavirin in the early phase of recurrent hepatitis C after orthotopic liver transplantation (OLT). In this prospective study, 26 patients transplanted for hepatitis C virus cirrhosis with recurrent hepatitis C were treated 3.4 ± 3.6 months after OLT and compared with an untreated historical control. PEG-IFN$_{\alpha\text{-}2a}$ was initiated as monotherapy, following stepwise dose escalation up to 180 μg/week and the addition of ribavirin up to 1200 mg/day or maximally tolerated doses for 48 weeks. In the intent-to-treat analysis, 38% showed an early virological response (EVR), 35% an end of treatment response (ETR) and 19% a sustained virological response (SVR). SVR was associated with EVR ($P = 0.0001$) and cumulative PEG-IFN$_{\alpha\text{-}2a}$ dose ($P = 0.04$). There was no significant histological improvement compared with untreated patients. There were no treatment-related serious adverse events. Adverse events included leucopenia (77%) and thrombocytopenia (46%). Three patients discontinued therapy due to side effects, fourteen were nonresponders and four relapsers. Treatment with PEG-IFN$_{\alpha\text{-}2a}$ and ribavirin in the acute phase of post-transplant recurrent hepatitis C yielded an EVR of 38% and an SVR of 19%. The combination was safe and well tolerated.

Introduction

Nearly 300 million people worldwide are chronically infected with the hepatitis C virus (HCV) and 20–30% develop liver cirrhosis within 20–30 years. HCV-related end-stage cirrhosis is currently the leading indication for liver transplantation in Europe [1]. HCV reinfection occurs almost universally after liver transplantation [2,3]. The severity of the recurrent infection ranges from minimal to severe liver damage [4,5] and can progress to clinical decompensation, graft loss and subsequent death [6]. HCV-reinfected patients show a reduced 5-year survival after transplantation compared with HCV-negative patients [7]. The course of HCV reinfection is accelerated in transplant recipients compared with immunocompetent patients, with reported 5-year rates of cirrhosis up to 28% [8]. Severe HCV recurrence is favoured by more potent immunosuppression regimens, especially by repetitive steroid bolus therapies [9,10].

Therefore, it seems reasonable to treat HCV reinfection after liver transplantation, particularly since there is evidence of high rates of sustained virological response (SVR) with pegylated interferon and ribavirin in the HCV-infected nontransplanted population [11]. However, patients with HCV reinfection of the graft differ from immunocompetent patients by higher viraemia [12–14] and a

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higher rate of genotype 1 infections; both factors are predictive for a lower virological response rate [15]. Moreover, interferon therapy has been occasionally associated with severe rejection of the graft [16–18]. Additionally, the optimal time point to start treatment after liver transplantation is still undefined. Most published data report experience with small patient groups and various treatment regimens. Larger clinical trials considering the immunosuppressive regimens and the state of pretransplant antiviral treatment are needed to improve the outcome of HCV reinfection.

Based on the favourable results of therapy for acute hepatitis C in the nontransplanted population [19], we decided to treat our HCV-reinfected transplanted patients starting in July 2000, as soon as acute HCV reinfection was proven by biochemical and histological criteria.

We aimed to evaluate the efficacy and safety of an escalating dose regimen of pegylated interferon α-2a (PEG-IFN$_{\alpha\text{-}2a}$) plus ribavirin for 48 weeks in the early phase of HCV reinfection after liver transplantation, with serologically and histologically confirmed hepatitis C of the graft.

Patients and methods

Patient characteristics

Among the 313 cadaveric liver transplantations performed between 1997 and 2005 at the University of Mainz, Germany, 72 recipients presented with HCV reinfection of the graft. The current antiviral treatment regimen was introduced in July 2000, in all liver-transplanted patients with serological and histological HCV recurrence. Recurrence was defined as histological evidence of lobular hepatitis and no signs of acute or chronic rejection, biliary obstruction or ischaemic damage.

This report describes our cohort of 26 patients who fulfilled the following criteria for antiviral treatment: alanine aminotransferase (ALT) elevation, serum HCV-RNA detection by polymerase chain reaction (PCR), histological evidence of reinfection on a liver biopsy performed within 2 weeks before treatment initiation and no contraindications for therapy. Acute rejection was ruled out when there was no histological evidence of venous endothelialitis, cholangitis or portal inflammation with activated lymphocytes. Cytomegalovirus (CMV) infection was ruled out by the absence of CMV inclusion bodies, negative immunohistochemistry in the liver and CMV pp65 negativity in blood lymphocytes. Exclusion criteria were: persistently normal ALT values, previous treatment with interferon after transplantation, retransplantation for rejection or chronic hepatitis C of the graft, presence of hepatocellular carcinoma after transplantation, coinfection with HBV or HIV, histological features of rejection on screening biopsy, severe post-transplantation complications like unresolved biliary complications, serum creatinine level >2 mg/dl; γ-glutamyltransferase level >20 × N (upper limit of normal), bilirubin level >5 mg/dl, neutrophil count <1500/mm^3, platelet count <50 000/mm^3, haemoglobin (Hb) level <10 g/dl for women and 10.5 g/dl for men, other organ transplantations.

Informed consent was given by each patient before the start of the treatment, and the study protocol followed the ethical guidelines of the Declaration of Helsinki. Treated patients were compared with a historical untreated control group, consisting of 19 patients, matched for baseline characteristics and diagnostic criteria, who had a post-transplant recurrence of hepatitis C before initiation of the current study.

Prognostic factors associated with SVR were studied by univariate analysis. We included virological variables, recipient characteristics, donor characteristics, immunosuppression-related variables and treatment parameters (cumulative interferon and ribavirin doses).

Treatment regimen and dose modification

Pegylated interferon α-2a and ribavirin were initiated within 2 weeks after confirmation of diagnosis and administered for 48 weeks. PEG-IFN$_{\alpha\text{-}2a}$ was given as monotherapy in a low-dose regimen (90 μg/week) for the first 4 weeks; starting with the fifth week of the therapy, PEG-IFN$_{\alpha\text{-}2a}$ doses were escalated in a stepwise manner (to 135 and 180 μg/week) according to individual tolerability, and ribavirin was initiated at 600 mg/day. The ribavirin dosage was then modified according to the Hb levels. If there was no drop in the Hb levels, the dosage was increased to 800–1200 mg/day after another 4 weeks of therapy.

PEG-IFN$_{\alpha\text{-}2a}$ was to be reduced to 90 μg/week when neutrophils dropped below 1.5×10^9/l or platelets dropped below 50×10^9/l. The indication for EPO was severe anaemia, defined as Hb values under 9 g/dl. The indication for G-CSF was severe neutropenia, defined as neutrophil count <1000/mm^3. Patients were followed up for 24 weeks after the completion of the therapy.

Safety monitoring

Data on patient history, physical examination and biochemical and haematological blood examinations were obtained weekly during the first 4 weeks and every month thereafter. Side effects were recorded, and the doses were adjusted according to the haematological parameters. In all patients, immunosuppression was reduced after the diagnosis of HCV recurrence. Patients received tacrolimus (target concentration 5–10 ng/ml depending on the time after OLT), cyclosporin (target concentration 70–125 ng/ml depending on the time after OLT) or sirolimus (target concentration 5–10 ng/ml depending on the time after

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OLT) as monotherapy or tacrolimus in combination with MMF (tacrolimus target concentration 3–5 ng/ml + MMF 2×500–1000 mg/day). Prednisolone was given for the first 6 months after OLT, in a maintenance dose between 0.05 and 0.1 mg/kg/day.

Liver biopsies at our transplant center are routinely performed at the time of OLT and every 12 months thereafter. Additional liver biopsies were performed whenever a biochemical abnormality was detected to diagnose recurrent hepatitis C or to rule out rejection. Patients with recurrent hepatitis C confirmed histologically were rebiopsied 1 year after confirmation; in these patients the routine yearly biopsy time point was exchanged with the above-mentioned. Specimens were evaluated by a single pathologist, who was blinded with respect to patient identification and time of the biopsy relative to treatment. Fibrosis was classified according to the Ishak score [20].

The control patients were followed monthly during the first 6 months and thereafter every 3 months or more frequently depending on their clinical status.

HCV-RNA was quantified before initiating antiviral treatment and at 4, 12, 48 and 72 weeks thereafter. HCV-RNA viral load in serum was determined by a quantitative assay [VERSANT HCV 3.0 (*bDNA*), (Bayer, Leverhusen, Germany), lower limit of detection: 600 IU/ml]; a qualitative assay [AMPLICOR Hepatitis C Virus (HCV) Test, version 2.0 (Roche, Greuzach-Whylen, Germany), lower limit of detection: 50 IU/ml] was used when HCV-RNA dropped below the detection limit of the quantitative test.

Efficacy results

Virological response was defined as HCV-RNA negativity confirmed by highly sensitive qualitative PCR [AMPLICOR Hepatitis C Virus (HCV) Test, version 2.0, Roche, lower limit of detection 50 IU/ml]. Early virological response (EVR) was defined as a 2 log or more decrease in viral load after 12 weeks of treatment, end of treatment response (ETR) was defined as the absence of HCV-RNA at week 48 and sustained viral response (SVR) was defined if HCV-RNA was persistently negative at 24 weeks of follow up after the end of the treatment. Other results were considered nonresponse (i.e. <2 log viral load drop at week 12) or relapse (i.e. positive PCR during the follow up after ETR).

Statistical analysis

All quantitative variables were expressed as mean ± SD. For categorical variables, between-group differences were analysed by the chi-squared test using Yates' correction. Between-group differences for quantitative variables were calculated using the Student's *t*-test. All tests were performed using a 5% level of significance (two-sided). The comparison between groups, the evaluation of side effects and the analysis of potential prognostic factors for SVR were performed with the SPSS program.

Results

Patient characteristics

Between 1997 and May 2005, in all 313 liver transplantations were performed at the Transplantation Center of the University of Mainz. Seventy-two patients had HCV-related liver cirrhosis. The 72 HCV-positive transplanted patients had a significantly lower survival rate than patients with orthotopic liver transplantation (OLT) performed for other underlying disorders ($P = 0.01$). Patients treated with interferon before OLT ($n = 31$) showed a significantly worse long-time survival compared with patients who had not been pretreated ($n = 41$; $P = 0.0072$). Patients with genotype 1 ($n = 66$) had a worse survival than patients with genotype 2 or 3 ($n = 6$; $P = 0.13$). Furthermore, individuals showing advanced Ishak score (4–6 points) in the liver biopsy 1 year after OLT ($n = 20$) showed a significantly worse long time survival compared to those with low Ishak scores (1–3 points; $n = 52$; $P = 0.0013$).

Among the 207 patients transplanted after July 2000, 53 (26%) had HCV cirrhosis. Among these, 30 fulfilled the criteria for treatment. The remaining 23 patients were not treated due to persistently normal ALT levels (<2-fold normal values; 12 cases), severe post-transplant complications (five cases), early death (four cases) or inability to adhere to treatment (two cases). As four patients are still under treatment, here we report the outcome of 26 HCV-infected patients after liver transplantation treated with the escalating PEG-IFN$_{\alpha\text{-}2a}$ and ribavirin therapy. Thirty-five percent of the patients (nine of 26) reached the intended dose of 135–180 μg/week PEG-IFN$_{\alpha\text{-}2a}$ and 800–1200 mg ribavirin/day, the mean doses were 105 μg/week PEG-IFN$_{\alpha\text{-}2a}$ and 645 mg ribavirin/day. Immunosuppressive treatment included prednisolone, combined with tacrolimus, sirolimus or cyclosporin. Four patients had treatment with low-dose tacrolimus and MMF. The control group comprised 19 untreated patients transplanted before June 2000, since the treatment of HCV reinfection was not established in transplanted patients. The selection process is illustrated in Fig. 1. Both groups were well matched with regard to the ALT level, the HCV genotype, baseline viral load, pretreatment prior to liver transplantation, time from transplantation to HCV recurrence and other parameters (Table 1).

Safety

Adverse events during treatment with PEG-IFN$_{\alpha\text{-}2a}$ and ribavirin are shown in Table 2. The most frequently

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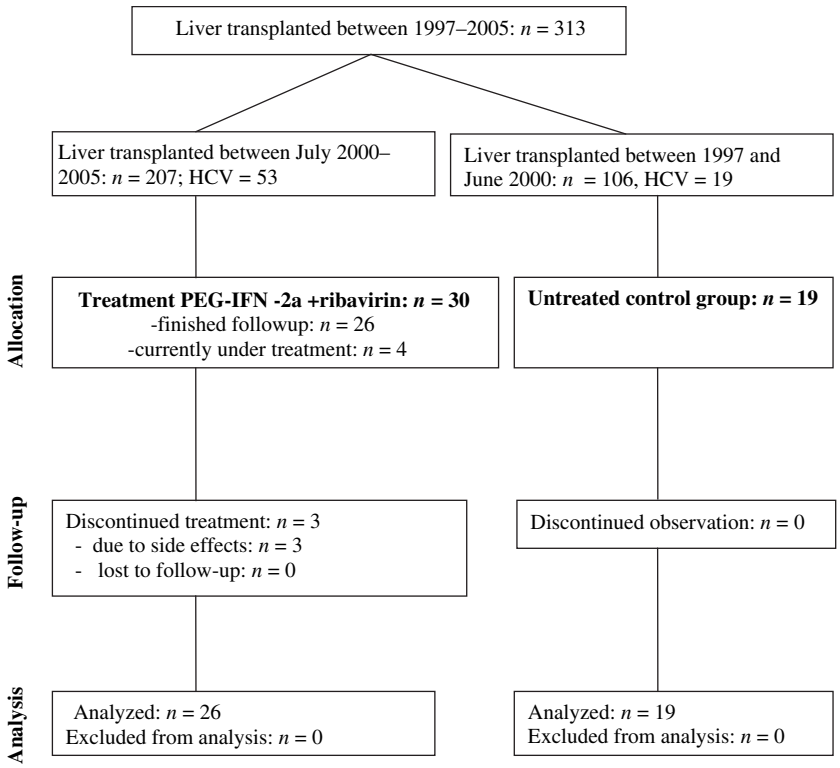

Figure 1 Treated and untreated transplanted patients with hepatitis C virus reinfection between 1997 and 2005: selection process.

Table 1. Patient characteristics.

Characteristics	Treated group	Control group	*P*-value
N	26	19	
Sex (M/F)	17/9	13/6	NS (0.83)
Age (mean ± SD; years)	57.9 ± 9.3	57.7 ± 8.2	NS (0.95)
Time from transplantation to diagnosis time point of recurrent hepatitis C (months)	3.43 ± 3.57	4.82 ± 4.04	NS (0.23)
Interferon-α pretreatment prior to liver transplantation	8/26	8/19	NS (0.59)
ALT at baseline (U/l)	157 ± 149	203 ± 182	NS (0.36)
ALT level at baseline (×normal)	7.47 ± 7.08	9.65 ± 8.69	
Bilirubin (mg/dl)	1.56 ± 0.99	1.72 ± 1.3	NS (0.65)
Creatinine (mg/dl)	1.08 ± 0.27	1.07 ± 0.4	NS (0.96)
Haemoglobin (g/dl)	12.22 ± 1.52	11.78 ± 1.54	NS (0.34)
Leukocytes (/mm^3)	6740 ± 2480	7760 ± 4080	NS (0.31)
Platelets (/mm^3)	190 960 ± 98 720	206 000 ± 117 000	NS (0.65)
BMI	23.67 ± 4.24	24.25 ± 3.18	NS (0.6)
Genotype 1	23 (15M/8F)	18 (13M/5F)	NS (0.63)
Genotype 3	3 (2M/1F)	1 (0M/1F)	
HCV-RNA (>2 million copies/ml)	9/26	6/19	NS (0.88)
Viral load at baseline (mean log HCV-RNA ± SD)	6.97 ± 7.37	6.44 ± 6.82	NS (0.27)
Histology at diagnosis (Ishak score)	2.43 ± 1.88	1.42 ± 1.00	NS (0.64)
HCC (in the explanted liver)	10/26	9/19	NS (0.70)

Analysis was performed with the Student's *t*-test for equal or unequal variables. Variation between groups was evaluated by the Levene test.
M, male; F, female; SD, standard deviation; ALT, alanine aminotransferase; BMI, body mass index; HCC, hepatocellular carcinoma.

reported complaints were asthenia, flu-like symptoms, diarrhoea, nausea, vomiting and haematological side effects. Remarkably, these symptoms were reported with similar frequency by patients in the control group. The only significant differences between the treated and untreated groups were leucopenia (77%), thrombocytopenia

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(46%) and anaemia (Hb: <12 g/dl; 62%). Severe anaemia (Hb: <9 g/dl) was not significantly more frequent in the treated group than in controls. No serious bacterial infections or episodes of bleeding were recorded under therapy, underscoring advantages of this escalating treatment regimen. In line with this good tolerability, only three of 26 patients (11.5%) discontinued therapy due to side effects [diarrhoea ($n = 1$), major depression ($n = 2$)]. Two patients received erythropoetin (EPO) and two other patients the granulocyte colony-stimulating factor (G-CSF) for anaemia/leucopenia. Dose reduction was necessary in 17 patients. One patient in the treated group displayed an episode of acute cellular allograft rejection.

Table 2. Adverse events in the treated and control group.

Type of event	Treated group	Control group	*P*-value
Asthenia	12/26	6/19	NS (0.45)
Myalgia	3/26	0/19	NS (0.25)
Arthralgia	2/26	0/19	NS (0.5)
Flu-like symptoms	9/26	3/19	NS (0.28)
Gastrointestinal symptoms	6/26	4/19	NS (1.0)
Anaemia			
Hb: <12 g/dl	16/26	6/19	**0.04 ($P < 0.05$)**
Hb: <9 g/dl + epoetin-β	2/26	1/19	NS (1.0)
Stop of ribavirin + transfusion	1/26	0/19	NS (1.0)
Leucopenia			
Leucopenia (<4500/mm^3)	20/26	7/19	**0.007 ($P < 0.05$)**
Neutrophils (<1000/mm^3) + G-CSF	2/26	0/19	NS (0.5)
Thrombocytopenia < 100 000/mm^3	12/26	2/19	**0.011 ($P < 0.05$)**
Weight loss (>10%)	2/26	0/19	NS (0.5)
Depression	6/26	2/19	NS (0.44)
Acute rejection	1/26	1/19	NS (1.0)
CMV infection under treatment	4/26	3/19	NS (1.0)
Renal failure (creatinine > 2 mg/dl)	1/26	0/19	NS (1.0)

P-values were calculated with the Fisher's exact test after chi-squared analysis. Significance was reached at $P < 0.05$, confidence interval at 95%.
Hb, haemoglobin; G-CSF, granulocyte colony-stimulating factor; CMV, cytomegalovirus.

Efficacy results

Virological response

Ten of the 26 treated patients (38%) showed an EVR at week 12 when compared with none of the patients in the control group ($P = 0.001$), nine of whom had negative HCV-RNA. The ETR was observed in nine of the 26 treated patients (35%) versus none in the control group ($P = 0.001$). SVR was noticed in five of the 26 patients

Table 3. Analysis of prognostic factors associated with SVR.

Factor	SVR ($n = 5$)	Relapsers ($n = 4$)	Nonresponder ($n = 16$) + breakthrough ($n = 1$)	*P*-value (SVR↔NR)
Donor age (years)	40.6 ± 8.38	46.75 ± 17.52	45.66 ± 16.85	NS (0.45)
Recipient age (years)	56.2 ± 11.71	49.00 ± 14.07	52.22 ± 11.78	NS (0.85)
Sex	5M/0F	3M/1F	9M/8F	NS (0.13)
Presence of hepatocellular carcinoma before OLT	2/5	0/4	8/17	NS (1.0)
Presence of acute rejection	0/5	0/4	1/17	NS (1.0)
Interval of time between liver transplantation and diagnosis of HCV reinfection (days)	64.80 ± 29.13	98.25 ± 68.72	114.05 ± 118.95	NS (0.23)
Administration of MMF + tacrolimus	1/5	0/4	3/17	NS (1.0)
Administration of cyclosporin	1/5	2/4	2/17	NS (1.0)
Administration of sirolimus	0/5	1/4	0/17	NS (1.0)
Administration of tacrolimus	3/5	1/4	12/17	NS (1.0)
Absence of corticosteroid boluses	3/5	4/4	12/17	NS (0.59)
Absence of CMV infection	4/5	2/4	16/17	NS (1.0)
HCV-RNA (IU/ml) before treatment	$7.1 \times 10^5 \pm 6.9 \times 10^5$	$2.3 \times 10^6 \pm 2.3 \times 10^6$	$1.2 \times 10^7 \pm 2.5 \times 10^7$	NS (0.42)
HCV-RNA (IU/ml) before transplantation	$5.1 \times 10^5 \pm 1.4 \times 10^5$	$4.2 \times 10^5 \pm 5.8 \times 10^5$	$2.9 \times 10^5 \pm 3.3 \times 10^5$	NS (0.42)
Decrease of 2 or more log HCV-RNA at week 12	5/5	4/4	1/17	**0.0001 ($P < 0.05$)**
Genotype 1	3/5	4/4	16/17	NS (0.1)
Genotypes 2 and 3	2/5	0/4	1/17	NS (0.1)
Cumulative PEG-IFNα-2a dose (μg per patient/48 weeks)	6552 ± 1682	6165 ± 1530	4341 ± 2299	**0.04 ($P < 0.05$)**
Mean cumulative PEG-IFNα-2a dose (μg/patient/week)	(137 ± 35)	(128 ± 32)	(90 ± 48)	
Cumulative ribavirin dose (mg per patient/44 weeks)	218 400 ± 42 279	231 000 ± 30 800	185 129 ± 54 563	NS (0.19)
Mean cumulative ribavirin dose (mg/patient/day)	(709 ± 137)	(750 ± 100)	(601 ± 177)	
Full dose	3/5	2/4	4/17	NS (0.36)
IFN-pretreatment (before OLT)	1/5	0/4	7/17	NS (1.0)

For analysis we used the Student's *t*-test for equal and unequal variables, after verifying the variation between groups with the Levene test. Significance was reached at $P < 0.05$, confidence interval at 95%.
MMF, mycophenolate mofetil; NR, nonresponder; SVR, sustained virological response; RL, relapser.

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(19%). Univariate analysis revealed the decrease of 2 or more log HCV-RNA at week 12 and the cumulative PEG-IFN dose as prognostic factors of SVR (Table 3). Five of the nine patients with ETR tolerated full doses of PEG-IFN$_{\alpha\text{-}2a}$ and ribavirin. During the follow up, four of the nine patients with ETR showed a relapse (44%).

Biochemical response

Patients in both groups showed high ALT levels at the diagnosis of HCV reinfection, with no significant differences between the groups (Table 1). Patients in the control group presented persistently high ALT values comparable with nonresponders (NR) in the treatment group. Patients with SVR had significantly lower ALT values than NR at the end of treatment ($P = 0.04$).

Histological results

Ten of the 26 (38%) of the patients with PEG-IFN$_{\alpha\text{-}2a}$ plus ribavirin showed an unchanged fibrosis Ishak score (20) at the end of the treatment compared with two of the 19 (11%) patients in the control group after an observation period of 1 year. Patients in the treated group had an average progress in the Ishak score of +0.8 at the end of the treatment, compared with +1.8 in the untreated group. Patients with SVR had a mean progress in the Ishak score of +0.4 compared with NRs (+1.1) after 48 weeks of treatment ($P = 0.24$).

Discussion

Reinfection of the graft is the leading cause of morbidity and mortality after liver transplantation in HCV-infected patients [2,8]. Almost all the patients presented a recurrence and approximately 70–80% developed mild to severe chronic hepatitis. The high incidence and fast development of cirrhosis [21], the negative impact of this complication on patient and graft survival [7], as well as the unsatisfying results of various treatment regimens and the need to discontinue therapy due to severe side effects [22] make research into new therapeutic strategies very important.

This is the first study evaluating the effect of an escalating dose regimen of combination treatment with PEG-IFN$_{\alpha\text{-}2a}$ and ribavirin started in the early stages of recurrent hepatitis C in liver transplant recipients.

From the 313 liver transplantations performed at the University of Mainz between 1997 and 2005, 72 patients presented a HCV reinfection. Factors with negative impact on post-transplant long-term survival were HCV infection as the underlying condition for transplantation, infection with genotype 1, interferon treatment before OLT and advanced Ishak scores (4–6 points) in the liver biopsy 1 year after OLT. These data are in line with other reports from the literature [2,7,8,21].

Our escalating dose regimen proved safe and tolerable, with only three of the 26 (11.5%) patients discontinuing the therapy. The most frequently reported side effects were asthenia, flu-like symptoms and gastrointestinal side effects. None of these was significantly more frequent in the treated group compared with the control group. The only significant differences between the groups were anaemia, leucopenia and thrombocytopenia, without severe bacterial infections or bleedings. There were only two patients who needed G-CSF administration. Overall, anaemia (Hb: <12 g/dl) was significantly more frequent in the treated group (16 of 26) when compared with the control group (six of 19). Severe anaemia, with indication for the administration of EPO (Hb: <9 g/dl), was encountered in only two treated patients. Lack of significance regarding severe anaemia compared with controls might be due to the small number of patients (Table 2). Furthermore, only one patient experienced an episode of acute rejection in the treated group, in contrast to prior studies with interferon monotherapy [22]. However, one patient in the control group also presented an acute rejection. The discontinuation rate in our group was one of the lowest reported until now among other PEG-interferon–ribavirin combination studies, where rates were between 12.5% and 63% and administration of G-CSF was necessary in 20–58% [23,24]. Recent studies using PEG-IFN$_{\alpha\text{-}2a}$ and ribavirin had revealed a high incidence of mainly severe haematological side effects [25–27] with 36–44% of the patients needing G-CSF or EPO treatment [28]. The good tolerability and the low rate of discontinuation highlight the advantage of this escalating treatment regimen. Furthermore, early reduction of the dosage before risking severe and potentially fatal haematological side effects to occur in patients post-OLT and the exclusion criteria for the selection of patients might additionally contribute to the low side effect profile.

At present, the optimal timing for the initiation of the HCV therapy and the duration of the treatment are not clearly defined. Based on the favourable results attained with antiviral therapy in the acute phase of HCV infection in immunocompetent patients [29], we introduced treatment in 26 liver transplant recipients in the early phase of the HCV reinfection, as soon as it was detected, in an attempt to limit the histological progression of the disease. This is in contrast to prior studies, where treatment was started only when recurrent cirrhosis was present [24] and in line with only two other recent reports of combination therapy with interferon α-2b and ribavirin, in which inclusion started in July 2000 [28] and April 2001 [25]. The optimal duration of therapy is still under debate. Different studies recommend a treatment duration of 6 months [30], more recent data underline the advantage of a 12-month protocol [25,31]. Our patients were

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treated for 48 weeks and followed up for at least 6 months thereafter. Virological response was as follows: EVR 38% (10 of 26), ETR 35% (nine of 26) and SVR 19% (five of 26). Spontaneous resolution of the HCV reinfection is exceptional in transplanted patients [2]. None of the patients in the control group showed a spontaneous resolution of the HCV reinfection. The virological efficacy is comparable with the most combination treatment studies with PEG-IFN$_{\alpha\text{-}2a}$ plus ribavirin, such as the data reported by Samuel *et al.* [24], who find an ETR of 32% and an SVR of 21%; however, with a discontinuation rate of 43% due to side effects. The better results reported by Castells *et al.* [25] (EVR 63%, ETR 58%, discontinuation rate 0% and SVR 35%) after a 48-week trial of interferon α-2b and ribavirin were accompanied by worse haematological side effects like severe anaemia (13%) and severe neutropenia (25%). Similar data are reported by Dumortier *et al.* (27). Fernández *et al.* report an SVR of 21% with severe anaemia in 36% of the patients, severe neutropenia in 13% and a discontinuation rate of 21% [32]. Chalasani *et al.* report a lower SVR of only 12% in the treated group; however, the patients received only PEG-IFN$_{\alpha\text{-}2a}$ without ribavirin [33].

These variable results could be explained by the different treatment regimens and pretransplant interferon treatments. In our treated group eight of the 26 patients were pretreated with interferon; seven of these were NRs and only one belonged to the SVR group (pretreatment with 3 × 3 MioIE unpegylated interferon α-2b/week and ribavirin). Here, the SVR of 19% in our group could be explained by the fact that our treatment addresses already 'hard to treat' patients. We hoped that an escalating dose regimen, with progressive increase of PEG-IFN$_{\alpha\text{-}2a}$ and later introduction of ribavirin will lead to an increased rate of SVR due to a better tolerance to treatment. However, early dose reductions were performed in order to prevent severe and potentially fatal haematological side effects in patients post-OLT (only nine of 26 reached the target dose). It can be speculated that the prolongation of the treatment for more than 48 weeks could potentially result in improved SVR by decreasing the number of relapsers. Further studies are needed to prove this hypothesis.

Statistical analysis identified only the decrease of 2 or more log HCV-RNA at week 12 and the cumulative PEG-IFN$_{\alpha\text{-}2a}$ dose as prognostic factors associated with SVR. It is worth mentioning that in our study, all the five patients with a SVR were tested HCV-RNA-negative at week 12, whereas none of the patients without an EVR achieved an SVR. This illustrates the effectiveness of the HCV-RNA quantification at week 12 of the treatment in immunosuppressed patients post-OLT to predict SVR. The known effect of genotype with worse outcomes in patients with genotype 1 is not reproduced by our group. This is most probably due to the small number of patients.

With respect to the histological outcome, there was no progression of fibrosis in 10 of 26 (38%) patients in the treated group compared with two of 19 (11%) patients in the control group during this short observation period. Overall, the patients in the treated group had a slower advance in the Ishak score (+0.8), compared with the untreated group (+1.8), which suggests fibrosis to be a predictor of treatment efficacy and pleads for the role of therapy in the early phase of post-transplant HCV reinfection. The results are in line with a recent study of combination therapy of PEG-IFN$_{\alpha\text{-}2b}$ and ribavirin in patients with HCV reinfection with the start of the treatment at a median of 3.2 years after liver transplantation, where the yearly fibrosis progression rate during the interferon therapy was 0.2 (Scheuer and Desmet score) and therefore lower than the fibrosis progression rate prior to the peginterferon α-2b treatment (0.48) [28].

In conclusion, treatment with PEG-IFN$_{\alpha\text{-}2a}$ and ribavirin for 48 weeks, started in the early phase of recurrent hepatitis C after liver transplantation yielded an EVR of 38% and an SVR of 19%. This dosage adapted and stepwise escalating regimen was well tolerated, with a low rate of discontinuation and acute rejection. Regarding the histological outcome, the progression of fibrosis was slower in the treated patient group. This treatment regimen seems to be safe and effective. Of course, further controlled studies are needed to establish to what extent early antiviral treatment in the acute phase of recurrent hepatitis C can improve the long-term patient survival rate and prevent the development of severe histological lesions.

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