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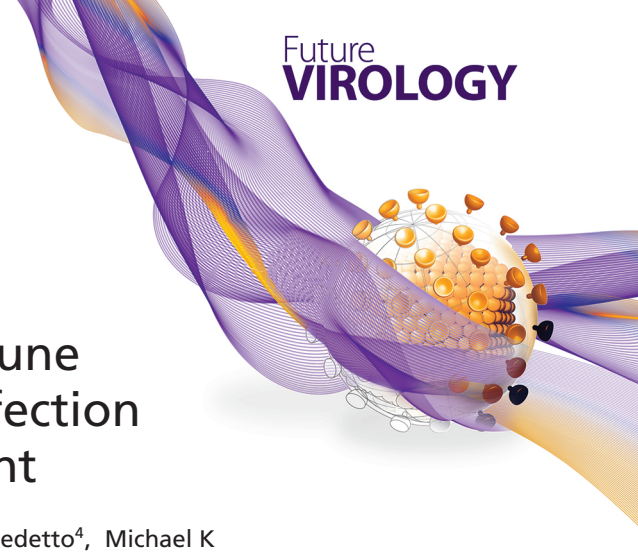

Efficacy and safety of Niuliva® immune globulin to prevent hepatitis B reinfection in *de novo* orthotopic liver transplant

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Aims: To determine efficacy and safety of intravenous hepatitis B immune globulin (Niuliva®, Grifols) to prevent reinfection in *de novo* orthotopic liver transplantation. **Patients & methods:** In a nonrandomized, noncontrolled and Phase III clinical trial, 15 adult patients (12 men) were treated with Niuliva from the anhepatic phase (10,000 IU/daily 1 week postsurgery) up to 6 or 12 months (5000 IU/weekly 1 month; 5000 IU/monthly thereafter). **Results:** No patients showed reinfection throughout the study. Niuliva was effective in maintaining antibody titers above the thresholds recommended by the European Medicines Agency (EMA) to prevent reinfection (100–150 IU/l). Four serious adverse events were reported in three patients (none related to the study product). There were no seroconversions and no deaths. **Conclusion:** Long-term, high-dose Niuliva administration was safe and effective to prevent graft reinfection in the tested patients.

First draft submitted: 9 August 2018; Accepted for publication: 29 November 2018; Published online: 31 January 2019

Keywords: anhepatic phase • HBIG • hepatitis B virus • intravenous • Niuliva® • orthotopic liver transplantation • recurrence • reinfection

The hepatitis B virus (HBV) infection is a major public health problem and a cause of infectious disease worldwide. Approximately, a third of the world's population presents serologic evidence of past or present HBV infection and approximately 240–400 million are chronically infected. Also, over 1 million people die annually from HBV-related complications (including cirrhosis, end-stage liver disease and hepatocellular carcinoma [HCC]) [1]. Patients suffering these conditions account for about 5–10% of liver transplantations (LT) among adults in the USA and Europe [2,3].

The use of HBV immune globulin (HBIG) after LT was the first major milestone in the prevention of post-LT HBV recurrence, producing a dramatic reduction in the risk of reinfection from 75 to 36% and improving overall patient survival [4].

Early European experience in the late 1980s and early 1990s suggested that maintenance of trough serum antihepatitis B surface antigen (HBs) titers above 100 IU/l is effective in preventing HBV recurrence after LT [5,6]. Indeed, long-term and high-dose HBIG prophylaxis has proven to be a highly efficient way of preventing HBV reinfection [7]. However, controversy still exists on the optimal dosing regimen to prevent HBV recurrence and the ideal anti-HBs titer to prevent post-LT HBV recurrence [8,9].

While HBIG was a major advance, post-LT prophylaxis failure may result from HBIG escape mutants which escape the limited diversity of anti-HBs contained in HBIGs [10]. The advent of potent antiviral therapy with nucleos(t)ide analogs (NAs) in combination with HBIG greatly enhanced post-LT prophylaxis. Evidence from meta-analysis indicated that combination prophylaxis was significantly superior to NAs or HBIG alone in preventing

HBV recurrence [11] likely by virtue of complementary modalities of suppression both against replication and viral entry of the hepatocyte target [4,12,13].

The LT in patients with HBV infection is particularly problematic since the causative agent is not completely eradicated [14]. The risk of reinfection may vary substantially depending on the patient's pre-LT HBV replication status, presence of fulminant HBV, hepatitis D virus coinfection, presence of HCC at transplant and HCC recurrence [4,15–20]. Early recurrence of HBV is more frequent in patients with a high level of pre-LT HBV replication, whereas late recurrences are usually caused by the emergence of mutations involving the a-determinant of the HBs gene [10].

Niuliva® (Instituto Grifols S.A., Barcelona, Spain) is a 5% liquid HBIG solution for intravenous administration that has been registered in Spain and Italy for the prevention of HBV reinfection after LT for HBV-induced liver failure, during the maintenance phase in nonreplicating patients. Niuliva has been reported to be well tolerated and safe for this indication [21]. However, Niuliva has not been clinically evaluated throughout the perioperative period.

The aim of this clinical trial was to evaluate the efficacy and safety of Niuliva in the prophylaxis of HBV recurrence after LT in *de novo* orthotopic LT recipients by reaching and maintaining target anti-HBs titer levels during the first 6 and 12 months post-LT.

Patients & methods

Study design & objective

This study was an open, multicenter, nonrandomized and noncontrolled Phase III clinical trial performed in four hospitals in Italy. The main aim of the trial was to determine the efficacy and safety of Niuliva in preventing HBV-recurrence in *de novo* LT recipients transplanted for HBV-related liver disease by reaching and maintaining target anti-HBs titer levels during the first 6 and 12 months after transplantation.

In the overall design, *de novo* LT recipients were enrolled within 3 months before transplantation and treated with Niuliva from the anhepatic phase up to 6 or 12 months (patients completing the 6 months treatment were offered to participate in an optional 6-month extension).

All participants provided written informed consent. The trial was designed and monitored in compliance with the ethical principles of the World Medical Assembly (Helsinki, 1964) and subsequent revisions. National and local independent ethics committee approved both the protocol and the associated informed consent and information sheets.

Patient population

The eligible patients were adults (18–70 years of age) of both sexes with end-stage liver disease that required LT due to acute or chronic infection and cirrhosis caused by HBV infection. Only patients that were HBsAg-positive, HBV DNA-negative and HBeAg negative prior to transplantation were allowed to participate in the clinical trial.

Patients were excluded according to the following criteria: retransplantation; participation in a clinical trial in the previous 3 months; history of active alcohol or drug abuse; known allergies to any component of Niuliva; possibility of treatment with other products containing anti-HBs during the study duration; unknown HBV replication status (HBV DNA and HBeAg serological determinations); unknown viral status for HCV, hepatitis A virus and HIV type 1 and type 2; selective immune globulin A (IgA) deficiency; history of serious adverse events (SAEs) or frequent adverse events (AEs) related to the administration of human blood-derived products; known medical, surgical or psychiatric condition or laboratory abnormality that may increase the risk associated with the study participation or interfere with the interpretation of the study results; any hemostatic abnormality contraindicating intravenous injection and inability to give informed consent personally. Pregnant women, nursing mothers or women expecting to be pregnant during the study duration were also excluded.

Study product characteristics & administration

Niuliva is a sterile, pasteurized, highly purified preparation containing a high titer of specific antibodies against HBsAg or anti-HBs (250 IU/ml) in vials of 5000 IU/20 ml and 10,000 IU/40 ml. The total protein content is 50 g/l of which at least 97% is IgG. Niuliva also contains D-sorbitol in a concentration of 5% as stabilizer.

Participants received Niuliva according to the following schedule: one dose of 10,000 IU during the anhepatic phase (day 0), daily doses of 10,000 IU during 1 week after transplantation (days 1–7), 1 weekly dose of 5000 IU at weeks 2, 3 and 4 (±1 day) and a monthly maintenance dose of 5000 IU from months 2 up to 12 after LT. Patients

were not permitted to be administered any HBIG other than the investigational product during the clinical trial period.

Study monitoring

Patients were followed during a minimum of 7 and a maximum of 13 months after the first investigational product administration. A physical examination and medical history data registration were done during the baseline visit and just prior to transplantation. Blood samples were drawn at different time points: baseline visit (within 3 months before transplantation), during the anhepatic phase or day 0 (before, during and after infusion transplantation), daily during 1 week after transplantation (pre- and post-infusion), weekly at weeks 2, 3 and 4 (pre- and post-infusion) and monthly at months 2 to 12 post-LT (pre- and post-infusion).

Blood samples were used to determine general hematological and coagulation parameters (hemoglobin, hematocrit, red cell count, white cell count, platelet count, aPTT, PT and fibrinogen), biochemistry parameters (bilirubin, ALT, AST, blood urea nitrogen, creatinine BUN), HBV viral markers (HBV DNA, HBeAg and HBsAg), total IgG and anti-HBs titers.

Patients were permitted to use over the counter or prescription medications throughout the duration of the study if they are approved by the treating physician. Concomitant treatment with nucleoside analogues (e.g., lamivudine) was permitted throughout the study.

Efficacy assessments

The percentage of patients with no HBV-recurrence during the first 6 and 12 months after transplantation was the first coprimary end point. The HBV-recurrence was defined by a positive determination of serum HBV-DNA (using a DNA PCR-amplification assay) and HBsAg (using a specific ELISA assay). The expected rate of reinfection without the intervention was considered to be of 50% [4].

The percentage of patients with anti-HBs preinfusion levels (trough levels) considered as protective according to the EMA recommendations [22], was the second coprimary efficacy end point. The following anti-HBs titers were targeted: ≥500 IU/l from day 3 to 7 during the 1 week post-LT, ≥250 IU/l from week 2 to 1 month post-LT and ≥150 IU/l during months 2 up to 12 post-LT.

Secondary efficacy end points included the proportion of patients with active viral replication assessed by positive HBV-DNA and/or positive HBeAg determinations at each visit (day 0–7; week 2–4 and months 2–12).

Safety assessments

Safety and immediate tolerance to the product during and after each product infusion were secondary end points for safety. Clinical safety and tolerability were assessed by monitoring vital signs, biochemistry, hematological parameters and AEs before, during and after each infusion and by recording possible discomforts associated with the product administration, until the final follow-up visit. Risk for bleeding was assessed by monitoring hematocrit levels and any clinical AEs for bleeding.

The AEs were categorized by the investigator according to their severity (mild, moderate, severe), seriousness (serious, nonserious) and cause–effect relationship to the study product (definitive, probable, possible, doubtful, unrelated).

Statistics

The number of patients, study design and follow-up period were not based on statistical considerations but rather on the recommendations of the EMA in force to evaluate the use of human normal immunoglobulin for intravenous administration [22,23]. Thus, ten patients undergoing LT due to HBV-liver disease were considered sufficient to evaluate Niuliva treatment efficacy. However, 15 patients were included in the study in order to compensate for possible dropouts.

All participants treated with at least one administration of the study product were assessed for safety, from enrollment to the end of the follow-up period or early dropout. For efficacy assessment, the intention-to-treat (ITT) population (all subjects enrolled who received at least one administration of the study product, with baseline evaluation and with at least one postbaseline efficacy measurement) was analyzed. The Per Protocol population (all ITT subjects that met all eligibility criteria and followed the protocol without relevant deviations) was also analyzed but considered as supportive.

Table 1. Baseline demographic and clinical characteristics of interest.

Characteristic	All patients (n = 15)
Age, years (median, range)	57 (26–65)
Sex: male (n, %)	12 (80.0)
Ethnicity: Caucasian (n, %)	15 (100)
Height, cm (mean ± SD)	170.3 ± 8.1
Weight, kg (mean ± SD)	73.7 ± 12.1
Alcohol consumption, abstinent (n, %)	15 (100)
Chronic HBV (n, %)	15 (100)
Previous medical conditions (n, %):	5 (33.3)
– Hepatocellular carcinoma	3 (20.0)
– Biliary complications	2 (13.3)
– Surgeries	2 (13.3)
– Gastrointestinal adenoma	1 (6.7)
– Inguinal hernia	1 (6.7)
– Typhus infection	1 (6.7)
– Intervertebral disc protrusion	1 (6.7)
Ongoing medical conditions (n, %):	12 (80.0)
– Gastrointestinal disorders	4 (26.7)
– Hepatocellular carcinoma	4 (26.7)
– Hepatobiliary complications	4 (26.7)
– Hepatic cirrhosis	3 (20.0)
– Hypertension	3 (20.0)
– Diabetes mellitus	2 (13.3)
– Active tobacco consumer	1 (6.7)
– Anemia/thrombocytemia	1 (6.7)

HBV: Hepatitis B virus; SD: Standard deviation.

According to their distribution, continuous variables were reported as mean, standard deviation, median, minimum and maximum values and 95% CI of the mean, while categorical variables were reported as number of patients and percentages.

Results

Study population

The study included 15 Caucasian patients: 12 men and 3 women, with a median age of 57 years. Five participants reported previous medical disease, mostly neoplasms (n = 4). Total of 12 patients reported ongoing pathologies, mostly gastrointestinal disorders, hepatobiliary complications and hepatic malignant neoplasms (n = 4 each). Further demographic and clinical data are shown in Table 1. No patients received any pre-LT HBIG prophylaxis. Total of 12 patients had received at least one pre-LT antiviral therapy (i.e., nucleoside/nucleotide analogues), one patient was treated solely with interferon therapy, and two did not receive pre-LT antiviral therapy. Post-LT antiviral therapy was maintained throughout the study for all patients from the anhepatic phase (day 0), with the exception of one patient who initiated antiviral treatment on day 7 post-LT and one patient that was treated with HBIG monotherapy. All patients were treated with immunosuppressives since the anhepatic phase and maintained this therapy during the study conduct. No patient was administered any polyvalent intravenous immune globulin.

All 15 participants completed the originally planned 6-month follow-up period and three patients consented to complete an extended treatment and follow-up up to 12 months. No patients dropped out or were withdrawn from the study although one patient was excluded from the ITT population since active viral replication prior to anhepatic phase visit was found present after consent. Figure 1 shows the flow of patients through the study.

Analysis of efficacy

No patients (0%) receiving the study HBIG presented HBV recurrence after LT throughout the perioperative period and during the 6-month (0 out of 15 patients) and 12-month (0 out of three patients) follow-up period. At day

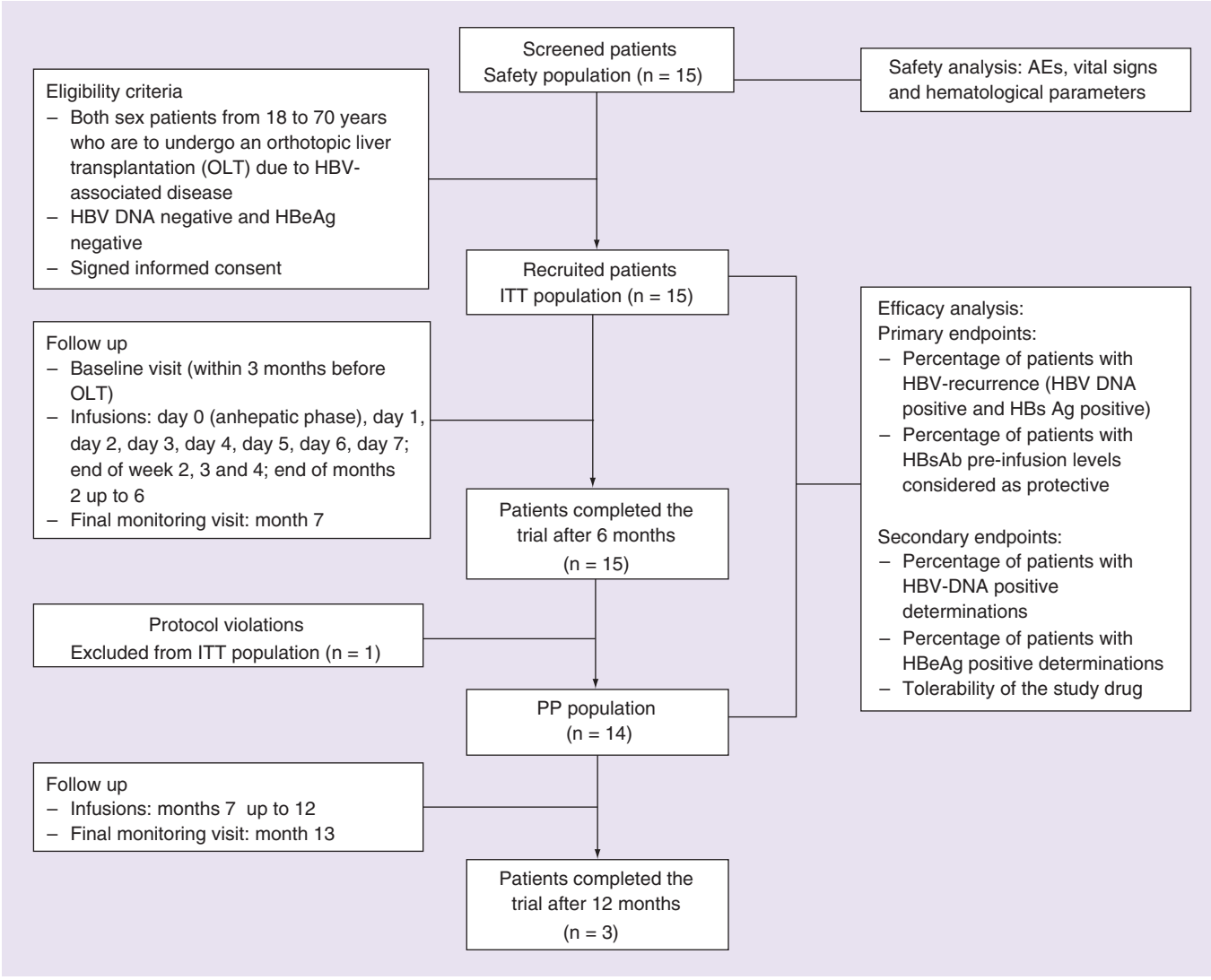

Figure 1. Flow of patients through the study.
AE: Adverse event; HBsAb: Hepatitis B surface antigen; HBeAg: Hepatitis B envelope antigen; HB: Hepatitis B; HBV: Hepatitis B virus; ITT: Intention-to-treat; OLT: Orthopic liver transplantation; PP: Per protocol.

0, one patient presented a transient positive HBV-DNA value and during days 1 and 2, another patient presented transient positive HBV-DNA values. However, subsequent determinations were all found negative throughout the follow-up period in all tests performed.

Targeted anti-HBs levels after LT were reached and maintained above the specified limits from day 3 to 7 (≥500 IU/l) with the exception of two patients that transiently fell short from the latter on day 4 and 5, respectively. Both patients did not develop any clinical signs of reinfection at any time point throughout their study participation and currently recommended protective thresholds set by the EMA (i.e., >100 IU/l) were reached at day 3 or 4 post-LT.

From 1 week post-LT to 1 month post-LT (≥250 IU/l) and throughout months 2–6 or 12 post-LT (≥150 IU/l) targeted anti-HBs levels were also reached and maintained for all patients with available data. However, two patients transiently presented titers slightly below the 150 IU/l threshold at 4 months. Detailed IU/l values of anti-HBs and percentages of patients with protective levels are shown in Table 2.

Analyses of secondary efficacy variables showed that, with the exception of a patient with positive HBV-DNA and HBeAg at pre-LT baseline visit, all participants presented nonactive viral replication from the baseline visit and throughout the 6- or 12-month follow-up period after LT.

Table 2. Anti-HBs titers and percentage of patients above targeted levels.

Anti-HBs target	Visit	Anti-HBs titer (IU/l)		Patients above targeted anti-HBs level n (%)
		Mean ± SD	95% CI	
>500 IU/l	Day 3	765 ± 376	496–1034	8 (80.0)
	Day 4	911 ± 282	709–1113	9 (90.0)
	Day 5	970 ± 96	901–1038	10 (100)
	Day 6	1000 ± 0.0	N/A	12 (100)
	Day 7	1000 ± 0.0	N/A	12 (100)
>250 IU/l	Week 2	1074 ± 244	910–1238	11 (100)
	Week 3	945 ± 250	740–1097	13 (100)
	Week 4	1029 ± 175	928–1130	14 (100)
>150 IU/l	Month 2	593 ± 260	442–743	14 (100)
	Month 3	439 ± 218	313–565	14 (100)
	Month 4	398 ± 273	247–549	13 (86.7)
	Month 5	352 ± 179	253–451	15 (100)
	Month 6	369 ± 183	264–475	14 (100)
>150 IU/l	Month 7	278 ± 89	56–499	3 (100)
	Month 8	304 ± 50	180–429	3 (100)
	Month 9	266 ± 35	180–325	3 (100)
	Month 10	301 ± 62	147–455	3 (100)
	Month 11	273 ± 118	-20–566	3 (100)
	Month 12	284 ± 137	-57–626	3 (100)

SD: Standard deviation; Anti-HB: Anti-hepatitis B.

Table 3. Summary of adverse events.

Type of AE	AEs, n = 57 n (%)	Patients, n = 15 n (%)
Patients with at least one AE	57 (100)	14 (93.3)
Patients with potentially treatment-related AEs	10 (17.4)	5 (33.3)
– Possible	2 (3.5)	2 (13.3)
– Unlikely/doubtful	8 (14.0)	4 (26.7)
AEs severity		
– Mild	31 (54.4)	13 (86.7)
– Moderate	25 (43.9)	11 (73.3)
– Severe	1 (1.8)	1 (6.7)
AEs seriousness		
– Serious	4 (7.0)	3 (20.0)
– Nonserious	53 (93.0)	12 (80.0)

AE: Adverse event

Analysis of safety

The total cumulative HBIG dosage infused over the study duration was 1,890,000 IU and the total number of infusions was 257. All infusions were rated as well tolerated in all patients. A total of 57 AEs were reported in 14 patients (93.3%) during the study. Events typically associated with the surgical procedure were not reported.

As summarized in Table 3, there were two AEs that were considered possibly related to the treatment (arthralgia and renal failure), while eight were considered unlikely related to the treatment (leukopenia, transplant rejection, hepatic enzyme increase, hypokinesia, two acute renal failures, hypertension, pharyngeal culture positive).

The severity of the AEs was prevalently mild (31/57, 54.4%), with only one AE rated as severe (biliary fistula). Four SAEs were reported in three patients (pyrexia, biliary fistula, liver transplant rejection and abnormal liver function test), but none of them was considered to be related to the study drug. All SAEs were resolved or improved. Further details are shown in Table 3.

Table 4. Frequency of adverse events by system organ class, preferred term occurring in patients.

Type of AE	AEs, n = 57 n (%)	Patients, n = 15 n (%)
Abnormal laboratory parameters	11 (19.3)	6 (40.0)
Gastrointestinal	9 (15.8)	5 (33.3)
Hepatobiliary	5 (8.8)	4 (26.7)
Nervous system	5 (8.8)	4 (26.7)
Blood and lymphatic system	5 (8.8)	4 (26.7)
Renal/urinary	4 (7.0)	4 (26.7)
Infections/infestations	3 (5.3)	3 (20.0)
Vascular	3 (5.3)	3 (20.0)
Administration site	3 (5.3)	3 (20.0)
Psychiatric symptoms	3 (5.3)	3 (20.0)
Immune system	2 (3.5)	2 (13.3)
Metabolism/nutrition	2 (3.5)	2 (13.3)
Musculoskeletal	1 (1.8)	1 (6.7)
Cardiac	1 (1.8)	1 (6.7)

AE: Adverse event.

The most frequent AE according to preferred term/system organ class was abnormal laboratory parameters (19.3%), mostly in blood biochemistry parameters, in six patients, and gastrointestinal disorders (15.8%), mostly nausea, in five patients, respectively (Table 4). Infection and infestations occurred in three patients (20.0%) and included biliary tract infection (n = 1), herpes virus infection (n = 1) and postoperative wound infection (n = 1). There was no seroconversion in patients previously negative to HCV or HIV and no clinical signs of seroconversion were reported. All patients were hepatitis A virus IgG positive at baseline. Mean levels of total IgG preinfusion during the anhepatic phase (day 0) was 13.0 ± 5.30 g/l and post-transplant measurements of total IgG were obtained pre- and post-infusion at subsequent visits: 9.7 ± 5.16 g/l (day 1), 9.3 ± 2.14 g/l (day 7); 10.6 ± 3.14 g/l (week 4); 10.2 ± 2.62 g/l (6 months) and 10.0 ± 1.30 g/l (12 months), respectively. No patient experienced an AE or SAE with a fatal outcome nor was withdrawn from the study.

Discussion

Liver transplantation in patients with HBV infection is particularly problematic since the causative agent is never completely eradicated, implying a high probability of recurrence that may lead to infection of the liver graft [4]. In this study, long-term, high-dose intravenous HBIG prophylaxis treatment with Niuliva in combination with oral antivirals was found effective and safe to prevent HBV recurrence and to achieve and maintain protective serum anti-HBs titers during the anhepatic phase and the first 6 and 12 months post-LT.

The majority of participants coincided with the available data on HBV pathology, which indicates a higher frequency in males. The primary end point set to assess the efficacy of the study HBIG in the prevention of HBV recurrence after LT was achieved as no patients presented HBV recurrence throughout the perioperative period and during the 6- or 12-month follow-up period. These results are aligned with those described in previous studies using other HBIG [24–28]. The three cases of positive HBV-DNA occurring during 1 week post-LT were transient and negative throughout the remaining follow-up. Four patients with evidence of HCC in the transplanted liver were included despite the latter being a known risk factor for postoperative HBV recurrence. However, all patients with HCC successfully completed the trial and did not present a higher reinfection rate as no recurrences, evaluated by the reappearance of HBsAg and HBV-DNA, were recorded throughout the perioperative period and during the 6- and 12-month follow-up period.

Treatment with HBIG was also effective in maintaining protective preinfusion levels of anti-HBs above the established thresholds recommended by the EMA for the prevention of HBV recurrence post-LT [22]. There is a direct relationship between HBV viral load at transplantation and the rate of HBV recurrence [16,29–31]. Targeted anti-HBs levels were achieved and maintained above the specified limits from day 3 to 7 (≥ 500 IU/l) with the exception of two patients that did not reach the desired target on day 4 and 5, respectively. Importantly, both slow-responding patients had positive qualitative HBsAg determinations until post-LT day 3. Both patients did not develop any clinical signs of reinfection at any time point throughout their study participation. Moreover, the

currently recommended protective threshold set by the EMA (i.e., >100 IU/l) were reached at day 3 or 4 post-LT. The residual HBV antigen load after LT is likely to be variable between patients, and thus the amount of HBIG to maintain HBV antigen free serum should also likely differ between patients. With this in mind, many prophylaxis protocols currently recommend following a tailored dose regimen based on the HBV replication status prior to transplant and/or the patient's anti-HBs levels post-LT as opposed to a fixed dose regimen.

From 1 week post-LT to 12 months post-LT targeted anti-HBs levels were achieved and maintained for all patients. However, two patients transiently presented anti-HBs titers slightly below the 150 IU/l threshold at month 4, but in all cases higher than protective threshold currently set by the EMA for nonreplicator patients (i.e., >100 IU/l). The effect was also evident from the analyses of secondary variables. Except for the HBV-DNA and HBeAg-positive patient at the baseline visit, all participants presented nonactive viral replication from the baseline visit and throughout the 6- or 12-month follow-up period.

It is also important to mention that the preinfusion anti-HBs titer thresholds defined by the study protocol were significantly higher than those set by the EMA for nonreplicator patients (>100 IU/l) [22]. In fact, four out of the five anti-HBs titer determinations deemed below the targeted levels in this trial were above the EMA recommendations. Therefore, it can be considered that there were no cases of nonefficiency of the study product in achieving anti-HBs protective titers.

Globally, this study confirmed for the anhepatic phase and following 6 or 12 months, the good efficacy results observed in a previous prospective study conducted with 20 adult patients, in which Niuliva was proven effective in the prophylaxis of recurrence as well as in providing adequate protective anti-HB serum levels during the maintenance phase after LT due to HBV-related liver disease [21].

The HBIGs are generally considered safe and AEs observed are typically minor [32]. In accordance with that the latter, the study product presented a suitable safety profile. The AEs considered to be related with the LT procedure were not reported in this clinical trial; a consistent comparison of total number of AEs with other studies [33] was not appropriate. A biliary fistula was the only SAE which can be considered likely after LT [34]. The two AEs considered as possibly related to the treatment (arthralgia and renal failure) were expected according to the product administration guidelines. The studied patient population is typically treated intensely with a wide spectrum of drug therapies, which may potentially contribute to the occurrence of renal dysfunction including substantial amounts of immunosuppressant drugs, opioids, anticoagulant/antithrombotic therapies, and antibiotic and antiviral prophylaxis, among others. Thus, the development of chronic kidney disease is frequently encountered in LT recipients, the prevalence of which may range from 30 to 80% in this patient population [35]. There were no deaths, study withdrawals or seroconversions.

The nonrandomization and open-label nature of the trial can be considered among the limitations of the study. In addition, not all values for efficacy evaluations were available from all patients.

Conclusion

Administration of high-dose intravenous Niuliva HBIG was a safe and effective treatment to prevent HBV recurrence as well as to provide HBsAg protective levels in *de novo* orthotopic LT recipients, throughout the perioperative period to 1 year follow-up period.

Summary points

- This was a nonrandomized, noncontrolled, Phase III clinical trial.
- Niuliva® is an intravenous 5% liquid hepatitis B virus immune globulin.
- Liver transplant recipients were treated with Niuliva from the anhepatic phase.
- Adequate antiviral titers to prevent recurrence were achieved.
- No hepatitis B reinfection was observed up to 12 months.
- None of the four serious adverse events reported was related to the study product.
- There were no seroconversions and no deaths.

Financial & competing interests disclosure

This study was funded by Grifols, manufacturer of Niuliva®. P De Simone has received speaker's fees from Grifols. M Barceló, MK Woodward and A Páez are employees of Grifols. M Salizzoni, U Cillo and F Di Benedetto declare no conflict of interest. The authors

have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

J. Bozzo has provided medical writing and editorial assistance.

Ethical conduct of research statement

All participants provided written informed consent. The trial was designed and monitored in compliance with the ethical principles of the World Medical Assembly (Helsinki, 1964) and subsequent revisions. National and local independent ethics committee or institutional review board approved both the protocol and the associated informed consent and information sheets.

Data sharing statement

The authors certify that this manuscript reports original clinical trial data (ClinicalTrials.gov identifier: NCT01131065; EudraCT number: 2010-020931-37). Data reported in this manuscript are available within the article or posted publicly at www.clinicaltrials.gov and www.clinicaltrialsregister.eu, according to the required timelines. Additional data from the study (e.g., study protocol) are available upon reasonable request.

Open access

References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1. WHO. Hepatitis B fact sheet (2016). www.who.int/en/news-room/fact-sheets/detail/hepatitis-b
2. Ganem D, Prince AM. Hepatitis B virus infection – natural history and clinical consequences. *N. Engl. J. Med.* 350(11), 1118–1129 (2004).
3. Lok AS, McMahon BJ. Chronic hepatitis B. *Hepatology* 45(2), 507–539 (2007).
4. Samuel D, Muller R, Alexander G *et al.* Liver transplantation in European patients with the hepatitis B surface antigen. *N. Engl. J. Med.* 329(25), 1842–1847 (1993).

•• **Milestone paper on the use of hepatitis B virus (HBV) immune globulin after liver transplantation dramatically prevented reinfection and improved patient survival.**

5. Lauchart W, Muller R, Pichlmayr R. Long-term immunoprophylaxis of hepatitis B virus reinfection in recipients of human liver allografts. *Transplant. Proc.* 19(5), 4051–4053 (1987).
6. Samuel D, Bismuth A, Mathieu D *et al.* Passive immunoprophylaxis after liver transplantation in HBsAg-positive patients. *Lancet* 337(8745), 813–815 (1991).
7. Shouval D, Samuel D. Hepatitis B immune globulin to prevent hepatitis B virus graft reinfection following liver transplantation: a concise review. *Hepatology* 32(6), 1189–1195 (2000).

•• **Review on long-term and high-dose HBV immune globulin prophylaxis as highly efficient way of preventing HBV reinfection.**

8. Jiang L, Yan L, Li B *et al.* Prophylaxis against hepatitis B recurrence posttransplantation using lamivudine and individualized low-dose hepatitis B immunoglobulin. *Am. J. Transplant.* 10(8), 1861–1869 (2010).
9. Gane EJ, Angus PW, Strasser S *et al.* Lamivudine plus low-dose hepatitis B immunoglobulin to prevent recurrent hepatitis B following liver transplantation. *Gastroenterology* 132(3), 931–937 (2007).
10. Ghany MG, Ayola B, Villamil FG *et al.* Hepatitis B virus S mutants in liver transplant recipients who were reinfected despite hepatitis B immune globulin prophylaxis. *Hepatology* 27(1), 213–222 (1998).
11. Sarin SK, Kumar M, Lau GK *et al.* Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. *Hepatol. Int.* 10(1), 1–98 (2016).
12. Nkongolo S, Ni Y, Lempp FA *et al.* Cyclosporin A inhibits hepatitis B and hepatitis D virus entry by cyclophilin-independent interference with the NTCP receptor. *J. Hepatol.* 60(4), 723–731 (2014).
13. Petersen J, Dandri M, Mier W *et al.* Prevention of hepatitis B virus infection *in vivo* by entry inhibitors derived from the large envelope protein. *Nat. Biotechnol.* 26(3), 335–341 (2008).

• **Mechanisms of action of HBV recurrence prevention by suppression of viral replication and viral entry of the hepatocyte target.**

14. Pruett T. Primary transplant treatment of hepatitis B: hepatitis B immunoglobulin (passive immunization). *Liver Transpl. Surg.* 3(5 Suppl. 1), S13–S15 (1997).
15. Faria LC, Gigou M, Roque-Afonso AM *et al.* Hepatocellular carcinoma is associated with an increased risk of hepatitis B virus recurrence after liver transplantation. *Gastroenterology* 134(7), 1890–1899; quiz 2155 (2008).

16. Degertekin B, Han SH, Keeffe EB *et al.* Impact of virologic breakthrough and HBIG regimen on hepatitis B recurrence after liver transplantation. *Am. J. Transplant.* 10(8), 1823–1833 (2010).

• **Description of the direct relationship between HBV viral load at transplantation and the rate of HBV recurrence.**

17. Yi NJ, Suh KS, Cho JY *et al.* Recurrence of hepatitis B is associated with cumulative corticosteroid dose and chemotherapy against hepatocellular carcinoma recurrence after liver transplantation. *Liver Transpl.* 13(3), 451–458 (2007).

18. Chun J, Kim W, Kim BG *et al.* High viremia, prolonged Lamivudine therapy and recurrent hepatocellular carcinoma predict posttransplant hepatitis B recurrence. *Am. J. Transplant.*10(7), 1649–1659 (2010).

19. Saab S, Yeganeh M, Nguyen K *et al.* Recurrence of hepatocellular carcinoma and hepatitis B reinfection in hepatitis B surface antigen-positive patients after liver transplantation. *Liver Transpl.* 15(11), 1525–1534 (2009).

20. Hu TH, Chen CL, Lin CC *et al.* Section 14. Combination of entecavir plus low-dose on-demand hepatitis B immunoglobulin is effective with very low hepatitis B recurrence after liver transplantation. *Transplantation* 97(Suppl. 8), S53–S59 (2014).

21. Mas A, Castells L, Abradelo M *et al.* Evaluation of anti-HBs serum levels and pharmacokinetic profile after intravenous administration of Niuliva, a new hepatitis B immunoglobulin, following liver transplantation. *Transplant. Proc.* 41(10), 4253–4258 (2009).

•• **Clinical trial that reports Niuliva® to be well tolerated and safe for the prevention of HBV reinfection after liver transplantation during the maintenance phase in nonreplicating patients.**

22. European Medicines Agency (EMA). Committee for Medicinal Products for Human Use (CHMP). Guideline on the clinical investigation of hepatitis B immunoglobulins (EMA/CHMP/BPWP/585257/2009) (2015). www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2015/07/WC500190207.pdf

23. European Medicines Agency (EMA). Committee for Medicinal Products for Human Use (CHMP). Guideline on the clinical investigation of human normal immunoglobulin for subcutaneous and/or intramuscular administration (SCIg/IMIg) (EMA/CHMP/BPWP/410415/2011 rev 1) (2012). www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/12/WC500135705.pdf

24. Markowitz JS, Martin P, Conrad AJ *et al.* Prophylaxis against hepatitis B recurrence following liver transplantation using combination lamivudine and hepatitis B immune globulin. *Hepatology* 28(2), 585–589 (1998).

25. Marzano A, Salizzoni M, Debernardi-Venon W *et al.* Prevention of hepatitis B virus recurrence after liver transplantation in cirrhotic patients treated with lamivudine and passive immunoprophylaxis. *J. Hepatol.* 34(6), 903–910 (2001).

26. Rosenau J, Bahr MJ, Tillmann HL *et al.* Lamivudine and low-dose hepatitis B immune globulin for prophylaxis of hepatitis B reinfection after liver transplantation possible role of mutations in the YMDD motif prior to transplantation as a risk factor for reinfection. *J. Hepatol.* 34(6), 895–902 (2001).

27. Steinmuller T, Seehofer D, Rayes N *et al.* Increasing applicability of liver transplantation for patients with hepatitis B-related liver disease. *Hepatology* 35(6), 1528–1535 (2002).

28. Han SH, Ofman J, Holt C *et al.* An efficacy and cost–effectiveness analysis of combination hepatitis B immune globulin and lamivudine to prevent recurrent hepatitis B after orthotopic liver transplantation compared with hepatitis B immune globulin monotherapy. *Liver Transpl.* 6(6), 741–748 (2000).

29. Marzano A, Gaia S, Ghisetti V *et al.* Viral load at the time of liver transplantation and risk of hepatitis B virus recurrence. *Liver Transpl.* 11(4), 402–409 (2005).

30. Mutimer D, Pillay D, Dragon E *et al.* High pre-treatment serum hepatitis B virus titre predicts failure of lamivudine prophylaxis and graft re-infection after liver transplantation. *J. Hepatol.* 30(4), 715–721 (1999).

31. Zheng S, Chen Y, Liang T *et al.* Prevention of hepatitis B recurrence after liver transplantation using lamivudine or lamivudine combined with hepatitis B Immunoglobulin prophylaxis. *Liver Transpl.* 12(2), 253–258 (2006).

32. Verna EC. Hepatitis viruses and liver transplantation: evolving trends in antiviral management. *Clin. Liver Dis.* 18(3), 575–601 (2014).

• **An approach to the safety profile of HBV immune globulins, which adverse events observed are typically minor.**

33. Yahyazadeh A, Beckebaum S, Cicinnati V *et al.* Efficacy and safety of subcutaneous human HBV-immunoglobulin (Zutectra) in liver transplantation: an open, prospective, single-arm Phase III study. *Transpl. Int.* 24(5), 441–450 (2011).

34. Nemes B, Gaman G, Doros A. Biliary complications after liver transplantation. *Expert. Rev. Gastroenterol. Hepatol,* 9(4), 447–466 (2015).

35. Ojo AO, Held PJ, Port FK *et al.* Chronic renal failure after transplantation of a nonrenal organ. *N. Engl. J. Med.* 349(10), 931–940 (2003).