Research Article

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Safety and efficacy of protease inhibitors to treat hepatitis C after liver transplantation: A multicenter experience

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Background & Aims: Protease inhibitors (PI) with peginterferon/ribavirin have significantly improved SVR rates in HCV G1 patients. Their use to treat HCV recurrence after liver transplantation (LT) is a challenge.

Methods: This cohort study included 37 liver transplant recipients (male, 92%, age 57 ± 11 years), treated with boceprevir (n = 18) or telaprevir (n = 19). The indication for therapy was HCV recurrence (fibrosis stage ⩾F2 (n = 31, 83%) or fibrosing cholestatic hepatitis (n = 6, 16%).

Results: Eighteen patients were treatment-naive, five were relapsers and fourteen were non-responders to dual therapy after LT. Twenty-two patients received cyclosporine and fifteen tacrolimus. After 12 weeks of PI therapy, a complete virological response was obtained in 89% of patients treated with boceprevir, and 58% with telaprevir ($p = 0.06$). The end of treatment virological response rate was 72% (13/18) in the boceprevir group and 40% (4/10) in the telaprevir group ($p = 0.125$). A sustained virological response 12 weeks after treatment discontinuation was observed in 20% (1/5) and 71% (5/7) of patients in the telaprevir and boceprevir groups, respectively ($p = 0.24$). Treatment was discontinued in sixteen patients (treatment failures (n = 11), adverse events (n = 5)). Infections occurred in ten patients (27%), with three fatal outcomes (8%). The most common adverse effect was anemia (n = 34, 92%), treated with erythropoietin and/or a ribavirin dose reduction; thirteen patients (35%) received red blood cell transfusions. The cyclosporine dose was reduced by 1.8 ± 1.1-fold and 3.4 ± 1.0-fold with boceprevir and telaprevir, respectively. The tacrolimus dose was reduced by 5.2 ± 1.5-fold with boceprevir and 23.8 ± 18.2-fold with telaprevir.

Conclusions: Our results suggest that triple therapy is effective in LT recipients, particularly those experiencing a severe recurrence. The occurrence of anemia and drug-drug interactions, and the risk of infections require close monitoring.

Keywords: Boceprevir; Drug-drug interaction; Early virological response; HCV recurrence; Liver transplantation; Protease inhibitors; Sustained virological response; Telaprevir.

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Abbreviations: ALT, alanine aminotransferase; AFEF, French Association for the Study of the Liver; AUC, area under the curve; BID, twice daily (*bis in die*); BOC, boceprevir; cEVR, complete early virological response; CNI, calcineurin inhibitors; CYP, cytochrome P450; EPO, erythropoietin; EOT, end of treatment response rate; EVR, early virological response; F, female; FCH, fibrosing cholestatic hepatitis; G1, genotype 1; G-CSF, granulocyte colony stimulating factor; GGT, gamma-glutamyl transferase; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IL, interleukin; INR, International Normalized Ratio; IS, immunosuppressive drugs; kg, kilogram; LT, liver transplantation; M, male; MELD, Model for End-stage Liver Disease; MMF, mycophenolate mofetil; n.a., not available; NR, non-response; PCR, polymerase chain reaction; PegIFN, pegylated interferon; PI, protease inhibitors; QD, once a day (*quaque die*); RBV, ribavirin; RVR, rapid virological response; SVR12, sustained virological response 12 weeks after the end of therapy; TBC, trough blood concentration; TID, three times a day (*ter in die*); TVR, telaprevir; VB, virological breakthrough; VL, viral load; VR, virological response.

Introduction

Hepatitis C virus (HCV) infection is the main indication for liver transplantation (LT) in most countries [1]. All patients who

undergo LT with detectable serum HCV ribonucleic acid experience graft re-infection [2]. HCV recurrence is the most frequent cause of death and accounts for two-thirds of graft failures [3]. HCV recipients have a shorter survival than other recipients [4]. Re-transplantation may be the only option in a context of severe recurrence [5]. Until 2011, pegylated interferon (PegIFN)/ribavirin (RBV) was the standard of care. A sustained virological response (SVR) was obtained in 30% of recipients [6–8]. In a randomized, double-blind multicenter study, 37.5% of genotype 1 (G1) patients treated with PegIFNα2a/RBV achieved SVR [9]. Obtaining SVR after LT guarantees a major survival benefit [10]. Using more efficient therapy in patients with a severe recurrence is a necessity. The addition of the protease inhibitors (PI) such as boceprevir (BOC) or telaprevir (TVR) enhances the efficacy of PegIFN/RBV therapy in G1 patients. Phase III trials have shown that PegIFN/RBV plus BOC or TVR increased SVR rates in naive and previously treated G1 non-transplant patients [11–14]. Using such drugs in a context of HCV recurrence is a challenge in the LT field. One limitation is the potential for interactions with calcineurin inhibitors (CNI) [15]. It was demonstrated in healthy volunteers that BOC increased the area under the curve (AUC) of cyclosporine and tacrolimus by 2.7 and 17-fold, respectively [16]. TVR in healthy volunteers increased cyclosporine and tacrolimus exposure by 4.6 and 70-fold, respectively [17]. We and others recently demonstrated that BOC and TVR could be administered safely in liver transplant recipients [18,19]. BOC induced a reduction in the estimated oral clearance of cyclosporine of 50% (n = 3), of tacrolimus of up to 80% (n = 2) and of everolimus of 50% (n = 1) [18]. When using TVR, the doses of cyclosporine, sirolimus and tacrolimus were reduced by 2.5, 7, and 22-fold, respectively [19].

During this multicenter cohort study, we assessed the efficacy and safety of PI and PegIFN/RBV in patients with chronic HCV G1 infection who presented with an HCV recurrence after LT.

Patients and methods

Study design

This cohort study was approved by the AFEF Transplantation Prospective Group in October 2011 and all the patients selected gave their written informed consent. Five French transplant centers (Centre Hépato-Biliaire in Villejuif, centers in Lyon, Grenoble, Marseille and Montpellier) agreed to participate. Data were collected up to and including 18 September, 2012.

Patients studied

We studied transplanted patients who experienced a G1 HCV recurrence and were treated with PegIFN/RBV with BOC or TVR between March 2011 and May 2012. The indications for antiviral therapy were individualized, based on biopsy-proven chronic hepatitis defined using the METAVIR score [20]. All the patients included had fibrosis stage ⩾F2 or suffered from fibrosing cholestatic hepatitis (FCH), which were always histologically proven and defined according to the following criteria: i.e., the presence of extensive, dense portal fibrosis with immature fibrous bands extending into the sinusoidal spaces, ductular proliferation, cholestasis and moderate mononuclear inflammation [20]. The criteria for exclusion were HIV co-infection and the presence of biopsy-proven acute rejection.

Antiviral therapy regimen

All patients received PegIFN/RBV. The dose and choice of PegIFNα were decided by the senior referent, PegIFNα2a (Pegasys®; Roche) or PegIFNα2b (Viraferon-peg®; Schering-Plough). The RBV dose was adjusted to renal function parameters (Copegus®; Roche, Rebetol®; Schering-Plough) and could be escalated to maximally tolerated levels or reduced, depending on the degree of cytopenia and overall tolerance. The choice of PI was left to the discretion of the investigator, with BOC, drug-drug interactions may be weaker but the triple regimen is longer, while with TVR, interactions are stronger but the triple regimen is shorter. BOC (800 mg *tid*) was initiated after a 4-week (W) lead-in phase with PegIFN/RBV. TVR (750 mg *tid)* was introduced at the same time or after a 4-W lead-in phase. The use of lead-in phase with TVR was requested by physicians who wanted to assess hematological and renal tolerance before the introduction of TVR. The intended duration of therapy was 48 weeks. The stopping rule applied was failure to achieve a reduction in HCV viral load (VL) to less than 100 IU/ml at W12 in the BOC group, and to less than 1000 IU/ml in the TVR group; in the event of such a lack of response, all treatment was discontinued.

For assessment of efficacy, viral load was monitored in plasma using the Abbott Real Time HCV assay (Abbott Molecular, USA; lower limit of detection, 12 IU/ml), at baseline and then at weeks 4, 8, 12, 24, and 48. Genotypes were determined using phylogenetic analyses of the NS5B region [21]. A rapid virological response (RVR) was defined as an undetectable VL at W4 of triple therapy. At W12, a complete EVR (cEVR) was defined as undetectable. An end of treatment therapy response (EOT) was obtained when the VL remained negative at the time of treatment discontinuation. A sustained virological response 12 was defined as a negative VL 12 weeks after the end of treatment. All the virological responses mentioned here were based on intention-to-treat results. Viral breakthrough (VB) was defined as achieving an undetectable VL but the subsequent occurrence of a detectable VL higher than 2-$\log_{10}$ IU/ml, or by a 1-$\log_{10}$ IU/ml increase of VL over time. In the event of VB or a non-response to triple therapy, the whole NS3 region was analyzed by sequencing, and PI resistance mutations were recorded [22].

Data were collected concerning the testing of recipient DNA for interleukin (IL) 28B polymorphism rs12979860 C/T using the ABI TaqMan allelic discrimination kit and the ABI7900HT Sequence Detection System (Applied Biosystems, Carlsbad, CA).

Safety assessments

Patients were hospitalized the day before PI initiation to enable strict clinical monitoring and daily controls of CNI trough blood concentrations (TBC). Data concerning clinical and biological parameters were collected during the lead-in phase and the first three months of triple therapy. Creatinine clearance was estimated using the Cockcroft-Gault formula. The dosing regimens of immunosuppressive drugs (IS) were adjusted to reach a therapeutic range that differed depending on the time elapsing since LT. TBC ranged from 50 to 150 ng/ml for cyclosporine, from 5 to 10 ng/ml for tacrolimus and from 3 to 8 ng/ml for everolimus. Blood samples were drawn before the intake of IS to measure TBC at a steady-state after LT, at the end of the lead-in phase (W0), on the day of PI initiation and every day thereafter until a steady-state was obtained. During triple therapy, TBC were closely monitored. At the end of PI therapy, TBC were monitored daily. CNI doses were adjusted to reach the target range. Whole blood concentrations were assayed using a chemiluminescent microparticulate immunoassay (CMIA) on an architect autoanalyzer for CNI, and LCMSMS (liquid chromatography coupled to tandem mass spectrometry) for everolimus. The laboratory was a participant in an international external quality control scheme (Analytical Services International Ltd, London).

Erythropoietin (EPO) (Neorecormon®; Roche) was administered to support the red blood cell count when hemoglobin levels dropped below 10 g/dl, or decreased by >1 g/dl/week, or when a transfusion had been required during prior antiviral therapy. RBV dose reduction could be required, depending on physician practices. Granulocyte colony stimulating factor (G-CSF) (Neupogen®, Amgen Europe BV) was administered to support the neutrophil count when it fell below 0.75 g/L despite PegIFN dose reduction. The investigator managed any adverse events according to AFEF guidelines [23].

Statistical analysis

Continuous variables were expressed as medians, means and ranges. Categorical variables were expressed as proportions. The Mann-Whitney test was used to compare continuous variables. The χ^2 and Fisher's exact tests were used to compare categorical characteristics. A p-value of <0.05 was considered to be significant. All analyses were performed using SPSS 16.0 statistical software (SPSS Inc., Chicago, IL).

Results

Characteristics of the study population

Thirty-seven patients were treated between March 2011 and May 2012 with triple therapy (BOC, n = 18; TVR, n = 19). All patients who received BOC and 8 (42%) patients in the TVR group completed a lead-in phase. The characteristics of the patients are shown in Table 1. The inclusion number of patients per center was as follows, 14 Villejuif, 3 Marseille, 1 Montpellier patients in the BOC group and 9 Villejuif, 7 Lyon, 3 Grenoble patients in the TVR group. The mean delay between LT and triple therapy was 49.8 ± 25.1 months [0.6–104]. Nineteen patients (51%) had previously been treated with PegIFN/RBV. Eighteen patients (49%) received PegIFNα2a and 19 (51%) received PegIFNα2b. The mean RBV dose was 11.3 ± 3.6 μg/kg/day [3–19]. Twenty-two patients (59%) received cyclosporine and 15 (41%) tacrolimus. In nine patients (24%), MMF was given at a dose ranging from 0.5 to 2 g/day. Twelve patients (32%) received prednisone at a dose ranging from 3 to 12.5 mg/day. At the time of writing the present manuscript, 28 patients (76%) had completed their treatment. Sixteen patients (43%) discontinued therapy because of a treatment failure or intolerance. Fig. 1 summarizes the outcomes of the patients enrolled in this study.

Biochemical, histological and virological parameters at baseline

Thirteen patients (35%) had elevated bilirubin levels prior to treatment (mean 88.2 ± 124.3 μmol/L [17–333]). Mean creatinine clearance was 73.9 ± 22.6 ml/min [39.2–135.4]. There were no differences in terms of blood count, liver and kidney functions between the BOC and TVR groups, except for higher ALT levels in the BOC group ($p = 0.08$). All patients underwent a liver biopsy prior to therapy. The fibrosis stages were F2 (38%), F3 (46%) and F4 (16%). Concerning cirrhotic patients, the mean MELD score was 11.6 ± 4.5 [6–19]. As for the Child Pugh score, four patients were classified as A and two were classified as B (7 and 8). 6 patients were suffering from FCH. Nine and twenty-six patients were infected with G1a and G1b, respectively (undetermined in two patients). Five of the sixteen patients tested (28%) displayed *IL28B* CC polymorphism in the BOC group, and one of the fifteen tested patients (5%) in the TVR group ($p = 0.05$). At baseline (W-4 or W0, depending on the antiviral therapy regimen), the mean VL was 6.5 ± 1.3 $\log_{10}$ IU/ml [3.1–8.3].

Efficacy

Biochemical, virological responses and predictors of response

All patients with abnormal bilirubin findings normalized their levels at W12. ALT levels remained abnormal in three patients at W12, more than 2-fold lower than the normal level.

During the lead-in phase, twenty-three patients (62%) experienced a >1 log decrease in VL (BOC, 61%; TVR, 63%; p = n.s.). At W4 of triple therapy, RVR was obtained in nineteen patients (51%) (BOC, 56%; TVR, 47%) (Fig. 2). A complete EVR was achieved in twenty-seven patients (73%), (BOC = 89%; TVR = 58%). The EOT was 72% among eighteen patients in the BOC group and 40% among ten patients in the TVR group. An SVR12 was obtained in one of the five eligible patients (20%) in the TVR group and five of the seven eligible patients (71%) in the BOC group.

Six patients (16%) experienced a VB (median delay, 35 weeks [16–44]). Five patients were non-responders (BOC = 1; TVR = 4, Table 2). One patient experienced a relapse in the BOC group, 8 weeks after discontinuation of antiviral therapy. The median duration of follow-up was 52 weeks [21–95]. Among patients with cirrhosis (n = 6), RVR was observed in 67%, cEVR in 83% of patients and EOT could be achieved for three out of five patients (60%). Among FCH patients (n = 6), RVR was achieved in 0%, cEVR in 33% and EOT in 33% of patients. Genotypes, *IL28B* polymorphism, fibrosis stages, baseline VL, PegIFN types, donor characteristics and CNI types did not have any impact on VR.

Virological resistance mutations

Complete NS3 sequence information was obtained for seven patients who experienced a treatment failure, a VB or a non-response. At least one mutation related to PI resistance was detected (Table 2).

Safety

Tolerability and adverse events

The median duration of treatment was 41 ± 16 weeks [2–48]. 10 patients (27%) developed an infection during antiviral therapy, leading to its discontinuation in 5 patients (Table 3). Three patients died in a context of sepsis. The first patient, receiving TVR and treated in a context of FCH 3 months after combined liver/kidney transplantation, died at W2 of septic shock resulting from a urinary tract infection. The second and third patient, in the BOC group, were 60 and 74-year old men treated for the presence of cirrhosis on the liver graft.

The most frequent adverse event was anemia, which affected 100% of patients in the BOC group and 84% in the TVR group (Fig. 3). 34 patients (92%) required EPO, (the mean interval prior to its introduction being 24.0 ± 16.7 days [0–46]); in 56% of patients, EPO was administered even though the hemoglobin level was up to 10 g/dl. Thirteen patients (35%) required red blood cell transfusions (BOC, n = 6; TVR, n = 7) with a median time to introduction of 6 weeks [2–24]). The median number of red blood cell units was 2 [2–6]. RBV reduction was required in twenty-six patients (70%) (median reduction percentage, 40% of the initial dosage).

Nine patients required a reduction in the PegIFN dose for neutropenia (n = 9) and/or thrombocytopenia (n = 2). Three patients (8%) received G-CSF for neutropenia, after two weeks of therapy. No platelet growth factors were used. There were no discontinuations or dose reductions of TVR or BOC.

Two patients required hospitalization for an acute flare-up of chronic kidney disease, and recovered after rehydration. A nadir of the glomerular filtration rate was observed at W24 (Fig. 4). Two patients developed *de novo* diabetes mellitus during BOC therapy. No serious dermatological adverse events (Grade 3/4) occurred.

One patient experienced a minimal acute rejection (Banff <3), assessed after a graft biopsy was performed in a context of elevated ALT and GGT. The outcome was satisfactory after a higher trough blood concentration of cyclosporine was targeted.

Table 1. Characteristics of patients at baseline, antiviral and immunosuppressive therapies.

	Boceprevir (n = 18)	Telaprevir (n = 19)	*p* value
Age (yr), mean ± SD	57.1 ± 12.1	57.5 ± 9.5	
Sex (M), No. (%)	16 (89)	17 (89)	
Body mass index (kg/m^2), mean ± SD	24.1 ± 3.6	24.7 ± 4.4	
LT indication, No. (%)			
Cirrhosis	9 (50)	5 (26)	
HCC	7 (39)	11 (58)	
HCV ReLT	2 (11)	3 (16)	
MELD score at listing, mean ± SD	16.5 ± 9.8	18.6 ± 9.8	
HBV co-infection, No. (%)	0 (0)	1 (5)	
Combined liver/kidney transplant, No. (%)	1 (6)	1 (5)	
CNI, No. (%)			
Cyclosporine	12 (67)	10 (53)	
Tacrolimus	6 (33)	9 (47)	
Others IS, No. (%)			
Corticosteroids	8 (44)	4 (21)	
MMF	4 (22)	5 (26)	
Everolimus	1 (6)	0 (0)	
Previous course of dual therapy pre-LT, No. (%)			
Naive of therapy	9 (50)	4 (21)	
Non-responders	7 (39)	13 (74)	
Relapsers	2 (11)	1 (5)	
Previous course of dual therapy post-LT, No. (%)			0.02
Naive of therapy	8 (44)	10 (53)	
Non-responders	5 (28)	9 (47)	
Relapsers	5 (28)	0	
Interval between LT/antiviral therapy (mo), mean ± SD	77.6 ± 90.4	34.6 ± 34.0	
HCV genotype, No. (%)			
1a	5 (28)	4 (21)	
1b	13 (72)	15 (79)	
Recipient *IL28B* genotype, No. (%)			0.05
CC	5 (28)	1 (5)	
CT	5 (28)	9 (47)	
TT	6 (33)	3 (16)	
Fibrosis stage ≥F3*, No. (%)	9 (50)	8 (42)	
Fibrosis stage =F4*, No. (%)	5 (28)	1 (5)	
Fibrosing cholestatic hepatitis, No. (%)	2 (12)	4 (21)	
Total bilirubin (mmol/L), mean ± SD	49.8 ± 89.8	39.2 ± 78.9	
ALT (IU/L), mean ± SD	170.1 ± 207.2	97.1 ± 55.0	0.08
INR, mean ± SD	1.01 ± 0.11	1.01 ± 0.11	
Creatinine clearance (ml/min), mean ± SD	75.0 ± 22.5	73.9 ± 22.6	
Hemoglobin (g/dl), mean ± SD	13.0 ± 1.7	13.3 ± 1.7	
Neutrophil count (G/L), mean ± SD	3.5 ± 1.9	2.9 ± 1.8	
Platelet count (G/L), mean ± SD	154.4 ± 74.2	136.3 ± 53.2	
HCV viral load (log_{10} IU/ml), mean ± SD	7.0 ± 0.7	6.5 ± 1.3	
PegIFN, No. (%)			0.03
α2a	3 (17)	15 (79)	
α2b	15 (83)	4 (21)	
Ribavirin dosage (mg/kg/d), mean ± SD	11.3 ± 4.0	11.4 ± 4.0	

*Only significant *p* values are shown.
CNI, calcineurin inhibitors; HBV, hepatitis virus B; HCC, hepatocellular carcinoma; HCV, hepatitis virus C; LT, liver transplantation; M, male; MMF, mycophenolate mofetil; PegIFN, pegylated interferon.

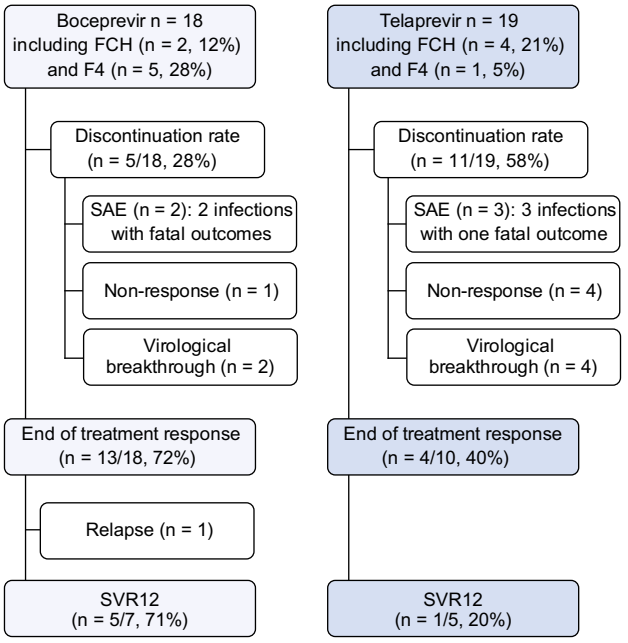

Fig. 1. The 37 liver transplant patients treated with boceprevir or telaprevir plus PegIFN/RBV. The median duration of therapy was 41 weeks [2–48]. Treatment response rates and treatment failures are represented as a function of antiviral regimen.

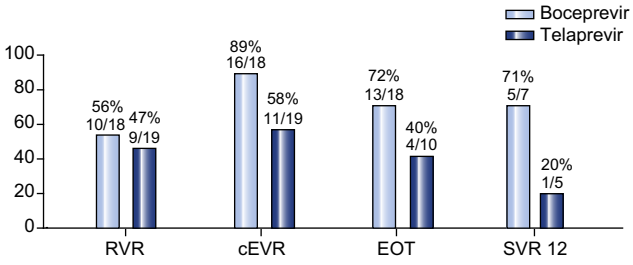

Fig. 2. Virological responses during triple therapy after liver transplantation. Virological responses at weeks 4, 12, 48 of triple therapy. An RVR (rapid virological response) means an undetectable HCV RNA level at week 4. A complete early virological response (cEVR) was observed when the HCV viral load was undetectable at week 12. An EOT (end of treatment response) was achieved when HCV RNA was undetectable at week 48. A sustained virological response 12 (SVR 12) was defined as undetectable HCV RNA 12 weeks after the discontinuation of antiviral therapy.

Management of immunosuppressive drugs with protease inhibitors

All patients achieved a steady-state of IS before the initiation of PI. In practice, the CNI dose was reduced on the day of PI introduction in line with the data available on healthy volunteer subjects and liver recipients. During the first week, the CNI dosing regimen had to be reduced in thirty-four patients (92%). In the BOC group, cyclosporine dose reductions were 1.8 ± 1.1 [1.0–5.0]-fold (or 36% of the initial dose), while they were 5.2 ± 1.5 [2.9–7.1]-fold (or 78% of the initial dose) with tacrolimus. In the TVR group, the dose reductions were 3.4 ± 1.0 [1.3–5] (or a 46% reduction) and 23.8 ± 18.2 [1.4–57.1]-fold (or a 95% reduction) with cyclosporine and tacrolimus, respectively. The steady-state of CNI was obtained in 5.4 ± 2.3 days with cyclosporine and in 6.5 ± 4.2 days with tacrolimus. The dose remained unchanged after a steady-state, although a rise in IS concentrations was observed, still within the target range. At the time of PI discontinuation, the CNI dose had to be increased in all patients. The CNI dose was superior to the baseline dose in ten patients (27%). The increases in cyclosporine and tacrolimus doses were 32% and 80% in the BOC group, respectively. In the TVR group, it was necessary to increase the doses of cyclosporine and tacrolimus by 47% and 95%, respectively. One patient received everolimus combined with cyclosporine in the BOC group. The everolimus dosage was reduced by half (1–0.5 mg/day).

Discussion

This study is the first to have provided such informative data on thirty-seven liver transplant patients infected with G1, treated with PegIFN/RBV plus BOC or TVR. Our analysis shows that the use of triple therapy achieved an EOT in 72% and 40% of patients treated with BOC and TVR, respectively. Although the data presented here constitute an interim analysis, the efficacy results seem encouraging in light of the comparison of EOT in 55.5% of G1 patients treated with standard PegIFN/RBV therapy (Roche *et al.* personal communication) according to a retrospective study, which included pan-genotypic patients [24]. Most of the patients enrolled in that cohort were of the particularly "difficult-to-treat" type. We initially treated patients with a poor short-term prognosis in the context of an early access program. Consequently, in more than half of these patients, triple therapy was a post-LT re-treatment that included 38% of non-responders to a prior course of PegIFN/RBV therapy after liver transplantation. In the present study, 46% of patients had an advanced fibrosis score of ⩾F3 (BOC, 50%; TVR, 42%, p = n.s.), 16% had histologically-proven cirrhosis and 16% had FCH.

In liver transplant patients, most studies have demonstrated that EVR is the principal predictive factor associated with SVR [24–26]. In the present study, a cEVR at week 12 was obtained in 89% and 58% of patients, with BOC and TVR, respectively. Even if the determination of predictive factors was not possible in this context because of the small number of enrolled patients, the cEVR in the BOC group seems very encouraging. Despite a lack of significant difference, the lower EVR obtained in the TVR group could be explained by: (1) the proportion of non-responders to dual therapy, (2) severe liver disease on the graft such as FCH, (3) the proportion of patients with the favorable CC *IL28B* genotype differing between the two groups. HCV resistance mutations were identified in most patients who experienced a treatment failure. A clearer understanding of these parameters is required, but the higher rate of treatment failures in the TVR group could suggest an inappropriate PI scheme in terms of dosage or duration. In the near future, determinations of PI TBC should be considered in order to better explore this issue.

One major benefit of PI therapy was its success in treating patients with FCH. This very severe complication affects between 5% and 8% of transplant recipients infected by HBV or HCV, and 20% of HIV/HCV co-infected patients [27]. Our findings in this population were remarkable, as an EOT was achieved in 33% of FCH patients.

The most common adverse event was anemia, thus confirming that transplant recipients are particularly susceptible to RBV-induced toxicity. According to AFEF guidelines, most patients received EPO during our study prior to RBV dose reduc-

Table 2. Characteristics of patients who experienced a treatment failure.

	VB 1	VB 2	VB 3	VB 4	VB 5	VB 6	NR 1	NR 2	NR 3	NR 4	NR 5
Age (yr), Sex	34, M	60, M	50, M	51, M	74, M	59, F	53, M	49, M	62, M	49, M	53, M
Time to occurrence (wk)	24	8	16	44	13	44	12	12	12	12	12
CNI	Cyclosporine	Tacrolimus	Cyclosporine	Cyclosporine	Tacrolimus	Tacrolimus	Tacrolimus	Tacrolimus	Cyclosporine	Cyclosporine	Tacrolimus
Others IS	Prednisone (5mg QD)	0	MMF (250 mg BID)	MMF (500 mg BID) + prednisone (10 mg QD)	0	0	Prednisone (10 mg QD)	0	Prednisone (5 mg QD)	Prednisone (5 mg QD)	0
Genotype	1b	1a	1a	1a	1b	1b	1a	1b	1a	1b	1b
Recipient *IL28B* genotype	n.a.	CT	CT	CT	n.a.	n.a.	CC	CT	CT	TT	CT
Activity (A)/ fibrosis stage (F) (METAVIR score) or FCH	A2/F2	A3/F3	A1/F2	FCH	A1/F3	A2/F2	A1/F4	A2/F2	A1/F2	FCH	A3/F3
Dual therapy pre-LT	Non-responder	Non-responder	Naive	Non-responder	Non-responder	Non-responder	Naive	Non-responder	Non-responder	Non-responder	Non-responder
Dual therapy post-LT	Naive	Naive	Non-responder	Relapser	Non-responder	Non-responder	Non-responder	Naive	Naive	Naive	Naive
Baseline HCV VL (log_{10} IU/ml)	8.27	6.66	6.20	8.49	7.8	5.45	7.35	7.6	7.17	7.93	6.1
PI	Boceprevir	Telaprevir	Telaprevir	Boceprevir	Telaprevir	Telaprevir	Boceprevir	Telaprevir	Telaprevir	Telaprevir	Telaprevir
Baseline PegIFN dosage	α-2a 135 mg/wk	α-2a 180 mg/wk	α-2a 180 mg/wk	α-2b 1.7 μg/kg/wk	α-2b 0.5 μg/kg/wk	α-2b 1.0 μg/kg/wk	α-2b 1.3 μg/kg/wk	α-2b 1.0 μg/kg/wk	α-2a 180 μg/wk	α-2a 180 μg/wk	α-2a 180 μg/wk
Baseline ribavirin dosage	9 mg/kg/QD	9 mg/kg/QD	19 mg/kg/QD	17 mg/kg/QD	3 mg/kg/QD	5 mg/kg/QD	10 mg/kg/QD	11 mg/kg/QD	14 mg/kg/QD	13 mg/kg/QD	11 mg/kg/QD
Lead-in phase	Yes	Yes	0	Yes	Yes	Yes	Yes	Yes	0	0	0
Complete VR	W8	W8	W4	W12	W8	W4	0	0	0	0	0
Ribavirin reduction (%)	100 (stop at W12)	0	83	33	0	50	30	0	66	0	0
Resistance mutations	T54A	V36M R155K	R155K	n.a.	n.a.	n.a.	n.a.	V36M R155K	T54A I170V	V36M R155K	T54S A156V I170V

CNI, calcineurin inhibitors; F, female; IS, immunosuppressive drugs; M, male; MMF, mycophenoloate mofetil; NR, non-response; VB, virological breakthrough; n.a., not available.

Table 3. Adverse events during triple therapy after liver transplantation.

	Boceprevir (n = 18)	Telaprevir (n = 19)
Death, No. (%)*	2 (11)	1 (5)
Infections, No. (%)ˣ	5 (27)	5 (26)
Hematological toxicity, No. (%)		
Anemia		
<10 g/dl	18 (100)	16 (84)
<8 g/dl	7 (39)	5 (26)
Neutropenia (<1 g/L)	11 (61)	4 (21)
Thrombocytopenia (<50 g/L)	9 (50)	3 (15)
Dermatological toxicity, No. (%)^	1 (5)	1 (5)
Renal failure, No. (%)	1 (5)	4 (21)
Diabetes mellitus, No. (%)	2 (10)	0
Rehospitalization rate	6 (33)	6 (32)

*Context of septic shock.
ˣCommunity-acquired pneumonia (n = 2, W3 and W4), cytomegalovirus infection (n = 1, W2), pneumocystosis and aspergillosis (n = 1, W20), urinary tract infection (n = 4, W2, W4, W24, and W25), erysipelas (n = 1, W16), peritonitis (n = 1, W20).
^Anal itching (Grade 1).

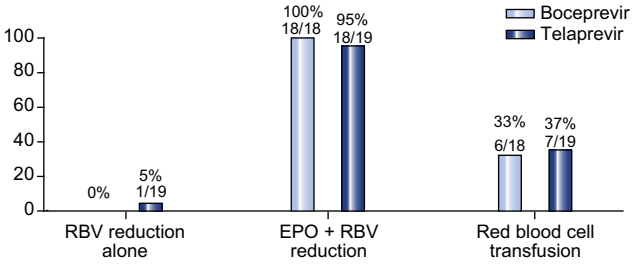

Fig. 3. Management of anemia during triple therapy after liver transplantation. Anemia is the most frequent adverse event during triple therapy in liver transplant patients. The percentages presented included all treatment periods, even after PI discontinuation. EPO, erythropoietin; RBV, ribavirin.

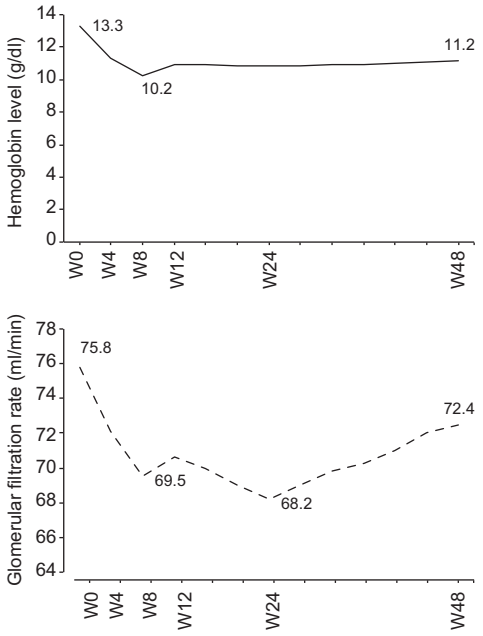

Fig. 4. Mean hemoglobin levels and glomerular filtration rates (GFR) during antiviral therapy. The GFR was estimated according to Cockcroft-Gault formula. The nadir of GFR was observed at W24. The mean GFR difference between baseline and W48 was −3.8 ml/min. The nadir of hemoglobin was achieved at W8 and remained stable thereafter.

tion in this difficult-to-treat population [23]. Despite this, more than a third of patients required red blood cell transfusions. This observation confirms the findings in non-transplanted patients treated with PI, whose anemia worsened [28]. The underlying mechanism for anemia was not due to hemolysis alone but was thought to result from a bone-marrow suppressive effect. In non-transplant patients, triple therapy has been associated with a 20% increase in the incidence and severity of anemia when compared to PegIFN/RBV alone. The frequency of anemia was 50% under triple therapy with BOC and 40% with TVR [11–14]. Recent communications have suggested that anemia does not impact the SVR rates achieved with PI [29]; during our study, anemia did not seem to impact the EVR either, and did not require a discontinuation of therapy.

The most severe adverse event was infection, which reached a rate of 27% in our study. Three patients died in a context of septic shock; in both cases the underlying liver disease was FCH or cirrhosis. The prevalence of infection remains unknown during triple therapy in "real-life". Infectious diseases may be an important issue in difficult-to-treat patients, when considering the recent data. In cirrhotic non-responding patients, severe infections (Grade 3/4) have been reported in 2.4% and 6.5% of patients during BOC and TVR therapy, respectively [30]. One patient treated with TVR after LT experienced bacterial pneumonia in the series reported by Werner *et al.* [19]. These severe complications were not predicted in the absence of neutropenia; the presence of severe underlying liver disease might contraindicate the introduction of PI or justify the initiation of preventive antibiotic therapy. This finding and the satisfactory results of studies using dual therapy administered at a moderate fibrosis stage on the liver graft argue in favor of an early introduction of triple therapy.

Interactions with IS were a major concern before the patients were enrolled in the cohort. PI are also potent inhibitors of the CYP3A4 enzyme [31]. Numerous drug-drug interactions have been described when using CNI [32]. Although data in healthy volunteers assessed the potential CNI-PI interactions, the application of such a regimen in a transplant population might produce different results [16,17]. We previously reported on the practical management of BOC initiation after LT in five patients [18]. Estimated oral CNI clearance rates fell by 50% with cyclosporine and >80% with tacrolimus, requiring constant reductions in CNI dose. Werner recently published an interim analysis of the use of TVR after LT, four patients received cyclosporine which had to be reduced 2.5-fold, four received tacrolimus and the reduction was 22-fold, and one received sirolimus with a reduction of 7-fold [19]. Based on these experiences, the patients were monitored daily regarding their TBC of CNI. A reduction in the CNI dose was also required during the present study. With BOC, the average reductions were about 2-fold and 5-fold with cyclosporine and tacrolimus, respectively, while with TVR, the interactions were more potent and the mean reductions were around 3-fold and 23-fold with cyclosporine and tacrolimus, respectively. Our

results cannot replace a targeted pharmacological study. They need to be confirmed because inter-patient variations in the potency of drug-drug interactions are a well-known phenomenon and we only treated a small number of patients. Nevertheless, our study confirmed that CNI-PI interactions impacted the monitoring of patients, but could be managed. We recommend reducing the CNI dose the day of PI initiation and then checking the TBC each day until a steady state is reached (around 5 days). At the time of PI discontinuation, the CNI dose at the steady-state was superior to the baseline dose in 27% of patients. This may have resulted from an improvement in liver function during therapy. This finding also emphasizes a need for the close monitoring of TBC following PI discontinuation. We also recommend particular caution at the time of PI discontinuation, increasing the CNI dose the next day and then checking the TBC at least every 48 h until a steady state has been achieved once more.

This cohort study did have some limitations. Firstly, it was not a randomized study, which did not allow an analysis of predictive factors in terms of efficacy or safety. The second drawback was the heterogeneity of immunosuppressive and antiviral regimens, which depended on the practices of different physicians.

In conclusion, 72% and 40% of the liver transplant recipients in our cohort achieved EOT after 48 weeks of BOC and TVR therapy, respectively. Although anemia was the most common adverse event, and interactions between PI and CNI were constant, the introduction of PI could be managed easily if close monitoring was ensured. More importantly, these very encouraging results in terms of feasibility represent an important step towards development in the near future of new protocols with novel antiviral drugs appropriate to the context of LT.

Conflict of interest

A. Coilly, Speaking and teaching, Astellas, Novartis, Janssen, Merck, Gilead, BMS; B. Roche, Grant/Research support, Roche, BMS; J. Dumortier, Board Membership, Novartis, Astellas, Roche; Consulting, Novartis; Grant/Research support, Novartis, Astellas, Roche, MSD, GSK; V. Leroy, Board Membership, Roche, Merck, Gilead, BMS. Consulting, Janssen; Grant/Research support, Roche, Gilead, BMS. Speaking and teaching, BMS, Merck, Gilead, Roche; G.P. Pageaux, Advisory committees/Review panels, Roche. Board Membership, Astellas; S.N. Si-Ahmed, Grant/Research support, BMS, Gilead, Schering Plough, Roche; D. Samuel, Consulting, Astellas, MSD, BMS, Roche, Novartis, Gilead, LFB, Janssen-Cilag; J.C. Duclos-Vallée, Speaking and teaching, Astellas, Novartis, Janssen, Merck, BMS, Gilead.

Authors' contributions

A. Coilly, study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; statistical analysis; study supervision. B. Roche, acquisition of data; critical revision of the manuscript for important intellectual content. J. Dumortier, acquisition of data; analysis and interpretation of data; critical revision of the manuscript for important intellectual content. V. Leroy, acquisition of data; analysis and interpretation of data; critical revision of the manuscript for important intellectual content; study supervision. D. Botta-Fridlund, acquisition of data. S. Radenne, acquisition of data. G.P. Pageaux, acquisition of data; analysis and interpretation of data; critical revision of the manuscript for important intellectual content. S.N. Si-Ahmed, acquisition of data; analysis and interpretation of data. O. Guillaud, analysis and interpretation of data. T.M. Antonini, analysis and interpretation of data. S. Haïm-Boukobza, acquisition of data; technical, or material support. A.M. Roque-Afonso, acquisition of data; technical, or material support. D. Samuel, critical revision of the manuscript for important intellectual content. J.C. Duclos-Vallée, study concept and design; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; study supervision.

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