Retrospective Data Collection (RDC) Documentation Plan

Biotest AG

Title: A retrospective data collection to increase the knowledge base of posttransplant treatment with the human hepatitis B immunoglobulin Zutectra or Hepatect CP in liver transplanted patients

Version incl. date: **1.0 (14 Nov 2014)**

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1 SIGNATURE PAGE

This retrospective data collection is carried out in compliance with applicable regulatory authority requirements. It is confirmed that the retrospective data collection will be carried out and documented in accordance with this RDC documentation plan.

Head Corporate Clinical Research
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Date, signature

Sen. Scientific [redacted]

Date, signature

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Signature Page for Investigators

Declaration of the Participating Investigator

I have read and understood this RDC documentation plan and agree to the following:

- To adhere to the local laws and regulations, and the applicable regulatory requirements.
- To conduct the RDC as set out in the documentation plan.
  This includes:
  - To wait until I have received approval from the appropriate Independent Ethics Committee / Institutional Review Board (IEC/IRB) before documenting data of any subject in this RDC, if applicable.
  - To obtain informed consent from all subjects prior to providing access to their patient source notes.
  - To permit RDC related monitoring, audits, IEC/IRB review, and regulatory authority inspections.
  - To provide direct access to all RDC patient related records, source documents, and subject files for the monitor, auditor, IEC/IRB, or regulatory authority upon request.
  - To understand that changes to the RDC documentation plan must be made in the form of an amendment that has the prior written approval of Biotest AG and, as applicable of the appropriate IEC/IRB and regulatory authority.
  - To comply with the reporting obligations for Adverse Drug Reactions (related Adverse Events) according to the RDC documentation plan.

I understand that all documentation that has not been previously published will be kept in the strictest confidence. This documentation includes the RDC documentation plan, Case Report Forms, and other scientific data.

Investigator

_____

Date, signature

Investigator stamp:

2 RDC SYNOPSIS

Title	A retrospective data collection to increase the knowledge base of post-transplant treatment with the human hepatitis B immunoglobulin Zutectra® or Hepatect® CP in liver transplanted patients
Clinical Phase	n/a (retrospective data collection, non-interventional)
RDC Objectives	• The effectiveness of long-term protection from HBV-recurrence after liver transplantation (LT) using subcutaneous Zutectra® or iv Hepatect® CP and / or a further hepatitis B immunoglobulin (HBIg) in the same patient. • If HBV-HCC (hepatocellular carcinoma) is the reason for LT or HBV-HCC is detected in the explanted liver the rate of HBV-HCC recurrence.
RDC Design	Retrospective, non-interventional, uncontrolled, single-arm, international, multi-centre, post-approval
RDC Population	Male and female adult patients after LT for HBV-induced liver failure
Inclusion Criteria	1. Patients 18 years or older 2. Patients with LT for fulminant Hepatitis B, Hepatitis B –cirrhosis, or HBV-HCC inside the Milan criteria, or with liver re-transplantation except due to HBV recurrence 3. Treatment with any HBIg for at least 1 year from LT onwards including at least a treatment of 6 months with Hepatect® CP or Zutectra® 4. LT after the year 2000 5. Written informed consent from patients alive to allow data collection and data transfer to third party
Number of RDC Patients	400
Countries/Number of Sites	Up to 20 sites in Germany, Great Britain, Italy, Switzerland, The Netherlands.
Medication	Zutectra® and Hepatect® CP with or without a nucleoside analog (NUC) combination.
Dosage and Mode of Administration	As prescribed by physician and as administered by physician, nurse, family member or patient in accordance with the SPC.
Duration of Documentation	A time period of at least 1 year up to 10 years from LT onwards with any HBIg including at least a treatment of 6 months with Hepatect® CP or Zutectra®.

Criteria for Evaluation ***Effectiveness***	• Proportion and absolute number of HBV-liver transplant patients free of hepatitis B virus recurrence as assessed by non-detectability of HBsAg and / or HBV-DNA in patients' sera. • The rate of HBV-HCC recurrence, if HBV-HCC is the reason for LT or HBV-HCC is detected in the explanted liver.
Safety	• The rate of adverse drug reactions (ADR) to Zutectra® and / or Hepatect® CP reported as individual case safety reports (ICSR) within routine pharmacovigilance.
Biometrical Concept	Evaluation using descriptive statistical methods. Effectiveness related proportions are reported with exact (Pearson-Clopper), one sided, upper 95%-confidence limit.
Start of Documentation *(planned)*	Q1 2015
Date of Analysis*(planned)*	Q2 2016

Confidentiality Statement

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4 LIST OF ABBREVIATIONS

ADR	adverse drug reaction
AE	adverse event
Anti-HBs	antibodies against hepatitis B-surface antigen
BMI	body mass index
BT088	human hepatitis B immunoglobulin (Zutectra®)
CDS	Corporate Drug Safety
CPMP	Committee for Proprietary Medicinal Products
CRF	case report form
CRO	Contract Research Organisation
DNA	desoxyribonucleic acid
eCRF	electronic case record form
EDC	electronic data capture
e.g.	for example
EMA	European Medical Agency
ETV	entecavir
EU	European Union
GGT	gamma glutamyl transferase
FTC	emtricitabin
HB	human hepatitis B
HBIg	human hepatitis B immunoglobulin
HBsAb	antibody against hepatitis B surface antigen
HBsAg	hepatitis B virus surface antigen
HBV	hepatitis B virus
HBV-HCC	hepatitis B virus induced hepatocellular carcinoma
HBV-LT	liver transplantation due to HBV-induced terminal liver failure
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HDV	hepatitis delta virus
HIV	human immunodeficiency virus
hgbNuc	high genetic barrier nucleos(t)id
i.e.	that is
ICSR	individual case safety reports
ICU	intensive care unit
IEC/IRB	Independent Ethics Committee / Institutional Review Board
IgA	immunoglobulin A
IgG	immunoglobulin G
IgM	immunoglobulin M
im	intramuscular
ISF	investigator site file
IU	international unit(s)
iv	intravenous
LAM	lamivudine
L	liter
LT	liver transplantation
MAH	marketing authorisation holder

mg	milligram
min	minute
ml	millilitre
NIS	Non-interventional Study
NUC	nucleos(t)id
nvCJD	new version of Creutzfeld Jacob Disease
PASS	Post-Authorisation-Safety Study
PSUR	perioditc safety update report
RDC	retrospective data collection
rAE	related adverse events
rSAE	related serious adverse event
SADR	severe adverse drug reaction
SAE	serious Adverse Event
SAP	statistical analysis plan
sc	subcutaneous
SD	standard deviation
SOP	standard operating procedure
SPC	summary of product characteristics
TDV	tenofovir
w/v	weight / volume

5 INTRODUCTION

Worldwide about 15 to 30% of the more than 300 million chronic carriers of the **h**epatitis B **v**irus (HBV) are at risk of developing end-stage liver disease (Fattovich G et al 2008). Today **l**iver **t**ransplantation (LT) is generally accepted as the most reliable medical intervention to rescue patients suffering from life-threatening decompensated liver disease due to chronic Hepatitis B as well as HBV-induced **h**epato**c**ellular **c**arcinoma (HBV-HCC).

The prerequisite for that successful intervention was the introduction of **h**epatitis **B** **i**mmuno**g**lobulin (HBIg) fractionated from human plasma which reduced the rate of HBV re-infection of the transplanted liver from $\geq$ 80% if no prophylaxis was performed to 20% to 30% (Müller et 1991; Samuel et al 1993; Shouval D, Samuel D 2000; Lok A 2002). The next significant improvement was the introduction of lamivudine (LAM) combined with HBIg. The combination treatment quickly turned out to be the worldwide "gold-standard" for HBV re-infection prophylaxis by reducing the risk of HBV re-infection to less than 10% as long as no HBV resistance to LAM pre-existed in the patient (Rosenau J et al 2001; Lok A 2002; Zheng S et al 2006; Dickson RC et al 2006). The disadvantage of LAM as mono-prophylaxis was the high risk of generating escape mutants of the YMDD motif (after 12 months: 10%; after 36 months 22% - 50%; Grellier L et al 1996; Malkan G et al 2000; Mutimer D et al 2000; Perrillo RP et al 2001; Lo CM et al 2001). The availability of the so called high genetic barrier nucleos(t)ide analogs (NUCs), **e**ntecavir (ETV) and **t**enofovir (TDV), succeeded in further reduction of the HBV re-infection rate below 4% (Cholongitas E, 2013) if combined with HBIg (ETV+HBIg: 3 / 197; TDV+HBIg 0/106). However, the use of ETV is not recommended for those patients having shown resistance to LAM treatment before switch to ETV because of a significant increase of HBV-escape mutations to ETV (40% to 50%) within 5 years of mono-virostatic treatment (Sherman M et al 2006). Mono-virostatic maintenance HBV re-infection prophylaxis using ETV, TDV, TDV+LAM and TDV+emtricitabine (FTC) after cessation of combination prophylaxis with HBIg showed HBV recurrence in 5 of 103 patients (Cholongitas E et al 2012). Currently it is too early to calculate a reliable statistical significance due to the low number of patients treated with HBIG/NUCs during the maintenance phase after LT. Today the chance to protect the allograft from being re-infected with HBV is higher than it has ever been before.

New strategies for HBV re-infection prophylaxis focus on patient individualized treatment regimens at which patients are classified according to risk criteria for HBV-recurrence. Those criteria are the HBV load at time of transplantation (Marzano A et al 2005), co-infection with other viruses e.g. HDV, HCV or HIV (Fox AN 2012), hepatocellular carcinoma (in particular outside the Milan criteria) and in particular for mono-virostatic prophylaxis, the patients' adherence to the treatment recommendations of their physicians. In addition increasing financial pressure in almost all national health systems result in strenuous efforts for cost reduction. Thus, a constant dosage regimen will rather be converted to an individualized regimen considering the patient's individual situation as a basis. However, even after more than 20 years of steadily improving HBV re-infection prophylaxis there is still no general consensus on the optimal treatment and dosage regimen in particular for HBIg.

As HBV-HCC is steadily increasing as an indication for LT (Burra P et al 2013) clinical studies in the USA (Saab S et al 2009; Campsen J et al 2013) and Taiwan (Wu TJ et al 2013) have investigated the recurrence rate of HBV-HCC in relation to HBV re-

infection prophylaxis using a combination of HBIg and virostatic drugs. In particular the dosage regimen and duration of treatment differed significantly. Thus, the optimal treatment regimen still needs to be established in this patient group. Therefore, it is of interest to evaluate whether the recurrence rate of HBV-HCC after LT can be reduced using dosage and treatment schedules for Hepatect® CP and / or Zutectra® and / or competitor HBIgs in combination with a modern HBV- virostatic drug applied in European liver transplant centers.

5.1 Aim of the Study

The aim of the present study is to increase the knowledge base by retrospective documentation of the rate of HBV-recurrence in patients after LT due to HBV-induced end-stage liver disease using Hepatect® CP and / or Zutectra® and intermittent other HBIg preparations with or without combination with virostatic drugs against HBV.

In addition, if HBV-HCC is the reason for LT the rate of HBV-HCC, recurrence will be analyzed.

5.2 Technical and Clinical Data on Hepatect® CP and Zutcetra®

Both Hepatect® CP and Zutectra® are well established HBIgs licensed for HBV re-infection prophylaxis after LT. Whereas Hepatect®CP is licensed for iv administration for both the early and maintenance phase after LT, Zutectra® is the only HBIg registered in the EU for subcutaneous (sc) administration in the post- LT maintenance phase in adults.

Both sterile liquid and ready-to-use HBIg preparations are manufactured according to the same technology from selected human plasma of voluntary donors actively immunized against HBV. The plasma donations are exclusively collected from countries free of any history of new version of Creutzfeld Jacob Disease (nvCJD). Both HBIg preparations have more than 96% (w/v) human IgG. Hepatect® CP is a 5% (w / v) HBIg solution with a potency of 50 IU/mL. Zutectra® is concentrated to 15% (w/v) IgG and a potency of HBsAb of 500 IU/mL. For both HBIg preparations glycin buffers are used. Hepatect® CP is available in filling sizes of 10 mL, 40mL and 100 mL. Zutectra® is offered as a 1 mL ready-to-use prefilled syringe.

5.3 Licenses for Hepatect® CP and Zutectra®

Hepatect® CP was first licensed in Germany on April 12, 2000 and thereafter in EU-member States according to the mutual recognition procedure. Hepatect® CP has been approved for the following indications:

1. Prevention of hepatitis B virus re-infection after LT for hepatitis B induced liver failure.

2. Immunoprophylaxis of hepatitis B
   - In case of accidental exposure in non-immunized subjects (including persons whose vaccination is incomplete or status is unknown).
   - In haemodialysed patients, until vaccination has become effective.
   - In the newborn of a hepatitis B virus carrier-mother.

- In subjects who did not show an immune response (no measurable hepatitis B antibodies) after vaccination and for whom a continuous prevention is necessary due to the continuous risk of being infected with hepatitis B.

Hepatect® CP had to be administered intravenously by the treating physician or trained medical personnel and according to national regulations.

Zutectra® received marketing authorization throughout the European Union via the centralized procedure in 2009 as well as for Norway, Iceland and Liechtenstein It is marketed in Austria, Belgium, Germany, France, Ireland, Italy, Portugal, Romania, Singapore, Spain, Sweden, the Netherlands, and United Kingdom. In addition, Zutectra is authorized in Switzerland and Israel.

- Prevention of hepatitis B virus (HBV) re-infection in HBV-DNA negative patients ≥ 6 months after liver transplantation for hepatitis B induced liver failure.
- Zutectra® is indicated in adults only.

Zutectra® can be subcutaneously administered by the patient, a trained family member or medical personnel either at patient's home, family doctor's practice or hospital.

5.4 Clinical Studies for Hepatect® CP and Zutectra®

The safety, pharmacokinetic and efficacy of both Hepatitis B Immunoglobulins have been demonstrated in several clinical studies:

Pharmacokinetic and Safety Study for Hepatect® CP

1. Thürmann P.A. et al: Pharmacokinetics of viral antibodies after administration of intravenous immunoglobulin in patients with chronic lymphocytic leukemia or multiple myeloma. *Eur. J.Clin.Pharmacol. (2001) 57: 235-241*

10 CLL and 5 multiple myeloma patients received 160 ml (= 8,960 IU) each within a mean infusion time of three hours as intravenous infusion. The maximum bio-availability (C_{max} = 3.01+/- 0.87 IU/mL) of HBs-antibodies were reached within a mean of 4 hours (3.62 +/- 1.06 hrs). The total body clearance came to 0.14+/- 0.08 mL/min and the elimination half-lives were in the range of 25.34+/-8.34 days. The mean residence time was determined 21.73+/- 3.41 days. Hepatect® CP was well tolerated with only mild adverse events such as mild headache in one patient.

Pharmacokinetic and Safety Study for Zutectra®

2. Thürmann P.A. et al: Pharmacokinetics and safety of a novel anti-HBs-enriched immunoglobulin in healthy volunteers after subcutaneous and intramuscular administration. *Eur J Clin Pharmacol (2006)* ***62****: 511–512*

In this open, randomized parallel study investigating safety and pharmacokinetic (pk) characteristics of Zutectra® after subcutaneous or intramuscular application in 30 male and female volunteers with a single dose of 30 IU/kg body weight presented pk-characteristics which were comparable to human intravenously administered HBIg. However, the maximum bio-availability was reached after four days as compared to 4 hrs following intravenous administration of HBIg e.g. Hepatect® CP. The mean

elimination half-life of three to four weeks corresponds to those of natural IgG. The administration of Zutectra® was well tolerated and safe.

Clinical Phase III Study for Zutectra®

3. Yahyazadeh A. et al: Efficacy and safety of subcutaneous human HBV-immunoglobulin (Zutectra®) in LT: an open, prospective, single-arm phase III study. *Transplant International (2011)* ***24****: 441-450*

In this open, prospective single-arm phase III study, 23 liver transplant patients on maintenance treatment with Hepatect® CP and a HBV virostatic drug were converted to Zutectra® according to the individual scheduled dosing interval, i.e. approximately three to four weeks after the last iv administration of Hepatect®CP. In this study individual trough levels in these LT patients obtained with Zutectra® were determined for 18 weeks with a facultative extension to 24 weeks in order to assess if serum anti-HBs concentrations of ≥ 100 IU/l. were maintained after weekly subcutaneous injections of 500 IU or 1000 IU of Zutectra®. As a result, a relatively constant mean serum HBs-antibody concentration within the range of 350 IU/l to 400 IU/l was achieved. At all weekly and fortnightly assessments, serum HBs antibody concentrations in all patients treated with Zutectra® were above the 100 IU/l minimum level required to offer effective protection against HBV re-infection of the transplanted liver. Additionally, no patient showed a HBV-related infection which confirms that effective protection against HBV re-infection was provided by subcutaneous administration of Zutectra®. More than 50% of the study patients were able to safely self-administer Zutectra® after a training period of three weeks and after the 120 days study period 22 of 23 patients performed self administration at home. The one patient who failed showed non-adherence to the study protocol and returned again to iv administration of HBIg.

Clinical Phase III Study for Zutectra®

4. Filipponi F, Salizzoni M, Verga G, Di Costanzo GG, De Carlis L, Rossi G and Calise F. Conversion to subcutaneous anti-hepatitis B immunoglobulins (ZutectraTM) in maintenance liver transplant patients: preliminary results of a multicenter, prospective, single-arm study. Poster 974. ILTS Valencia 2011.

This open, prospective, single-arm phase III study was conducted to investigate the feasibility of home self-treatment, efficacy and safety of Zutectra after subcutaneous application in liver transplanted patients. Results showed that treatment with Zutectra was well tolerated and safe. A total of 72 patients were screened, 66 patients were included and 58 patients were treated with Zutectra according to protocol with weekly injections. After completion of 24 weeks of treatment patients were offered to continue therapy with Zutectra for another 24 weeks. All 58 patients completed this facultative extension phase according to protocol. The results indicate that Zutectra is effective in the prophylaxis of HBV re-infection in liver transplanted patients. Serum HBs antibody concentrations in all patients were above the 'safety level' of 100 IU/l and no patient showed a hepatitis B related infection. Furthermore, a good compliance of the patients with regard to subcutaneous self-administration of Zutectra was observed in this study.

Investigator Initiated Clinical Trial with Zutectra®

5. Di Constanzo G.G. et al: Safety and efficacy of subcutaneous hepatitis B immunoglobulin after LT: an open single-arm prospective study. *American Journal of Transplantation (2013)* ***13****, 348–52*

In an open single-arm clinical study 135 stable post-LT patients who were transplanted more than 12 months ago were switched from standard HBIg to Zutectra®. All patients received a combination treatment with HBIg and a HBV virostatic drug before being converted to Zutectra®. Patients received Zutectra® according to body weight that is 500 IU for patients below 75 kg and 1000 IU for those with ≥ 75 kg body weight. HBsAg- and HBsAb-serotiter were assessed biweekly during the first two months and thereafter in monthly intervals. The total study period was 48 weeks per patient. The median serotiter of HBsAb after the last iv administration of HBIg was 407 IU/l (211-869 IU/l) and after 48 weeks of self administration of Zutectra® the median serotiter of HBsAb was 232 IU/l (115 – 566 IU/l). Serum of all study patients had undetectable HBsAg and HBV-DNA. There were no serious adverse events (SAEs) detected and adverse events (AEs) attributable to Zutectra® were mild hematomas (15 of 9296 sc injections).

Post-Authorisation Study for Zutectra®

6. Klein C.G. et al: Compliance and tolerability of subcutaneous hepatitis B immunoglobulin self-administration in liver transplant patients: A prospective, observational, multicenter study. *Ann Transplant, 2013;* ***18****: 677-684*

In a German multicenter post-authorisation safety study (PASS) subcutaneous self-administration of Zutectra® for maintenance HBV re-infection prophylaxis was followed up with 61 patients for a median of 18 weeks (range 14 – 27,9 weeks). The median time from LT was 5.7 years. The indication for LT was HBV-induced liver cirrhosis in 82% of the patient population and concomitant HCC in 37.7%. A total of three visits were planned during the observation period of 18 weeks, these visits included the starting visit, one intermediate and one final visit after the end of the observation period. The median time for the first home administration of Zutectra® was eight days, the maximum delay 27 days. The majority of patients were already experienced with self-administration at home. Only five patients were not self-administering Zutectra® at home at study entry. Fourty-one out of 60 patients used Zutectra® in combination with an oral HBV-virostatic drug. There were no serious adverse effects and only non-serious adverse drug reactions. Compliance failure which was defined as ≥ 1 HBs-antibody serotiter below 100 IU/l was detected in four patients. Of these, three patients received a lower dose than recommended for their body weight. The mean HBs-Ab level at the first visit was 248 IU/l with a SD of 97 IU/l. At the final visit the mean patient HBsAb titer was 255 IU/l with a SD of 104 IU/l. In 91.8%, patient compliance was rated as very good or good. At the end of the study all patients' serum HBsAb levels exceeded 100 IU/l and no patient experienced HBV-re-infection. No patient discontinued the study.

According to the core SPC for human plasma derived hepatitis B immunoglobulin for iv use (CPMP/BPWG/4027/02) for the marketed iv immunoglobulin preparations antibody levels above 100-150 IU/l should be maintained in HBV-DNA negative patients. For

prevention of hepatitis B recurrence after LT the dosage for adult patients is individually adapted.

The results of the performed clinical studies (Yahyazadeh A. et al 2011, Di Constanco et al, 2013) showed that trough levels of serum HBIg > 100 IU/l were achieved during weekly sc administration of Zutectra® in HBsAg negative patients after LT for HBV induced liver failure. Using weekly doses of 500 to 1,000 IU HBsAb (1-2 ml) Zutectra® no patient experienced trough anti-HBs levels < 100 IU/l and no HBV re-infection according to HBsAg and HBV-DNA determination and clinical signs were observed. Thus, it could be demonstrated that Zutectra® can be used for long-term maintenance treatment in patients after LT for HBV induced liver failure starting 6 month after LT.

Additionally, the results of study 974 demonstrate that trough levels well above 100 IU/l can be achieved with the home self-treatment of Zutectra®. Sc self-administration of Zutectra® was maintained continuously throughout 48 weeks.

6 RDC OBJECTIVES

6.1 Primary objectives

Effectiveness

- Proportion and absolute number of HBV-liver transplant patients free of hepatitis B virus recurrence as assessed by non-detectability of HBsAg and / or HBV-DNA in patients' sera.

6.2 Secondary objectives

Effectiveness

- Documentation of HBV re-infection in relation to
    - the dosage regimen and treatment duration used for HBIgs
    - the virostatic drugs administered in combination with HBIgs
    - the severity of the indication for LT
    - the immuno-suppressive treatment applied

- Documentation of:
    - recurrence of HBV-HCC in relation to pre-transplant and post-explantation histopathology
    - trough anti-HBs levels
    - individual tolerability issues related to changes of HBIg preparations

Safety

There are no primary or secondary safety objectives.

Adverse drug reactions (ADR) to Zutectra® and / or Hepatect® CP are reported as individual case safety reports (ICSR) within routine pharmacovigilance. Re-infections will be reported as SAEs immediately to the MAH.

7 ETHICAL AND REGULATORY CONSIDERATIONS

This RDC documentation plan and any substantial amendments will be submitted to properly constituted **I**ndependent **E**thics **C**ommittees (IEC) / **I**nstitutional **R**eview **B**oard (IRB) and/or Regulatory Authorities, in agreement with applicable regulatory requirements, for formal approval (if required by law) of the RDC. A copy of these approvals (if applicable) must be submitted to Biotest before initiation of the RDC and each site needs to keep a copy of these documents.

8 RDC DESIGN

This is a non-interventional, retrospective, single-arm, international multi-centre data collection after approval of the medication under investigation.

Hepatitis B treatment-related information from patients with LT will be collected.

There is no safety signal requiring the conduction of a safety study with the registered products. It is proven that HBV re-infection can be effectively prevented by administration of HBIg in combination with NUCs and the study medication is safe.

Patients treated with any HBIg after LT onwards for at least 1 year including at least a 6 months treatment with Zutectra® or Hepatect® CP in combination with or without a NUC should be evaluated for any HBV re-infection event. HBV re-infection is defined as detection of HBsAg and / or HBV-DNA in the patients' serum having used licensed diagnostic test systems for Hepatitis B Virus. Documentation should start at LT and should proceed as long as possible up to 10 years.

9 RDC POPULATION

9.1 RDC Population and Number of Patients

It is planned to collect data from about 400 patients under prophylaxis for HBV recurrence after LT as described in chapter 8. These are about 200 patients from participating centres in Group I (Germany, Netherlands, Switzerland and UK) as well as 200 in Group II (Italy). Group I and Group II encompass 8-10 transplant centres each. Thus, on average 20-25 patients are foreseen to be documented per transplant centre. If one centre documents less than 25 patients the missing patients can be documented by centres with higher patient numbers to get altogether 400 documented patients. The total number of patients per centre available for documentation shall be determined before starting the RDC.

9.1.1. Patient Selection Plan

If more than 25 patients fulfil the inclusion criteria a defined selection process should be followed in order to avoid selection bias and to get:

- long term documentations (up to 10 years)
- short term (1-4 years) documentations from treatments with most recent virostatic drugs in combination with HBIg

- data from patients with normal and complicated treatment course (e.g. HCC recurrence)

All available patients from one year should be documented before proceeding with the next year of LT. In detail, each centre should start screening all patients having the LT in the year 2004, then in the year 2010 and then should continue as outlined in Table 1, until the maximum number of patients per center is reached and documented.

Table 1)

Year of LT	Max. documentation time
2004	10
2010	4
2003	10
2011	3
2002	10
2012	2
2001	10
2013	1
2000	10
2008	6
2005	9
2007	7
2006	8

9.2 Inclusion Criteria

Only patients meeting all of the following inclusion criteria will be considered for the RDC:

1. Patients 18 years or older
2. Patients with LT for fulminant Hepatitis B, Hepatitis B –cirrhosis, or HBV-HCC inside the Milan criteria, or with liver re-transplantation except due to HBV recurrence
3. Treatment with any HBIg for at least 1 year from LT onwards including at least a treatment of 6 months with Hepatect® CP or Zutectra®
4. LT after the year 2000
5. Written informed consent from patients alive to allow data collection and data transfer to third party

9.3 RDC Patients Withdrawal Criteria and Procedures

The consent for the retrospective data collection can be terminated by an individual RDC patient due to his own request (e.g. personal reasons).

A RDC patient is entitled to discontinue participation in this RDC at any time without stating a reason.

9.4 RDC Patients' Information

The RDC patient will be informed about the RDC according to the legal requirements.

The RDC patient must have given written consent to allow the proposed data handling and access to their medical records by signing and personally dating the informed consent form before start of the RDC.

A duplicate of the signed and dated written informed consent form must be handed over to the RDC patient. Informed consent documentation will be kept at the site in order not to disclose the patients' identity to the sponsor or CRO.

Data from deceased patients who have not given informed consent beforehand will be documented after pseudonymization or anonymization as required by the respective national regulations.

10 COURSE OF THE RDC

10.1 Duration of the RDC

Start of RDC Data Collection *(planned)*	Q1 2015
Date of Analysis *(planned)*	Q2 2016

Individual RDC Patient

The individual documentation per patient starts at LT and includes a time period of at least 1 year up to 10 years from with any HBIg including at least a treatment of 6 months with Hepatect® CP or Zutectra®. All treatment related data that were recorded by the treating physician during this period will be included in the evaluation.

End of RDC

The end of the RDC will be defined as the last documentation performed in 400 patients.

11 PARAMETERS OF DOCUMENTATION

11.1 Baseline data

Baseline data to be recorded include demographic data (gender, year of birth, body height, body weight and body mass index (BMI)), medical history including concomitant liver disease other than hepatitis B relevant for LT (e.g. liver cirrhosis, HCC), viral status

(serum HBs-antibodies, HBsAg, HBV-DNA) and viral co-infections with HIV, HCV, HDV, liver function, kidney function, immunoglobulin levels and immuno-suppressive regime, as well as concomitant antiviral / hepatitis B-related medication.

For deceased patients who have not given informed consent beforehand, potentially identifiying data (e.g. gender, year of birth) will not be collected, if required by the respective national regulations.

11.2 Effectiveness Parameters: Serum HBsAb, HBsAg, HBeAg, HBV-DNA

All measurements of HBsAb, HBsAg, HBeAg, HBV-DNA performed pre-operative and after LT have to be documented. Documentation will occur in accordance to the standard of care at each participating centre.

All clinical signs of HBV re-infection (e.g. jaundice) have to be documented.

In addition the re-occurrence of HCC and the occurrence of new cancers will be documented, determined by clinical diagnostics and / or histopathology.

11.3 Safety Parameters

Adverse drug reactions (ADRs)

Following the ISPE Guideline 2008 (ISPE 2008) in this RDC no individual ADR reporting will be performed. Aggregated safety results from the study will be implemented in the Periodic Safety Update Report (PSUR).

No documentation of adverse drug reactions (ADRs) in addition to the routine, spontaneous ADR documentation by the treating physician will be performed. ADRs will not be documented in the eCRF. New safety complaints (AE, SAE) that became evident during the documentation will be reported by the treating physician within routine pharmacovigilance to Corporate Drug Safety (CDS) of Biotest for involvement of Zutectra®, Hepatect® CP or to other companies being the marketing authorisation holder (MAH) of the respective involved medication. A standard report form for the Biotest products Zutectra®, Hepatect® CP will be provided.

In case of a HBV re-infection a SAE report will be sent immediately by the physician to CDS Biotest for further processing.

12 STATISTICS

The statistical planning and evaluation of the RDC will be carried out by the contract research organization Syneed Medidata GmbH. A Statistical Analysis Plan (SAP) will be prepared before data base lock.

12.1 Analysis populations

Full Data Set defined as all patients with any data captured within this retrospective study.

Full Analysis Set defined as all patients according to the inclusion criteria.

12.2 Statistical methods

Results of an exploratory analysis are presented. Since there are no confirmatory analyses planned, hypotheses are not formulated, and p-values are not presented.

Primary and secondary endpoints

Proportions of patients free of hepatitis B virus recurrence, patients with a HBV recurrence after LT, and proportions of patients with a re-occurrence of cancer after LT will be presented as percentages (total: size of full analysis set) together with exact (according Pearson-Copper) 1-sided upper confidence limits, analyzed at full analysis set. For the occurrence of HCC a time to event analysis using the Log Rank Test will be applied.

Subgroup analyses will be performed to assess the influence on HBV recurrence by the virostatic drugs administered in combination with Zutectra®/ Hepatect CP® and other HBIg, the dosage regimen of Zutectra®/ Hepatect CP® and other HBIg applied and the immunosuppressive treatment used.

Descriptive statistics

Full analysis set is analyzed.

For continuously scaled variables, mean, standard deviation, minimum, maximum, median, 25% and 75% percentiles are tabulated. If relevant deviation from normal distribution is detected, only appropriate estimates are reported.

For categorically scaled variables, absolute and relative frequencies are tabulated.

Safety

The number of re-infections will be described in the effectiveness part.

Calculation of sample size, uncertainty of results

Sample size estimation is not applicable because this is a retrospective, non-controlled, data collection.

The population is determined by the number of available patients with Hepatect® CP and / or Zutectra® treatment after LT.

It is planned to evaluate about 400 patients.

13 DATA MANAGEMENT

13.1 Data Collection

Data will be entered to the electronic CRF (eCRF) at the study site. Data entries will be checked by automatic and manual queries according to the data validation plan. Corrections have to be entered into the eCRF at the study site.

The personnel responsible for data entry performance and controlling and specific data handling procedures will be defined upfront.

The final data will be transferred to the SAS-system for subsequent data analyses in accordance with the statistical analysis plan.

Concomitant medication will be coded by the **A**(natomical) **T**(herapeutical) **C**(hemical)-code, level <2>. MedDRA will be used for coding of concomitant diseases and medical history.

13.2 Missing Data

All available data will be included in the analyses and will be summarized as far as possible.

Unless otherwise specified there will be no substitution of missing data, i.e. missing data will not be replaced.

14 QUALITY CONTROL AND QUALITY ASSURANCE

14.1 RDC Initiation Activities

The investigator(s) will be informed about objectives and methods of the RDC by a visit of a member of the respective CRO. This will occur after a signed contract with the study site and an ethic approval of the study from the appropriate ethic commission is available. The visit will take place before the documentation is started.

14.2 Documentation and Filing

Electronic Case report form (eCRF)

All data to be recorded according to this RDC documentation plan must be documented in the eCRF. The investigator will be instructed how to use the EDC system for data entering.

Entries in the eCRF must only be made by the investigator or persons authorized by the investigator. An individual account for each authorized person will be created.

The investigator must verify that all data entries in the CRF are accurate and correct.

14.3 List of Patients (patient identification log)

The investigator is asked to keep a confidential list of names of all patients participating in the RDC, giving reference to the patients' records.

With the help of this list it must be possible to identify the patients and their medical records.

Entries for deceased patients who have not given informed consent beforehand must made unrecognisable for the monitor, if anonymized data collection is required by the respective national regulations.

14.4 Source data

Source data is all information in original records and certified copies of original records of medical findings, observations, or other activities necessary for the reconstruction and evaluation of the RDC. Source data are contained in source documents which comprise clinical documentation, data, and records (e.g., hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories and at medico-technical departments).

Source data of deceased patients who have not given informed consent beforehand must not be reviewed by the monitor, if anonymized data collection is required by the respective national regulations.

14.5 Investigator Site File

The CRO will provide an Investigator Site File to each RDC site. The ISF will include essential documents as per applicable local requirements.

The investigator will be responsible for the continuous update and maintenance of the investigator site file. In case of an audit by the sponsor or an inspection by the Regulatory Authorities these documents will be reviewed.
All RDC related documents are to be archived and stored according to legal requirements.

14.6 Monitoring

The monitor is responsible for checking the quality of data and adherence to RDC documentation plan, to legal and ethical requirements according to local laws.

RDC source data verification is an essential part of the monitoring process and the investigator must grant direct access to the RDC Patients' source data.

A combination of centralized and on-site monitoring will be applied to assure data quality in this RDC. The extent and nature of monitoring will be described in detail in the monitoring plan.

14.7 Audits and Inspections

Audits will be performed according to the corresponding audit program, including the possibility that a member of the sponsor’s quality assurance function may arrange to visit the investigator in order to audit the performance of the RDC at the centre, as well as all documents originating there. Audits may also be performed by contract auditors.

In this case, the sponsor's quality assurance function will agree with the contract auditor regarding the timing and extent of the audit(s). In case of audits at the investigational site, the monitor will usually accompany the auditor(s).

Inspections by regulatory authority representatives and IECs/IRBs are possible at any time, even after the end of RDC. The investigator is to notify the sponsor immediately of any such inspection. The investigator and institution will permit RDC related monitoring, audits, reviews by the IEC/IRB and/or Regulatory Authorities, and will allow direct access to source data and source documents for monitoring, audits, and inspections.

14.8 Archiving

After evaluation and reporting of the data, all documents relating to the RDC will be kept in the archives of the CRO or sponsor for at least 10 year in according to national and European law and the clinical site(s) according to applicable local regulatory requirements.

15 GENERAL REGULATIONS, AGREEMENTS AND ORGANISATIONAL PROCEDURES

15.1 RDC Administrative Structure

Details for the administrative structure are kept as a separate list filed in the abbreviated NIS Trial Master File.

15.2 Insurance

No study specific insurance is required for the RDC.

15.3 Data protection

Pseudonymization will be used to allow only with a list at the study center to determine patients identity. Only data after pseudonymization will be stored and forwarded for analysis. Each patient has to sign an agreement to give his consent to use his medical data. This will be part of the informed consent form which will get approval by the respective competent EC.
Data from deceased patients will be documented without informed consent after pseudonymization or anonymization as required by the respective national regulations.

15.4 Study conduct, data management and analysis

The study will be handled by the CRO Syneed Medidata GmbH and local, national subcontractors of the CRO. Details of the tasks and responsibilities are regulated in the study contract between the sponsor of the study Biotest AG and the CRO.

15.5 Written Agreements

A written agreement will be set up between Biotest AG or the CRO and each investigator setting out any arrangements on delegation and distribution of tasks and obligations and on financial matters.

15.6 Confidentiality

The objectives and contents of this RDC as well as its results are to be treated as confidential and may not be made accessible to third parties.

15.7 Final Report and Publication

The aim of this RDC is to provide additional long term treatment data for patients being treated with Zutectra® and Hepatect® CP after LT.

A RDC report will be produced. At the end of the RDC the sponsor will provide the competent authority with a summary of the final report within the required timelines. After data analysis on request anonymzed data of all RDC patients will be forwarded to the participating study centers.

A publication group including representatives from study centers form all countries involved will prepare a manuscript for publication.

Each investigator is obligated to keep data pertaining to the RDC secret. He/she must consult with the sponsor before any RDC data are published.

The legitimate interests of the sponsor, such as acquiring optimum patent protection, coordinating submissions to the health authorities or coordination with other studies in the same field that are underway, protection of confidential data and information, etc. will be given due consideration by all partners involved.

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