A prospective, randomised, open-label phase IIb clinical trial assessing the effect of pegylated Interferon alfa-2a (Pegasys®) 180 µg once weekly for 48 weeks in addition to an ongoing nucleos(t)ide based treatment on quantitative HBsAg levels in patients with chronic HBeAg-negative hepatitis B

PADD-ON

***P*eg-interferon *ADD*ed to an *O*ngoing *N*ucleos(t)ide based treatment in patients with chronic hepatitis B to induce decrease of HBs antigen**

EudraCT No: 2011-002812-10

Sponsor trial code: PADD-ON

Roche code: ML 27787

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List of abbreviations

AE	Adverse event
AFP	alpha-fetoprotein
ALT	alanine aminotransferase
AMA	anti-mitochondrial antibody
AMG	German drug law (Arzneimittelgesetz)
ANA	anti-nuclear antibody
ANC	absolute neutrophil count
ANCA	Anti-neutrophil cytoplasmic antibody
APC	antigen presenting cell
AST	aspartate aminotransferase
BMD	bone mineral density
BUN	blood urea nitrogen
CHB	chronic hepatitis B
CPK	creatine phosphokinase
CK	creatinine Kinase
CPT	Child-Pugh-Turcotte
CRF	Case report form
CV	Curriculum vitae
CT	Computed Tomography
dATP	deoxyadenosine triphosphate
DF	disoproxil fumarate
DMSO	Dimethyl sulfoxide
DNA	deoxyribonucleic acid
DSMB	Data safety monitoring board
EC/IEC	Ethics committee/Independent ethics committee
e-CRF	Electronic case report form
ECG	Electrocardiogram
EEG	electroencephalogram
EMEA	European Medicines Evaluation Agency
ETV	Entecavir
EU	European Union
FDA	(U.S.) Food and Drug Administration
FSI	First subject in
FTC	Emtricitabine
g	g-force
Gamma-GT	gamma-glutamyl transferase
GCP	Good clinical practice
GCP-V	GCP regulation
HBeAb	hepatitis B e antibody
HBeAg	hepatitis B e antigen
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HDL	high density lipoprotein
HDV	hepatitis D virus
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
ICH	International conference on harmonization of technical requirements for registration of pharmaceuticals for human use
IFN	interferon
IgG	immunglobulin G
IL-2	interleukin 2
IMP	Investigational medicinal product
IRB	Institutional review board
ISRCTN	International standard randomised controlled trial number
INN	International nonproprietary name
INR	international normalized ratio

ISF	Investigator site file
IUD	intra-uterine device
IV	Intravenous
LAM	lamivudine
LBW	lean body weight
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LFT	liver function test
LKP	Clinical Trial Director according to AMG (Leiter der Klinischen Prüfung)
LLN	Lower limit of normal
LSI	Last subject in
LSO	Last subject out
MedDRA	Medical dictionary for regulatory activities terminology
MELD	Model for End Stage Liver Disease
mITT	Intention to treat
MRI	magnetic resonance image
Non-IMP	Non investigational medicinal product
NSAID	Nonsteroidal anti-inflammatory drugs
Nuc	nucleos(t)ide analogon
PBMC	Peripheral blood mononuclear cells
PCR	polymerase chain reaction
PE	physical examination
Peg	pegylated
Peg-IFN	Pegylated interferon alfa-2a
PO	oral administration (per os, by mouth)
PT	prothrombin time
QD	once daily
RNA	ribonucleic acid
rpm	rounds per minute
RPMI	Roswell Park Memorial Institute medium
RR	Response Rate
rt	reverse transcriptase
SAE	Serious adverse event
SAP	Statistical analysis plan
SC	subcutaneous
SDV	Source data verification
SLA	anti-soluble liver antigen antibody
SMA	anti-smooth muscle antibody
SOC	standard of care
SPC	Summary of product characteristics
SUSAR	Suspected unexpected serious adverse reaction
TDF	tenofovir DF, tenofovir disoproxil fumarate
TID	three times a day
TMF	Trial master file
TNF	tumor necrosis factor
T-regs	regulatory T-cells
ULN	upper limit of the normal range
US	United States

Synopsis

Title	A prospective, randomised, open-label phase IIb clinical trial assessing the effect of pegylated interferon alfa-2a (Pegasys®) 180 µg once weekly for 48 weeks in addition to an ongoing nucelos(t)ide based treatment on quantitative HBsAg levels in patients with chronic HBeAg-negative hepatitis B
Short title	PADD-ON Peg-interferon ADDed to an Ongoing Nucleos(t)ide based treatment in patients with chronic hepatitis B to induce decrease of HBs antigen
EudraCT No	2011-002812-10
Sponsor trial code	PADD-ON
Roche code	ML 27787
Indication	Chronic viral hepatitis B, HBe-antigen negative
Phase	Phase IIb
Treatments	Test product: Pegylated interferon alfa-2a, s.c. 180 µg once per week in addition to nucleos(t)ide(s) Reference therapy: Nucleos(t)ide therapy
Primary objective	The primary objective of the trial is to investigate whether the add-on of pegylated interferon alfa-2a to a continued treatment with nucleos(t)ide analogues increases the percentage of patients who have significant decrease (≥1$\log_{10}$) of HBs antigen after 48 weeks.

Secondary objectives	• To evaluate safety and tolerability of pegylated interferon alfa-2a when combined with tenofovir, entecavir, lamivudine, adefovir, or a combination of lamivudine or entecavir with adefovir or tenofovir
	  ○ Adverse events
	  ○ Vital signs, physical examination
	  ○ Laboratory test abnormalities
	  ○ Laboratory test value changes over time
	• To identify biological variables predicting HBs antigen decrease:
	  ○ viral genotype
	  ○ time course of HBs antigen level

Scientific objectives	• To determine the effect of pegylated interferon on frequency and functional properties of HBV-specific cytotoxic and regulatory T-cells in responder and nonresponder patients • To identify potentially predictive biomarkers
Trial design	Prospective, randomised, open-label
Trial population	**Main inclusion criteria:** Subjects meeting all of the following criteria will be considered for enrolment to the trial: • Chronic hepatitis B, HBe antigen negative • Treatment with a stable oral antiviral treatment (not containing telbivudine) and a fully suppressed viral load for at least 12 months (below limit of detection in conventional HBV-PCR assays, e.g. <116 copies / mL or <20 IU/mL) • 18-70 yrs • willingness and ability to give informed consent and to follow study procedures • willingness to use adequate contraception **Main exclusion criteria:** Subjects presenting one of the following criteria will not be enrolled in the trial: • contraindications against treatment with pegylated interferon, e.g. depression, uncontrolled epilepsy, autoimmune diseases, pregnancy, leukocytopenia or thrombocytopenia at screening, etc. • active alcohol or drug abuse • preexisting polyneuropathy
Trial duration and dates	First subject in: ~~July~~ September 2012 Last Subject In: ~~July 2013~~ December 2014 Last subject out: ~~June 2014~~ November 2015 (end of follow-up ~~January 2015~~ May 2016) Duration of the trial ~~30~~ 44 months

Number of subjects	It is planned to enrol 170 subjects
Number of sites	~~10~~ 16-25 trial sites are planned to participate.
Primary endpoint	The primary endpoint is the objective response after 48 weeks of combination therapy. The response is defined as a confirmed reduction of ≥1log_{10} in HBs antigen compared to baseline.
Secondary endpoints	• Decline of quantitative HBs antigen at week 12 and 24 • Rate of patients with at least 10% HBs antigen loss at week 24 compared to baseline • HBsAg seroconversion defined as percentage of subjects who become HBsAg negative and anti-HBs positive during the observation period • Safety and tolerability of pegylated interferon alfa-2a when added to a continuing treatment with tenofovir, entecavir, lamivudine, adefovir, or a combination of those: Analysis of adverse events and laboratory data. • HBs antigen levels at all measurement times
Statistical analysis	The response rates will be compared between the treatment groups by Fisher's exact test. The primary analysis population is the modified Intention to treat population comprising all randomised patients for which the primary endpoint can be assessed. Further supportive analyses are planned (Per Protocol Analysis, Logistic Regression with relevant covariates). Secondary endpoints will be analysed using appropriate methods depending on the scale of the respective parameter. Analyses of secondary endpoints will be interpreted descriptively. Safety: Frequencies of subjects experiencing at least one adverse event (AE) will be displayed by body system and preferred term according to MedDRA terminology. A subject listing of AEs will be prepared. Listings will be prepared for each laboratory measure and will be structured to permit review of the data per subject as they progress on treatment. Summary tables will be prepared to examine the changes of laboratory measures over time. Additionally, shift tables will be provided to examine the changes of laboratory data from normal baseline to values outside the corresponding reference range during/after treatment.

Flow chart – Treatment group (Pegasys® + nucleos(t)ide)

	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12	Visit 13	Visit 14	Visit 15
	Screening	Baseline												EoT	EoFU
	Week -4 to 0	Week 0	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24	Week 28	Week 32	Week 36	Week 40	Week 44	Week 48	Week 72
Informed consent	x														
Medical history	x														
Eligibility criteria	x														
12 Lead ECG	x														
Virology	x														
Liver assessment	x														
Drug screening, urinalysis	x														
Pregnancy screening	x				x			x			x			x	
Randomisation		x													
Physical examination[1]	C	T	T	T	T	T	T	T	T	T	T	T	T	C	C
AEs[2], concomitant med.	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Routine laboratory tests[3]	III	I	I	I	II	I	I	II	I	I	I	I	I	II	II
HBV-PCR	x				x			x						x	x
HBs antigen / anti-HBs	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Immunology[4]		x	x	x										x	
Bio-[5] and genetic markers[6]		x[5,6]	x[5]	x[5]	x[5]			x[5]			x[5]			x[5]	x[5]

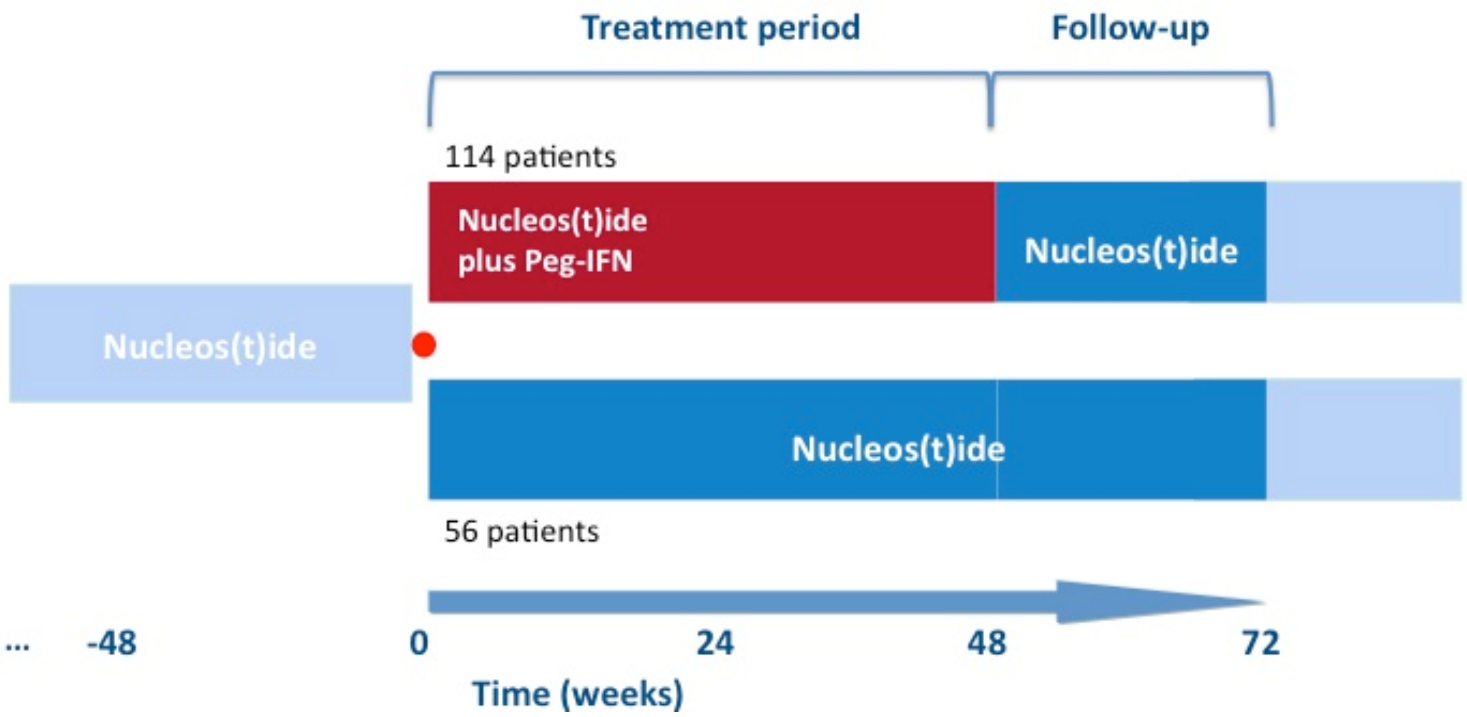
Flow chart – Control group (nucleos(t)ide only)

	Visit 1	Visit 2			Visit 3			Visit 4			Visit 5			Visit 6	Visit 7
	Screening	Baseline												EoT	EoFU
	Week -4 to 0	Week 0	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24	Week 28	Week 32	Week 36	Week 40	Week 44	Week 48	Week 72
Informed consent	x														
Medical history	x														
Eligibility criteria	x														
12 Lead ECG	x														
Virology	x														
Liver assessment	x														
Drug screening, urinalysis	x														
Pregnancy screening	x							x						x	
Randomisation		x													
Physical examination[1]	C	T			T			T			T			C	C
AEs[2], concomitant med.	x	x			x			x			x			x	x
Routine laboratory tests[3]	III	I			II			II			I			II	II
HBV-PCR	x				x			x						x	x
HBs antigen / anti-HBs	x	x			x			x			x			x	x
Immunology[4]		x												x	
Bio-[5] and genetic markers[6]		x[5,6]			x[5]			x[5]			x[5]			x[5]	x[5]

Footnotes:

1. Virology: HCV, HDV, HIV testing; HBe Ag, anti-HBe, anti-HBc
2. Physical examination: “C” is a complete physical examination. “T“ is a targeted physical examination including measurements of vital signs, and evaluation of organ systems particularly associated with adverse event(s) symptoms.
3. For patients with early withdrawal from medication, the End of follow-up visit is at 24 weeks after the End of treatment visit.
   I = erythroctes, hemoglobin, hematocrit, platelets, total leukocytes and differential, AST, ALT, total bilirubin, gamma-GT, alkanine phostphatase, total bilirubin (and conjugated bilirubin if total bilirubin is >1.5 x ULN), LDH, sodium, potassium, calcium, creatinine (and calculated clearance), blood urea nitrogen (BUN), uric acid, glucose, PT, INR
   II = erythroctes, hemoglobin, hematocrit, platelets, total leukocytes and differential, AST, ALT, total bilirubin, gamma-GT, alkanine phostphatase, total bilirubin (and conjugated bilirubin if total bilirubin is >1.5 x ULN), LDH, sodium, potassium, calcium, creatinine (and calculated clearance), blood urea nitrogen (BUN), uric acid, glucose, PT, INR, albumin, TSH, fT3, fT4
   III = erythroctes, hemoglobin, hematocrit, platelets, total leukocytes and differential, AST, ALT, total bilirubin, gamma-GT, alkanine phostphatase, total bilirubin (and conjugated bilirubin if total bilirubin is >1.5 x ULN), LDH, sodium, potassium, calcium, creatinine (and calculated clearance), blood urea nitrogen (BUN), uric acid, glucose, PT, INR, albumin, TSH, fT3, fT4, AFP, IgG, ANA, SMA, SLA, LKM, AMA, ANCA, ferritin, lipid profile (triglycerides, total cholesterol, HDL, LDL), HLA typing, urinalysis
4. Immunology/Immune monitoring: Blood samples (50 mL each) for PBMC purification and immune monitoring
5. Biomarkers: blood samples of 15-20 mL each, serum samples to be stored at -80 °C
6. Genetic Markers: 2 vials of 2 mL EDTA blood to be frozen in liquid nitrogen and stored at -80°C.

Treatment algorithm



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1 INTRODUCTION

1.1 Scientific background

Nucleos(t)ides are very successful in avoiding disease progression in patients with chronic hepatitis B by suppressing HBV-DNA replication. However, in most patients this requires long-term medication of undefined length. HBsAg seroconversion as a marker for endogenous immunological control is observed only in a minority of these patients, even under a long-term nucleos(t)ide therapy[12], and it almost never occurs in HBe-Ag negative hepatitis B[2].

This calls for more effective treatment regimens to achieve immunological control within a defined treatment duration.

An *acute* hepatitis B is effectively controlled in more than 95% of cases by the immune system. The “innate immunity” is the first and decisive mechanism as it activates specific cellular immune responses[3]. However, if this response fails, the immune system switches to a more or less anergic state, which allows chronic replication. Several parts of the immune system have been shown to be affected in chronic infection: HBV antigen-specific $CD4^+$ T-cells are less active against viral antigens[4], intrahepatic $CD8^+$ T-cells in general are functionally impaired[5], the expression of “programmed death-1”(PD-1)-receptors on lymphocytes is increased[6], and the number of $CD4^+CD25^+Foxp3^+$ T-cells (T-regs), is higher, leading to suppression of the specific immune response[7]. In addition, it was reported that HBs antigen directly inhibits antigen-presentation of professional APCs like macrophages[8].

The peripheral quantitative HBs antigen correlates with the amount of intracellular cccDNA which is the latent reservoir of HBV-DNA inside the hepatocytes. Some data show that HBsAg levels can be reduced by long-term nucleos(t)ide treatment[910], with the newer drugs being more effective[11].

Interestingly, it was shown that treatment with nucleos(t)ide analogues partly restores the number of hepatitis B-specific $CD4^+$ T-cells[12]. Most recent data further support the idea that viral load reduction re-increases immunological function of the innate immune system[13] and decreases PD-1 and PD-L1 expression on HBV-specific T-cell populations[14].

Interferon is known to be even more effective in reducing HBsAg[15]. It acts via induction of several proteins, which stimulate innate and adaptive immunity[16]. Effectivity of interferon seems to be higher if pre-treatment levels of IFN Type I response genes and proteins are not already elevated in chronic infection.

The faster the decline of quantitative HBs antigen under interferon treatment, the more often an HBsAg seroconversion was observed in the subsequent time[17]. The assessment of this decline may therefore be used to decide at an early time point which patient will profit from interferon treatment[18].

The potency of both interferon and modern nucleos(t)ide analogues to reduce HBsAg levels is suggestive to combine both substances. Previous trials using this combination were not successful, but in these studies, less potent nucleos(t)ides were used, and were always started at the same time as interferon[19,20], although it is known that it usually takes several months until HBV-DNA is completely suppressed. A recently published trial combined adefovir or lamivudine with peg-interferon but extended treatment duration for up to 96 months, reaching high rates of HBsAg seroconversion of 30% with lamivudine and 24% with adefovir[21].

Recently, a combination of peg-interferon and telbivudine showed the rate of HBsAg reduction is markedly more rapid with the combination than either drug alone. Unfortunately, the combination arm had increased rates of peripheral neuropathy; thus the study was terminated prematurely[22].

Pilot data

We monitored HBsAg levels of twelve patients who had decided to receive additional peg-interferon alfa-2a as an individualized therapy. Nine patients were male, mean age was 44 (range 25-60) years. Three patients were HBeAg positive. Cirrhosis was histologically proven in four

patients. Current treatment comprised lamivudine (one patient), lamivudine plus adefovir (two patients), entecavir (seven patients), or entecavir plus tenofovir (two patients). When starting additional interferon treatment, viral load had already been undetectable for an average of 24.6 (range 10-57) months. Mean baseline HBsAg was 4,695 (range 16-15,120) IU/ml and was negatively correlated with time of complete viral suppression (correlation coefficient: - 0.234).

In two patients HBsAg continuously declined by -2.6log_{10} and -3.58log_{10} to week 28 and 36 as compared to baseline. The slope started in week 8 and 16, respectively. The first patient was HBeAg negative, was infected with genotype D, had a very low initial HBsAg level, and HBV had already been suppressed by entecavir for 27 months. HBsAg dropped down to 0.04 U/l. The second patient was HBeAg-positive, infected with genotype A, and HBV was fully suppressed for ten months with entecavir plus tenofovir. She had an HBeAg seroconversion at week 24 and even developed anti-HBs of 10 U/l at week 32. In all other patients quantitative HBsAg only decreased by 0.07 log_{10} (range 0.01 - 0.25 log_{10}) over 8-24 weeks of combination therapy (mean 16 weeks), and therefore, interferon was stopped. Side effects were comparable to interferon monotherapy[30].

1.2 Trial rationale

In patients with HBeAg negative hepatitis B decrease or loss of HBs antigen occurs only rarely during therapy with either nucleos(t)ide analogues or interferon, even if suppression of viral replication (as indicated by an undetectable HBV-DNA) is achieved. As there are no known means to induce immune control of HBV yet, these patients often need long-time medication for many years.

The rationale of this trial is to assess whether, in patients with chronic HBeAg-negative hepatitis B, the addition of pegylated interferon alfa-2a to an ongoing therapy with nucleos(t)ide analogues increases the percentage of patients who have a significant decrease (≥ 1log_{10}) of HBs antigen after 48 weeks. This might then predict loss of HBsAg in the future and the option to terminate antiviral therapy.

1.3 Treatments and rationale for dose selection

Patients in the treatment group and in the control group will continue their oral medication with lamivudine, adefovir, entecavir, tenofovir or a combination thereof. In addition, patients in the treatment group will receive 180 µg of pegylated interferon alfa-2a once weekly for 48 weeks. The dosing and duration of the interferon therapy are the same as recommended for a monotherapy of chronic hepatitis B.

Due to the increased neurotoxicity of pegylated interferon alfa-2a when combined with telbivudine in a previous study, use of telbivudine is not allowed.

Previous studies on monotherapy with pegylated interferon have shown that decrease of HBs antigen after 12 to 24 weeks can be predictive of HBs antigen loss or seroconversion and may thus be used to determine treatment failure at an early timepoint. However, most of the patients in those studies had HBeAg positive hepatitis B. The kinetics of HBs antigen decrease in HBeAg negative patients might be different. In addition, the effect of the treatment with nucleos(t)ide analogues, especially when started months or years before, is unknown.

To account for these factors, interferon therapy in this trial should continue for 48 weeks in all patients in the treatment group independent of the occurrence of HBs antigen decrease or loss.

1.4 Summarized risk-benefit assessment

The effectiveness as well as possible risks and side effects of both pegylated interferon alfa-2a and nucleos(t)ide analogues are well described.

Side effects of interferon can be very disagreeable and, in the case of uncontrolled hematologic alterations, even dangerous; nevertheless in most cases these side effects resolve within a few months of the termination of interferon treatment. More severe side effects with a lasting impact on patients' health (e.g. contribution to the development of autoimmune disease, lasting depression) are rare.

Oral antiviral agents, nucleoside and nucleotide analogues that are used in the treatment of hepatitis B, are usually well tolerated. However, they must be taken regularly, and, in most cases, over a course of many years or even life-long. Risks of this kind of therapy are on the one hand side the possible development of resistance mutations, and on the other side effects that might develop over many years (e.g. nephropathy or osteoporosis).

In recent years, interferon-alfa has been used in combination with different nucleos(t)ide analogues in the setting of clinical trials, and for the combination with lamivudine or adefovir no significant additional toxicity or interactions were noted[19-21,23]. A study using a combination of peg-interferon with telbivudine however had to be terminated because of a higher incidence of polyneuropathy in the treatment group[22]. Therefore, patients on telbivudine treatment will not be included into this study, and all patients will be closely monitored for adverse events, especially for the development of polyneuropathy.

In summary, we feel that the prospect that a combination therapy might reduce HBsAg and ultimately lead to a loss of HBsAg in some patients, thereby allowing for them to stop all antiviral therapy (and the associated long-term side effects), compensates for the possible risks.

The investigators will be informed about any relevant or new findings related to combined therapy of peg-interferon and antiviral agents.

2 TRIAL OBJECTIVES

2.1 Primary objective

The primary objective of the trial is to investigate whether the add-on of pegylated interferon alfa-2a to a continued treatment with nucleos(t)ide analogues increases the percentage of patients who have significant decrease (≥1 $\log_{10}$) of HBs antigen after 48 weeks.

2.2 Secondary objectives

The secondary objective(s) of the trial are

- To evaluate the safety and tolerability of pegylated interferon alfa-2a when combined with tenofovir, entecavir, lamivudine, adefovir, or a combination of those,
  - Adverse Events (classified into mild/moderate/severe)
  - Tolerability, vital signs and physical examination
  - Laboratory test abnormalities
  - Laboratory test value changes over time
- To identify biological variables predicting HBs antigen decrease, e.g.:
  - viral genotype
  - time course of HBs antigen level

2.3 Scientific objectives

- To determine the effect of pegylated interferon on the frequency and functional properties of HBV-specific cytotoxic and regulatory T-cells in responder and non-responder patients
- To identify potentially predictive biomarkers

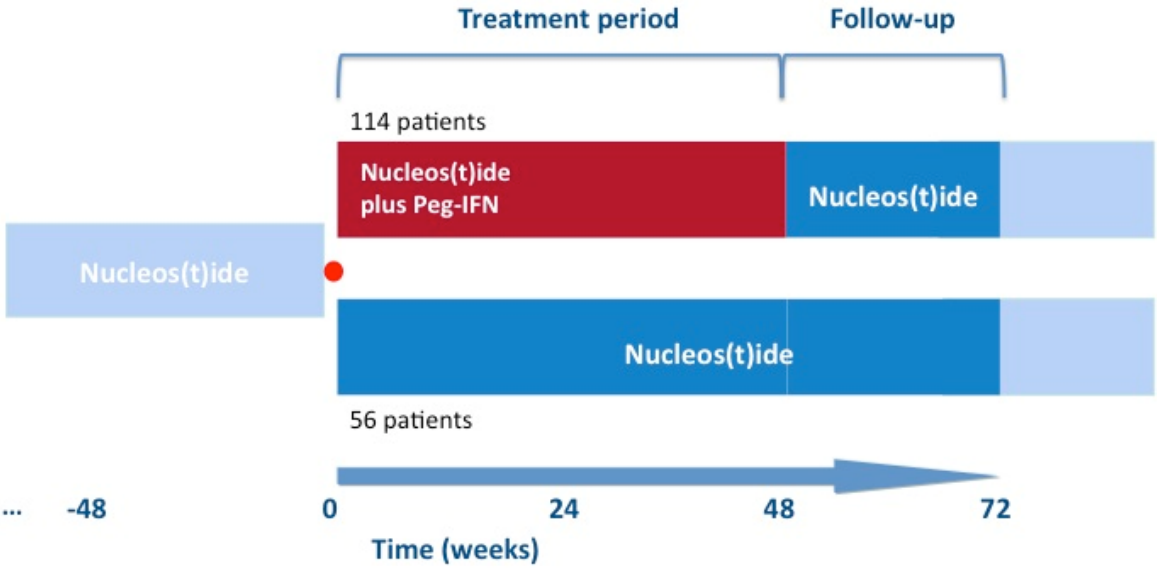
3 TRIAL DESIGN

3.1 Trial duration and schedule

The overall duration of this trial is expected to be ~~24~~ 44 months. The subject recruitment is proposed to start in ~~January~~ September 2012 and end in ~~June 2012~~ December 2014.

There will be an individual screening period of approximately 4 weeks. After randomisation, the treatment period will take 48 weeks. Follow-up will continue for another 24 weeks.

The actual overall trial duration or subject recruitment period may vary from this time period.



3.2 Number of subjects

It is planned to enrol 170 subjects in the clinical trial, i.e. 114 subjects in the treatment group and 56 subjects in the control group. Recruitment and treatment of subjects are expected to be performed in ~~10~~ 16-25 trial centers.

3.3 Primary endpoint

The primary endpoint is the objective response after 48 weeks of combination therapy. The response is defined as a confirmed reduction of ≥1$\log_{10}$ in HBs antigen compared to baseline.

3.4 Secondary endpoints

- Decline of quantitative HBs antigen at week 12 and 24
- Rate of patients with at least 10% HBs antigen loss at week 24 compared to baseline
- HBsAg seroconversion defined as percentage of subjects who become HBsAg negative and anti-HBs positive during the observation period
- Safety and tolerability of pegylated interferon alfa-2a when added to a continuing treatment with tenofovir, entecavir, lamivudine, adefovir, or a combination of those: Analysis of adverse events and laboratory data.
- HBs antigen levels at all measurement times

3.5 Measures taken to minimize/avoid bias

3.5.1 Randomisation

Subjects will be randomised after checking if all inclusion criteria are met and no exclusion criterion is met. Randomisation will be done in a 2:1 ratio (Pegylated interferon alfa-2a in addition to nucleos(t)ide(s) vs. Nucleos(t)ide therapy) using block randomisation stratified by HBsAg at screening (<20.000 IU/mL vs. ≥20.000 IU/mL). Randomisation lists will be generated at IZKS Mainz by means of a SAS program. The randomisation list will be kept in safe and confidential custody at IZKS Mainz.

A web based randomisation tool developed by IZKS Mainz will be used within this trial allowing investigators to randomise patients via a secure web interface. Role specific access rights and the need to confirm all details necessary for stratified randomisation (HBsAg at screening (<20.000 IU/mL vs. ≥20.000 IU/mL) are incorporated within the tool and will reduce the risk of misuse and

unintended randomisations. The randomisation tool will not be released for use within the study before all components implemented specifically for the trial have been sufficiently tested.

3.5.2 Blinding

This trial is an open controlled trial. Blinding of patients and study personal would be challenging because pegylated interferon alfa-2a is administered as subcutaneous injection. The lack of blinding is less problematic in this trial, since the primary endpoint is not prone to subjective influence.

3.6 Selection and withdrawal of subjects

No subject will be allowed to enrol in this trial more than once.

Enrolled patients who discontinue from the trial before intake of at least one dose of study medication will be replaced.

3.6.1 Inclusion criteria

Subjects meeting all of the following criteria will be considered for admission to the trial:

- Chronic hepatitis B, HBe antigen negative, confirmed by documented positive HBs antigen for > 6 months
- Treatment with a nucleos(t)ide regimen (lamivudine, adefovir, entecavir, tenofovir or one of the following combinations: lamivudine/adefovir, lamivudine/tenofovir, entecavir/adefovir or entecavir/tenofovir) and a fully suppressed viral load for at least 12 months (below limit of detection in conventional HBV-PCR assays, e.g. <116 copies/mL or <20 IU/mL)
- Quantitative HBsAg value available at least 180 days prior to screening
- HBsAg ≥~~1000~~ 100 IU/mL
- Normal retinal finding on fundoscopy within 6 months prior to Day 1 (baseline)
- 18-70 years
- Ability of subject to understand character and individual consequences of clinical trial
- Signed and dated informed consent of the subject must be available before start of any specific trial procedures.
- Women of childbearing potential have to be practicing a medically accepted contraception during trial and a negative pregnancy test (serum or urine) should be existent before trial.

3.6.2 Exclusion criteria

Subjects presenting with any of the following criteria will not be included in the trial:

- HBe antigen positive Hepatitis B
- Co-infection with HCV, HDV or HIV – as based on positive serology or PCR
- Ongoing antiviral treatment with telbivudine
- Contraindications against treatment with pegylated interferon, e.g. severe depression, epilepsy, autoimmune diseases, pregnancy, leukocytopenia or thrombocytopenia at screening, etc.
- Preexisting polyneuropathy
- ~~Histologically proven liver cirrhosis,~~ Decompensated liver disease, or history of decompensated liver disease, as evidenced by ascites, portal hypertension, jaundice or hepatic encephalopathy, coagulopathy, varices, history of varicose bleeding, or any other clinical evidence of decompensation. (Patients with stable liver cirrhosis are eligible for this study, if a history of decompensated liver disease as outlined above has been excluded.)
- History of alcohol or drug abuse other than cannabis within the past 12 months. Patients with documented drug and alcohol addiction free history of at least 12 months who are, in the opinion of the investigator, unlikely to relapse, may be enrolled in the study.

- Body mass index < 18 or > 35 kg/m^2
- Usage of any investigational drugs within ~~12~~ 3 months before enrolment; or the planned usage of an investigational drug during the course of the current study
- Known hypersensitivity to any ingredient of the study drugs
- A condition that is defined as one which in the opinion of the investigator may put the patient at risk because of participation in the study or may influence the results of the study or the patient's ability to participate in the study.
- Active malignancy or history of malignancy within the last 5 years (with the exception of appropriately treated basal cell carcinoma of the skin or in situ carcinoma of the uterine cervix). Suspected but yet unproven hepatocellular carcinoma.
- Alpha fetoprotein value >100 ng/mL at screening; if > 20 ng/mL and ≤ 100 ng/mL, patients can be included if there is no evidence of liver cancer in an appropriate imaging study (e.g., ultrasound, CT scan, MRI) within past 6 months of Day 1.
- Total bilirubin >2 x ULN with ratio of direct/indirect > 1 (Patients with Gilbert's syndrome are not excluded.)
- ALT or AST level >3 x ULN
- Prothrombin time INR (Institutional Normalised Ratio) prolonged to >1.5
- Hemoglobin < 11.5 g/dL for women and < 12.5 g/dL for men
- White blood cell count < 2,000 cells/mm^3
- Absolute neutrophil count < 1,500 cells/mm^3
- Platelet count < 90,000 cells/mm³
- TSH, fT3 and fT4 outside normal limits and no adequately controlled thyroid function; patients with TSH below the lower limit of normal may be enrolled if free T4 is normal and there is no clinical evidence of hyperthyroidism or hypothyroidism.
- Poorly controlled diabetes mellitus as evidenced by HbA1c > 7.5%
- Hemoglobinopathy (e.g., thalassemia major or sickle cell anemia)
- History of moderate, severe or uncontrolled psychiatric disease, especially depression, including a history of hospitalisation or prior suicidal attempt; patients with a history of mild, stable depression may be enrolled provided that a pre-treatment assessment of the patient's psychiatric disease supports that the patient is clinically stable.
- Clinical evidence of chronic cardiac disease (e.g., coronary artery disease, congestive heart failure, uncontrolled hypertension, significant arrhythmia)
- Clinically significant abnormalities on screening ECG
- Clinical evidence of chronic pulmonary disease (e.g., chronic obstructive pulmonary disease) associated with functional impairment
- Active autoimmune disease, including autoimmune hepatitis
- Organ transplant history, other than cornea or hair
- Active seizure disorder within the past 2 years; patients may be enrolled if on stable medication and seizures have not been experienced for more than 2 years before enrolment.
- Requirement for chronic systemic corticosteroids (nasal or pulmonary steroids will be allowed)
- Pregnancy and lactation
- Medical or psychological condition that would not permit completion of the trial or signing of informed consent

3.6.3 Withdrawal criteria

Subjects can withdraw their consent at their own request without given reasons at any time during the trial. This should be without any disadvantages for the subject. However the investigator should try to perform a final visit to get concluding findings of investigation.

In addition, subjects may be withdrawn from the trial for the following reasons

- At their own request.
- If, in the investigator's opinion, continuation of the trial would be detrimental to the subject's well-being.
- For women, if it becomes known that the subject is pregnant.
- If development of a toxicity or adverse event warrants drug discontinuation.
- If polyneuropathy develops and is confirmed by a neurologist, withdrawal will be discussed according to severity of the symptoms
- If the Patient has to take any concomitant drug interfering with the study medication
- If the Patient needs any excluded concomitant medication (see chapter 4.2)
- If Patient is no longer able to participate for other medical reasons (e.g., surgery, adverse events, pregnancy or other diseases).

In all cases, the reason for withdrawal must be recorded in the CRF and in the subject's medical records. In case of withdrawal of a subject at his/her own request, if possible the reason should be asked for and documented. The subject must be followed up and if possible, all examinations scheduled for the final trial day should be performed and documented.

For patients with early withdrawal from medication, the End Follow up visit is at 24 weeks after the End of Treatment visit. For patients with a detectable VL in the End of Follow-up visit after negative VL in the previous visits, an independent confirmatory measurement of viral load is required.

If a patient is withdrawn from the study prior to Visit 2 (Baseline at week 0), only data with regard to demographics, AEs and concomitant medications will be captured and reported.

Lost to follow-up:

A patient will be considered lost to follow-up if the investigator is not able to contact him despite multiple attempts (at least 2 telephone contacts plus 1 mailing). Any patient who prematurely discontinues from the study at any time must have their appropriate visit eCRF pages completed. If the patient terminates the trial early, Visit 14 or 6 (end of treatment) and the Trial completion Form must be completed (cf. section 5, End of follow-up). If the patient terminates the trial early and does not return for Visit 14, the Trial Completion from should be completed explaining the reason.

3.6.4 Premature closure of trial sites

Trial sites may be closed at any time during the trial at the discretion of the sponsor. Reasons for premature closing of a trial center include:

- Major protocol violations
- Violations of legal and ethical regulations (GCP)
- Poor recruitment
- Non-compliance of investigator.

3.6.5 Premature closure of the clinical trial

The following reasons the whole trial may be discontinued at the discretion of the sponsor:

- New risks for subjects become known.
- Inefficacy of the trial medication becomes evident.
- Occurrence of AEs unknown to date in respect of their nature, severity, and duration or the unexpected increase in the incidence of known AEs.

- Medical or ethical reasons affecting disadvantageous the continued performance of the trial.
- Difficulties in the recruitment of subjects.

The ethic committees (EC) and the competent authorities must then be informed. Should the trial be closed prematurely, all trial material (completed, partially completed, blank CRF, randomisation envelopes, investigational medicinal products, etc.) must be returned to the sponsor or fetched by the monitor.

4 TRIAL TREATMENTS

4.1 Investigational treatments

Pegylated interferon alfa-2a (Pegasys®) (IMP):

The treatment group will receive pegylated interferon alfa-2a (Pegasys®) 180 µg s.c. once weekly for 48 weeks.

The initial dose will be given in the clinic. Subjects will be taught self-administering injections at the baseline visit, and will be encouraged to self-administer all subsequent doses. Alternatively, a trained caregiver may administer peg-IFN injections per investigator discretion.

Patients will be instructed to return empty containers as well as unused study medication in the original packaging at each study visit. The investigators must instruct subjects on the safe storage and transport of used and unused syringes / PENs.

Temporary interruptions of study drug(s) administration are discouraged; patients are to remain on treatment for the entire duration of the trial; and the dose of peg-IFN should not be changed during the trial, unless dose reduction is indicated because of laboratory abnormalities/adverse events (c.f. Section 4.1.8 for instructions for dose reduction). The patients must be counselled regarding the importance of not missing doses of peg-IFN.

However, in the case that a patient does miss a dose, they should apply the next injection immediately. The following week, they are to proceed as below:

- If the peg-IFN injection was late by 1 or 2 days, the next dose should be administered on the usual day
- If the peg-IFN injection was late by 3-5 days, they should receive further injections every 5 to 6 days until their usual day for peg-IFN injection is reached
- If the peg-IFN injection was late by 6 days, the next application will be on the next day (1 dose is skipped)
- If the peg-IFN injection was late by ≥7 days, treatment can be restarted at any time by the investigator; if necessary, the following injections can be given at 5 or 6 day intervalls to achieve a convenient weekday

The control group won't receive pegylated interferon alfa-2a (Pegasys®) as add-on treatment.

Basic treatment (Non-IMP):

All patients are to continue their medication with lamivudine, adefovir, entecavir or tenofovir or a combination of those during the trial until at least the end of follow-up. The known dosing instructions, contraindications, side effects and risks for each drug must be taken into account according to the manufacturers' recommendations. Regular laboratory and clinical assessments throughout the course of this study are to provide adequate monitoring of potential toxicities.

4.1.1 General information about investigational medicinal product (IMP)

International nonproprietary name (INN)	Pegylated interferon alfa-2a

ATC Code	L03A B11
Formulation	180 µg/ 0,5 ml- Solution for injection in pre-filled syringe / pre-filled pen for single use only
Storage	in the refrigerator at 2 °C to 8 °C (36 °F to 46 °F)
Marketed Product	Pegasys®
Manufacturer	Roche Pharma AG
Ingredients	sodium chloride , polysorbate 80 , benzyl alcohol (10 mg/ 1 ml), sodium acetate , acetic acid , water for injections

4.1.2 Therapeutic effects

Pegylated recombinant human interferon alfa-2a is an inducer of the innate antiviral immune response. It stimulates the production of effector proteins such as serum neopterin and 2', 5'-oligoadenylate synthetase.

The biological activity of pegylated interferon alfa-2a is derived from its recombinant human interferon α-2a moiety. Peginterferon alfa-2a binds to the human type 1 interferon receptor leading to receptor dimerization. Receptor dimerization activates multiple intracellular signal transduction pathways initially mediated by the JAK/STAT pathway. Given the diversity of cell types that respond to interferon α-2a, and the multiplicity of potential intracellular responses to interferon receptor activation, peginterferon alfa-2a is expected to have pleiotropic biological effects in the body.

In the stable HCV cell culture model system (HCV replicon), ribavirin inhibited autonomous HCV RNA replication with an effective concentration (EC) value of 11-21 µm. In the same model, PEG-IFN α-2a also inhibited HCV RNA replication, with an EC value of 0.1 - 3 ng/mL.

4.1.3 Special warnings and precautions for use

Severe CNS effects, particularly depression, suicidal ideation and attempted suicide have been observed in some patients during Pegasys® therapy, and even after treatment discontinuation mainly during the 6-month follow-up period. Other CNS effects including aggressive behaviour (sometimes directed against others such as homicidal ideation), bipolar disorders, mania, confusion and alterations of mental status have been observed with alpha interferons. Patients should be closely monitored for any signs or symptoms of psychiatric disorders. If such symptoms appear, the potential seriousness of these undesirable effects must be borne in mind by the prescribing physician and the need for adequate therapeutic management should be considered. If psychiatric symptoms persist or worsen, or suicidal ideation is identified, it is recommended that treatment with Pegasys® be discontinued, and the patient followed, with psychiatric intervention as appropriate. Patients with existence of, or history of severe psychiatric conditions: If treatment with Pegasys® is judged necessary in patients with existence or history of severe psychiatric conditions, this should only be initiated after having ensured appropriate individualised diagnostic and therapeutic management of the psychiatric condition.

4.1.4 Known side effects of pegylated interferon alfa-2a

Serious adverse events:

Serious adverse events in hepatitis B and hepatitis C trials included neuropsychiatric disorders (homicidal ideation, suicidal ideation, suicide attempt, suicide, psychotic disorder and hallucinations), serious and severe bacterial infections (sepsis), bone marrow toxicity (cytopenia and rarely, aplastic anemia), cardiovascular disorders (hypertension, supraventricular arrhythmias and myocardial infarction), hypersensitivity (including anaphylaxis), endocrine disorders (including thyroid disorders and diabetes mellitus), autoimmune disorders (including idiopathic thrombocytopenic purpura, thrombotic thrombocytopenic purpura, psoriasis, lupus, rheumatoid arthritis and interstitial nephritis), pulmonary disorders (dyspnea, pneumonia, bronchiolitis obliterans, interstitial pneumonitis, pulmonary hypertension, and sarcoidosis), colitis (ulcerative and

hemorrhagic/ischemic colitis), pancreatitis, ophthalmologic disorders (decrease or loss of vision, retinopathy including macular edema and retinal thrombosis/hemorrhages, optic neuritis and papilledema), pancytopenia (in combination with azathioprine), and peripheral neuropathy (in combination with telbivudine).

Adverse reactions reported during post-approval use of therapy with pegylated interferon alfa-2a include dehydration, hearing impairment, hearing loss, serious skin reactions, including erythema multiforme major, infections (bacterial, viral and fungal), and serous retinal detachment, and pure red cell aplasia.

Most common adverse events:

The most common adverse events reported for pegylated interferon alfa-2a and ribavirin combination therapy observed in clinical trials were fatigue/asthenia (65%), headache (43%), pyrexia (41%), myalgia (40%), irritability/anxiety/nervousness (33%), insomnia (30%), alopecia (28%), neutropenia (27%), nausea/vomiting (25%), rigors (25%), anorexia (24%), injection-site reaction (23%), arthralgia (22%), depression (20%), pruritus (19%) and dermatitis (16%). In clinical trials of 48-week treatment duration, the adverse event profile of pegylated interferon alfa-2a in chronic hepatitis B was similar to that seen in chronic hepatitis C pegylated interferon alfa-2a monotherapy use, except for exacerbations of hepatitis. The most common adverse events reported for pegylated interferon alfa-2a, observed in clinical studies, were pyrexia (54%), headache (27%), myalgia (26%), fatigue (24%), alopecia (18%) and anorexia (16%).

Transient ALT elevations are common during hepatitis B therapy with pegylated interferon alfa-2a. Twenty-five percent and 27% of subjects experienced elevations of 5 to 10 x ULN and 12% and 18% had elevations of >10 x ULN during treatment of HBeAg negative and HBeAg positive disease, respectively. Flares have been accompanied by elevations of total bilirubin and alkaline phosphatase and less commonly with prolongation of PT and reduced albumin levels. Eleven percent of subjects had dose modifications due to ALT flares and <1% of subjects were withdrawn from treatment. ALT flares of 5 to 10 x ULN occurred in 13% and 16% of subjects, while ALT flares of >10 x ULN occurred in 7% and 12% of subjects in HBeAg negative and HBeAg positive disease, respectively, after discontinuation of pegylated interferon alfa-2a therapy.

Peripheral neuropathy has been reported when alpha interferons were given in combination with telbivudine. In one clinical trial, an increased risk and severity of peripheral neuropathy was observed with the combination use of telbivudine and pegylated interferon alfa-2a as compared to telbivudine alone. The safety and efficacy of telbivudine in combination with interferons for the treatment of chronic hepatitis B has not been demonstrated.

For details of adverse reactions see Summary of Product Characteristics[24].

Contraindications:

Pegylated interferon alfa-2a is contraindicated in patients with hypersensitivity to pegylated interferon alfa-2a or any of its components, autoimmune hepatitis, and hepatic decompensation (Child-Pugh score greater than 6; class B and C) in cirrhotic patients before or during treatment, with severe pre-existing cardiac disease, including unstable or uncontrolled cardiac disease in the previous six months. The combination of Pegasys® with telbivudine is also contraindicated.

4.1.5 Dosage schedule

In this trial the dosage and duration of Pegasys® is 180 micrograms once weekly for 48 weeks by subcutaneous administration in the abdomen or thigh which is consistent with the recommended dosage and duration for both HBeAg-positive and –negative chronic hepatitis B.

4.1.6 Treatment interruptions

Temporary interruptions of study drug(s) administration are discouraged; patients are to remain on treatment for the entire duration of the trial; and the dose of peg-IFN should not be changed during the trial, unless dose reduction is indicated because of laboratory abnormalities/adverse events

(c.f. Section 4.1.8 for instructions for dose reduction). The patients must be counselled regarding the importance of not missing doses of peg-IFN.

4.1.7 Overdose instruction

Overdoses involving between two injections on consecutive days (instead of weekly interval) up to daily injections for 1 week (i.e., 1260 micrograms/week) have been reported. None of these patients experienced unusual, serious or treatment-limiting events. Weekly doses of up to 540 and 630 micrograms have been administered in renal cell carcinoma and chronic myelogenous leukaemia clinical trials, respectively. Dose limiting toxicities were fatigue, elevated liver enzymes, neutropenia and thrombocytopenia, consistent with interferon therapy.

4.1.8 Dose Adjustment for adverse reactions

When a Pegasys® dose modification is required for moderate to severe adverse reactions (clinical and/or laboratory), initial dose reduction to 135 µg should be performed. However, in some cases, dose reduction to 90 µg may be needed.

Following improvement of the adverse reaction, re-escalation of the Pegasys® dose may be considered (refer to warnings, precautions, and adverse reactions in the Pegasys® medication guide).

Hematological:

Pegasys® Hematological Dose Modification Guidelines

Laboratory Values	Reduce Pegasys® Dose to:	Discontinue Pegasys® if
ANC	≥750/mm^3: Maintain 180 µg < 750/mm^3: Reduce to 135 µg	< 500/mm^3: Treatment should be suspended until ANC values return to more than 1000/mm^3 Re-institute at 90µg and monitor ANC
Platelet	≥ 50.000/mm^3: Maintain 180 µg < 50.000/mm^3: Reduce to 90 µg	<25.000/mm^3

Liver function:

In patients with progressive or persistent ALT increases > 10 x ULN and >2 x baseline values, the dose of Pegasys® should be reduced to 135µg and more frequent monitoring of liver function should be performed. If ALT increases are further progressive despite dose reduction or accompanied by increased bilirubin or evidence of hepatic decompensation, therapy should be immediately discontinued. After Pegasys® dose reduction or withholding, therapy can be resumed after ALT flares subside.

4.1.9 Treatment assignment

The trial medication will be administered only to subjects included in this trial.

Subjects withdrawn from the trial retain their identification codes (e.g. randomisation number). New subjects will always receive a new identification code.

4.1.10 Treatment after the end of the trial

After the end of the trial, patients who experienced a complete HBs-seroconversion (i.e. who become HBsAg negative and anti-HBs positive) will stop all antiviral therapy. For patients with HBe seroconversion a continuation of nucleos(t)ides for at least 6 months is recommended. HBeAg negative patients without HBs-seroconversion will continue therapy with nucleos(t)ides as previously.

4.1.11 Packaging and labelling

Packaging and labelling of trial medication will be conducted by Roche Pharma AG.

The trial medication will be labelled according to §5 GCP regulation.

Pegylated interferon alfa-2a 180 µg (Pegasys®) is provided as 0,5 ml- Solution for injection in pre-filled syringe / pre-filled pen for single use only. Each pre-filled syringe / pen is packed in a single covering box.

IMP assignment and traceability as required by GCP-V § 5 (1) will be realised by accurate documentation of drug accountability and compliance on an accompanying document.

4.1.12 Drug storage, supplies and accountability

The investigator will take inventory and acknowledge the receipt of all shipments of the trial medication. All trial medication must be kept in a locked area with access restricted to designated trial staff.

Pegasys® should be stored in the refrigerator at 2 °C to 8 °C (36 °F to 46 °F). Do not freeze or shake. Protect from light. Pre-filled syringes / pre-filled pens are for single use only. The investigator will also keep accurate records of the quantities of trial medication dispensed, used, and returned by each subject on the drug accountability form.

The site monitor will periodically check the supplies of trial medication held by the investigator to verify the correct accountability of all trial medication used. At the end of the trial, all unused trial medication will be completely returned to Roche Pharma AG in case no other procedure is agreed.

It will be assured that a final drug accountability report is prepared and maintained by the investigator.

4.1.13 Procedures for monitoring subject compliance

Trial medication will be dispensed to the subjects by the investigator.

Subjects will be instructed to bring all trial medication to the trial site at every visit (including all empty containers and unused trial medication). Compliance will be assessed by counting of empty containers and unused medication. All empty containers will be disposed by the trial center after verification by the monitor. Details will be recorded in the CRF and on the drug accountability form in the investigator site file.

4.2 Not permitted medication

The following concomitant treatments are not permitted during the trial:

- Telbivudine
- Systemic corticosteroids, if used for longer than 14 days, or other immunsuppressive medications (e.g. cyclosporines, tacrolimus, methotrexate etc.)
- Systemic cytostatic chemotherapy
- Immune-modulating agents such as IL-2, anti-TNF-antibodies or rituximab
- Nephrotoxic agents such as aminoglycoside antibiotics, amphotericin B, foscarnet, ganciclovir, NSAID, or other agents with significant nephrotoxic potential
- Hepatotoxic agents such as anabolic steroids, isoniacid, itraconazole, ketoconazole, rifabutin, rifampin, or other agents with significant hepatotoxic potential

If a subject needs treatment with any excluded concomitant medication, the sponsor should be consulted prior to the initiation of the new medication.

All concomitant medication, including vitamin supplements and herbal remedies must be recorded.

5 TRIAL SCHEDULE

After patients have been informed about the trial, written informed consent in accordance with GCP and the local legislation must be obtained prior to any study related procedures taking place. Patients will be assigned a patient screening number which must be recorded in eCRF.

Screening (visit 1) should take place within 28 days before baseline (visit 2). Patients should undergo a re-screening (visit 1.1) if start of treatment is delayed to more than 28 days after Visit 1. Patients who have a laboratory test value outside the range specified by the inclusion criteria may have the test repeated to determine eligibility; however, the result must be available prior to Visit 2 (Day 1).

(Screening) Visit 1 (week -4 to -1)

The following data will be obtained/checked and documented:

- Informed consent
- Evaluation of eligibility criteria
- Demographics: Age (date of birth), gender, ethnic origin/nationality, height and body weight
- Medical History: Possible mode and assumed year of infection (perinatal, injection drug use, sexual, occupational, transfusion, unknown, other)
- Recording of concomitant medication
- Alcohol consumption (regular alcohol consumption, average number of units/drinks per day)
- Prior and concomitant medication for HBV: Standard interferon, pegylated interferon, nucleoside/nucleotide analogues: start of therapy, dosage regimen (start and end dose), cessation of therapy. In addition herbal drugs and vaccination for HBV
- HBV disease characteristics: HBV genotype (A-H) (if available)
- Complete physical examination, including measurement of vital signs
- 12-lead resting-ECG measurement
- hepatic ultrasound or CT scan or MRI
- HBV-PCR
- HBs antigen / anti-HBs
- Virology:
    - HBeAg, anti-HBe, anti-HBc
    - Samples for serological assessment of HBV genotype (if not available)
    - HCV, HDV, HIV testing
- Routine laboratory test (hematology and clinical chemistry III)
- Urine drug screening and urinalysis
- Urine pregnancy test for women of childbearing potential
- Liver assessment (if available): Results from liver biopsy and documentation of the histopathological scoring system used (e.g. METAVIR, Ishak, Knodell, Scheuer), and from non-invasive methods, e.g. liver elastography (specify kPa assessed by FibroScan).

(Baseline) Visit 2 (week 0)

If upon completion of screening and, if necessary, re-screening the patient has been determined eligible by the investigator to enter the trial, the patient will be randomised and assigned a randomisation number. For this, the investigator has to request the treatment allocation via a web-based randomisation tool at Visit 2 (week 0). Sites must enter the treatment allocation on the appropriate eCRF pages.

- Targeted physical examination including vital signs
- Randomisation
- Routine laboratory tests (hematology and clinical chemistry I)
- HBs antigen / anti-HBs
- Samples for immunology
- Recording of AEs and concomitant medication

Start of treatment with pegylated interferon alfa-2a. Administration of first dose should be under the supervision of the investigator (or a designee) at the study center. This is to minimise dosing errors.

Visit 5, 8, (week 12, 24) – treatment group

Visit 3, 4 (week 12, 24) – control group

- Targeted physical examination
- Routine laboratory tests (clinical chemistry II)
- HBV-PCR
- HBs antigen / anti-HBs
- Urine pregnancy test for women of childbearing potential (Treatment group: visit 5, 8, control group: visit 4 only)
- Recording of AEs and concomitant medication

Visit 3, 4, 6, 7, 9, 10, 11, 12, 13 (week 4, 8, 16, 20, 28, 32, 36, 40, 44) – treatment group

Visit 5 (week 36) – control group

- Targeted physical examination
- Routine laboratory tests (clinical chemistry I)
- HBs antigen / anti-HBs
- Urine pregnancy test for women of childbearing potential (only treatment group: visit 11)
- Samples for immunology at week 4 and 8 for treatment group only
- Recording of AEs and concomitant medication

(End of treatment) Visit 14 (week 48) – treatment group

(End of treatment) Visit 6 (week 48) – control group

- Complete physical examination
- Routine laboratory tests (clinical chemistry II)
- HBV-PCR
- HBs antigen / anti-HBs
- Samples for immunology
- Urine pregnancy test for women of childbearing potential
- Recording of AEs and concomitant medication

(End of follow-up) Visit 15 (week 72) - treatment group

(End of follow-up) Visit 7 (week 72) - control group

- Complete physical examination
- Routine laboratory tests (clinical chemistry II)
- HBV-PCR
- HBs antigen / anti-HBs
- Recording of AEs and concomitant medication
- Trial completion form

6 TRIAL METHODS

Physical examination

Vital signs (body temperature, heart rate and blood pressure) and weight will be determined at every visit, height at screening only.
Blood pressure measurement should be performed in a consistent manner after the patient has been sitting for five minutes. A manual cuff should be used on the same arm each time blood pressure is measured.

Complete physical examinations will be performed at screening, Week 48, Week 72, and Early Discontinuation (if applicable). Symptom-directed physical examination will be performed at all other visits.

As an increased incidence of polyneuropathy was observed during combination therapy with pegylated interferon alfa-2a and telbivudine, special attention should be paid to the development of polyneuropathy or other neurological symptoms in subjects on combination therapy. Investigators need to question patients about development of hyp- or dysesthesia, muscle weakness or any other neurological symptoms. If neurological symptoms occur, they need to be documented as adverse events, and the patients will then be referred to a neurologist for further examination.

Ultrasound or CT scan

All subjects must undergo hepatic ultrasound or CT scan or MRI to rule out HCC within 6 months before taking part in this study.

Cardiologic Evaluations

A standard 12-lead ECG will be performed at screening visit.

Laboratory tests

Blood samples will be collected and assessed as scheduled (see section 5).

HBV-PCR, HBsAg and Anti-HBs are measured centrally at the department of clinical chemistry at University Medical Center of JoGu University Mainz (Zentrallabor der Universitätsmedizin Mainz). To this aim, samples of EDTA blood and serum (8-10 ml each) will be collected the trial site, frozen (- 80°C), and sent to Mainz via courier (overnight transport at room temperature).

All other laboratory tests, including HBeAg, anti-HBe, anti-HBc, HCV, HDV, HIV testing, routine hematology, clinical chemistry, and urine screening tests (drug screening and urinalysis) will be performed locally in each study center by an accredited laboratory (accredition by the Zentralstelle der Länder für Gesundheitsschutz bei Arzneimitteln und Medizinprodukten). Urine pregnancy test for women of childbearing potential will be performed using commercially available tests at the trial site.

Routine laboratory tests:

I = erythrocytes, hemoglobin, hematocrit, platelets, total leukocytes and differential, AST, ALT, total bilirubin, gamma-GT, alkanine phostphatase, total bilirubin (and conjugated bilirubin if total bilirubin is >1.5 x ULN), LDH, sodium, potassium, calcium, creatinine (and calculated clearance), blood urea nitrogen (BUN), uric acid, glucose, PT, INR

II = erythrocytes, hemoglobin, hematocrit, platelets, total leukocytes and differential, AST, ALT, total bilirubin, gamma-GT, alkanine phostphatase, total bilirubin (and conjugated bilirubin if total bilirubin is >1.5 x ULN), LDH, sodium, potassium, calcium, creatinine (and calculated clearance), blood urea nitrogen (BUN), uric acid, glucose, PT, INR, albumin, TSH, fT3, fT4

III = erythrocytes, hemoglobin, hematocrit, platelets, total leukocytes and differential, AST, ALT, total bilirubin, gamma-GT, alkanine phostphatase, total bilirubin (and conjugated bilirubin if total bilirubin is >1.5 x ULN), LDH, sodium, potassium, calcium, creatinine (and calculated clearance), blood urea nitrogen (BUN), uric acid, glucose, PT, INR, albumin, TSH, fT3, fT4, AFP,

IgG, ANA, SMA, SLA, LKM, AMA, ANCA, ferritin, lipid profile (triglyceride, total cholesterol, HDL, LDL), HLA typing, urinalysis

These laboratory studies include and surpass the manufacturers' recommendations (summary of product characteristics) for the surveillance of patients receiving lamivudine, adefovir, entecavir or tenofovir, especially concerning the development of nephropathy.

Additional tests

Immune monitoring / Immunology
Blood samples of 50 mL each for PBMC purification and immune monitoring should be acquired from all patients at specific time points. PBMC purification using Ficoll will be performed locally at each study center according to SOP of the sponsor. PBMC will be transported to Mainz on dry ice.

Bioinformatic analysis of genetic markers
In cooperation with a current study at our department at University Medical Center Mainz analysis of several genetic markers via microarray is planned in order to identify genes which may be associated with HBs antigen loss. For each patient 2 vials of 2 ml of EDTA-blood will be taken at baseline and frozen in liquid nitrogen and stored at -80°C. In addition, if available, histology samples (10 slices of 5 µm each) will be examined.

Biomarkers
Blood samples of15-20 mL each for further anaylsis of potentially predictive biomarkers will be collected at week 0, 4, 8, 12, 24, 36, 48 and 72 (treatment group) and 0, 12, 24, 36, 48 and 72 (control group). Serum samples will be retained and stored at -80°C.

6.1 Assessment of efficacy

Biological markers of hepatitis B

To address the primary objective of this study, the decline in quantitative HBsAg during 48 weeks of add-on therapy with peg-IFN versus nucleos(t)ides alone, quantitative HBs antigen will be measured every four or twelve weeks in the interferon or control arm, respectively.

To evaluate secondary objectives:

- Serological markers of Hepatitis B will be routinely tested as scheduled (quantitative HBs antigen, Anti-HBs, HBe antigen, Anti-HBe) as well as quantitative HBV-DNA and liver function tests.
- Since a genomic analysis of the virus is not possible in patients with non-detectable HBV-DNA serotypic determination of HBV type A-D will be performed[25]. This assay shows a coincident rate with genotyping of 95-100%.

6.2 Assessment of safety

6.2.1 Adverse events

6.2.1.1 Definitions

Adverse Event (AE)

According to GCP, an adverse event (AE) is defined as any untoward medical occurrence in a subject treated with a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal investigational product, whether or not related to that product.

An AE may be:

- a new symptom or medical condition
- a new diagnosis
- a change in laboratory parameters
- an intercurrent illness or accident
- worsening of a medical condition/diseases existing before the start of the clinical trial
- recurrence of a disease
- an increase in frequency or intensity of episodic diseases.

Surgical procedures themselves are not AEs; they are therapeutic measures for conditions that require surgery. The condition for which the surgery is required may be an AE. Planned surgical measures permitted by the clinical trial protocol and the condition(s) leading to these measures are not AEs, if the condition leading to the measure was present before inclusion in the trial. In the latter case the condition should be reported as medical history.

Change in laboratory parameters: The criteria for determining whether an abnormal test finding should be reported as an adverse event are as follows:

- Test result is associated with accompanying symptoms, and/or
- Test result requires additional diagnostic testing or medical/surgical intervention, and/or
- Test result leads to a change in trial dosing outside of protocol-stipulated dose adjustments, or discontinuation from the trial, significant additional concomitant drug treatment, or other therapy, and/or
- Test result is considered to be an adverse event by the investigator or sponsor.

Serious adverse event (SAE)

A serious adverse event (SAE) is one that at any dose (including overdose):

- results in death
- is life-threatening[1]
- requires subject hospitalization or prolongation of existing hospitalization[2]
- results in persistent or significant disability/incapacity[3] or
- is a congenital anomaly/birth defect
- is an important medical event[4].

[1]"Life-threatening" means that the subject was at immediate risk of death at the time of the serious adverse event; it does not refer to a serious adverse event that hypothetically might have caused death if it were more severe.

[2]If the admission is pre-planned (i.e., elective or scheduled surgery arranged prior to start of the trial) or not associated with an adverse event (e.g., social hospitalisation for purpose) or results in a hospital stay less than 12 hours, the serious criterion "hospitalisation" is not fulfilled. However, it should be noted that invasive treatment during a hospitalisation may fulfil the criteria of "medically important" and may be reportable as a serious adverse event dependent on clinical judgement.

[3]"Persistent or significant disability or incapacity" means that there is a substantial disruption of a person's ability to carry out normal life functions. The irreversible injury of an organ function (e.g., paresis, diabetes, cardiac arrhythmia) fulfils this criterion.

[4]Medical and scientific judgement should be exercised in deciding whether expedited reporting is appropriate in situations where none of the outcomes listed above occurred. Important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above should also usually be considered serious. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, convulsions that do not result in subject hospitalization, or the development of drug dependency or

drug abuse. A diagnosis of cancer during the course of a treatment should be considered as medically important.

Adverse Reaction (AR)

An adverse reaction is any noxious and unintended response to an investigational medicinal product (the causal relationship between the medicinal product and the adverse event is at least a reasonable possibility).

Serious Adverse Reaction (SAR)

If there is a causal relationship between a serious adverse event and trial medication then the event is called serious adverse reaction (SAR).

Suspected Unexpected Serious Adverse Reaction (SUSAR)

A SUSAR is a serious adverse reaction which is unexpected.

An unexpected serious adverse reaction is any adverse reaction, the nature or severity of which is not consistent with the applicable product information (i.e., the current SPC).

Clarification of the difference in meaning between "serious" and "severe":

The terms “serious” and “severe” are not synonymous but are often used interchangeably. The term ‘severe’ is often used to describe the intensity (severity) of a specific event; the event itself, however, may be of relatively minor significance (such as severe headache). This is not the same as “serious”, which is based on subject/event outcome or action criteria usually associated with events that pose a threat to a subject’s life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.”

6.2.1.2 Assessment of AEs by investigator

Subjects must be carefully monitored for adverse events by the investigator. The intensity of the adverse events and the causal relation to trial medication and/or procedures are to be assessed.

Intensity

The intensity of an AE will be assessed by the investigator as follows:

Mild: Temporary event which is tolerated well by the subject and does not interfere with normal daily activities.

Moderate: Event which results in discomfort for the subject and impairs his/her normal activity.

Severe: Event which results in substantial impairment of normal activities of subject.

Causal relation to trial medication/procedures

The assessment of the relationship of an adverse event to the administration of study drug is a clinical decision based on all available information at the time of the completion of the case report form. The investigator will evaluate the causal relationship of each adverse event with the administration of the investigational product(s) and/or trial procedures according to modified criteria of WHO 1991.

Certain: A clinical event, including laboratory test abnormalities, occurring in a plausible time relationship to drug administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be definitive pharmacologically or phenomenologically, using a satisfactory rechallenge procedure if necessary.

Probable: A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, unlikely to be attributed to concurrent

disease or other drugs or chemicals, and which follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge is not required to fulfil this definition.

Possible: A clinical event, including laboratory test abnormality, with a reasonable time sequence to administrations of the drug, but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.

Unlikely: A clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable, and in which other drugs, chemicals or underlying disease provide plausible explanations.

Not related: A clinical event, including laboratory test abnormality that does not follow a reasonable temporal sequence from trial participation and that is definitely caused by the subject's clinical state, other modes of therapy or other known etiology.

6.2.1.3 Period of observation

In this trial, the period of observation for collection of adverse events extends from the time the subject has signed the informed consent document up to visit 15 of the treatment group or visit 7 of the control group (week 72, End of follow-up).
If the investigator detects a serious adverse event in a trial subject after the end of the period of observation, and considers the event possibly related to the prior trial, he should contact the sponsor to determine how the adverse event should be documented and reported.

6.2.1.4 Documentation of AEs and Follow up

All AEs reported by the subject or detected by the investigator will be documented on the appropriate pages of the case report form (CRF). AEs must also be documented in the subject's medical records.

The following approach will be taken for documentation:

- All adverse events (whether serious or non-serious) must be documented on the "Adverse Event" page of the CRF.
- If the adverse event is serious (see Section 6.2.1.1), the investigator must complete, in addition to the "Adverse Event Page", a "Serious Adverse Event Form" at the time the serious adverse event is detected.

Every attempt should be made to describe the adverse event in terms of a diagnosis. If a clear diagnosis has been made, individual signs and symptoms will not be recorded unless they represent atypical or extreme manifestations of the diagnosis, in which case they should be reported as separate events. If a clear diagnosis cannot be established, each sign and symptom must be recorded individually.

All subjects who have adverse events, whether considered associated with the use of the investigational products or not, must be monitored to determine the outcome. The clinical course of the adverse event will be followed up according to accepted standards of medical practice, until a satisfactory explanation is found or the investigator considers it medically justifiable to terminate follow-up, but not longer than EoFU.

Should the adverse event result in death, a full pathologist's report should be supplied, if possible.

All questions on the completion and supply of adverse event report forms and any further forms issued to the investigator at a later date to clarify unresolved issues should be addressed to the sponsor.

6.2.1.5 Immediate reporting of SAEs by investigator

SAEs must immediately (within 24 hours of the investigator's awareness) be reported to:

IZKS Mainz

Safety Management

Langenbeckstr. 2

55131 Mainz

FAX-No.: 06131-17-9916

The initial SAE Report should be as complete as possible including the essential details of subject's identification (screening number, random number), the serious adverse event (medical term, diagnosis), the trial medication and the assessment of the causal relationship between the event and the trial medication. The SAE report must be reviewed and signed by the investigator.

The investigator should provide related additional information on the clinical course and the outcome of each SAE as soon as possible (Follow up report).

The "Serious Adverse Event Form" is provided in the Investigator Site File.

The investigator should also inform the trial monitor in all cases.

Worsening of a sign or symptom of the condition under treatment will normally be measured by efficacy parameters. However, if the outcome fulfils the definition of "serious adverse event", it must be reported as such.

6.2.1.6 Immediate Reporting of pregnancy by investigator

Any **pregnancy** diagnosed in a female subject or in the female partner of a male subject during treatment with the investigational product must be reported immediately using the "Pregnancy Reporting Form" to:

IZKS Mainz

Safety Management

Langenbeckstr. 2

55131 Mainz

FAX-No.: 06131-17-9916

Pregnancy occuring during the clinical trial, although not considered a SAE, must be reported within the same timelines as a serious adverse event. The outcome of a pregnancy should be followed up carefully and abnormal outcome of mother or child should be reported if any.

6.2.1.7 Safety evaluation and Reporting by sponsor

The sponsor will ensure that all legal reporting requirements are met. According to GCP the sponsor is responsible for the continuous safety evaluation of the investigational product(s) and the clinical trial.
On behalf of the sponsor IZKS Mainz will conduct the management of SAEs and the expedited reporting as required by German Drug Law (AMG) and GCP regulation (GCP-V). Suspected unexpected serious adverse reactions (SUSARs) and safety issues as defined by GCP-V are determined for expedited reporting: The competent authorities and the ethics committees should be notified as soon as possible but not later than 15 calendar days if the event is non-fatal and 7 calendar days if it was fatal.
All investigators will be informed too. The marketing authorization holder of the IMP (Roche Pharma AG) will also be informed.

Work flow and procedures concerning SAE management will be described in a separate document (e.g. Safety manual).

During the clinical trial the sponsor will submit the annual safety report including a list of all serious adverse reactions to the ethics committee(s) and the competent authorities once a year.

6.2.1.8 Emergency procedures

During and following a subject's participation in the trial, the investigator should ensure that adequate medical care is provided to a subject for any AEs including clinically significant laboratory values. The investigator should inform a subject when medical care is needed for intercurrent illness(es) of which the investigator becomes aware. There is no specific antidote or emergency treatment. Overdoses involving between two injections on consecutive days (instead of weekly interval) up to daily injections for 1 week (ie, 1260 micrograms/week) have been reported. None of these patients experienced unusual, serious or treatment-limiting events. Weekly doses of up to 540 and 630 micrograms have been administered in renal cell carcinoma and chronic myelogenous leukaemia clinical trials, respectively. Dose limiting toxicities were fatigue, elevated liver enzymes, neutropenia and thrombocytopenia, consistent with interferon therapy.

6.2.2 Other safety data

All observations pertinent to the safety of the study medication will be recorded on the CRF and included in the final report.
Safety variables are as follows: laboratory changes, changes in vital signs (blood pressure, heart rate and temperature) and, cardiologic evaluation by ECG.

6.3 Other assessments

6.3.1 Immune monitoring

Immune control in chronically HBV infected patients is dependent on HBV specific T cells. In chronically HBV infected patients low frequencies of IFN-γ producing CD8+ cells between 0.02 and 0.08% are observed, in contrast to patients after successful HBV clearance and immune control who develop significantly higher HBV specific T cell frequencies between 0.2 and 0.5%[26,27]. Moreover, CD8 T cell responses were more frequently observed in patients being able to control HBV replication after interruption of IFN treatment[28]. This observation indicates that T cell reactivity is essential for immune control of HBV infection and might be induced by IFN therapy.

Therefore, we will include a close monitoring during the treatment course which will require blood samples at baseline and after 4, 8, and 48 weeks of pegIFN treatment and at baseline and week 48 for the control group.

6.3.2 Bioinformatic analysis of genetic markers

In cooperation with a current study at our department at University medical Center Mainz analysis of several genetic markers via microarray is planned in order to identify genes which may be associated with HBs antigen loss.

6.3.3 Other biomarkers

Serum samples for further analysis of potentially predictive biomarkers will be acquired from all patients at week 0, 4, 8, 12, 24, 36, 48 and 72 (treatment group) and 0, 12, 24, 36, 48 and 72 (control group).

6.3.4 Prior and concomitant illnesses

Relevant additional illnesses present at the time of informed consent are regarded as concomitant illnesses and will be documented on the appropriate pages of the case report form (CRF).

6.3.5 Prior and concomitant treatments

Relevant additional treatments administered to the subjects on entry to the trial or at any time during the trial are regarded as concomitant treatments und must be documented on the appropriate pages of the CRF.

7 STATISTICS

Details of the statistical analysis of the data collected in this trial will be documented in a Statistical Analysis Plan (SAP) that will be generated by IZKS Mainz and finalized before closing the data base. The SAP is based on the protocol including all amendments. The document may modify the plans outlined in this protocol; however any major modifications of the primary endpoint definition and/or its analysis will also be reflected in a protocol amendment. Any deviation from the original statistical plan must be described and justified in the final report. The statistical analysis will be conducted by means of SAS®.

7.1 Sample size

Based on pilot data, it is assumed that 12% of the patients in the peg-IFN/Nuc group HBsAg will drop >1log_{10} after 48 weeks of combination therapy, while only 0.1% of the patients without additional interferon will reach this endpoint.

Studies in hepatitis C patients reported of a discontinuation rate due to tolerability and safety of approximately 10-15%[29]. As this rate was observed in combination with ribavirin we assume a drop-out rate of 10% due to better tolerability and safety in hepatitis B patients.

The assumed difference in HBsAg loss rates will be detected with a power of 80% by Fisher's exact test at a two-sided significance level of 5%, if 153 patients (102 in the interferon arm, 51 in the control arm) can be analysed. Taken into account a drop-out rate of 10%, 170 patients will be randomised. Patients with no HBs antigen measurement at week 48 will be analysed by their last HBs antigen measurement (last observation carried forward). This approach is considered slightly conservative but allows all randomised patients to be included into the confirmatory modified Intention to treat analysis.

7.2 Analysis populations

All subjects who signed informed consent / were assigned a randomisation number are considered as enrolled/randomised subjects, even if they did not receive any trial treatment.

All subjects who received at least one dose of trial treatment and with at least one available post-baseline assessment of the primary analysis variable will be included in the modified Intention-to-treat (mITT) population. This population is the primary analysis population. Within mITT population analyses subjects will be assigned to the treatment to which they were randomised.

To be eligible for the per protocol population, subjects must fulfil the following criteria:

- No violation of inclusion criteria
- Not meeting any exclusion criteria
- No concomitant treatment with not permitted medication (see section 4.2)
- Treatment compliance of at least 90%
- Trial treatment as randomised
- No deviation from planned visit schedules

The safety population comprises all subjects who received at least one dose of trial treatment. In analyses of the safety population subjects will be assigned to the treatment which they actually received.

7.3 Efficacy analyses

The primary population for the analyses of efficacy is the mITT Population.
All hypotheses will be tested on a two-sided level of significance α=0.05.

7.3.1 Definition and analysis of primary endpoint

Null hypothesis: $HBsAgRR_{Interferon} = HBsAgRR_{Control}$
Alternative hypothesis: $HBsAgRR_{Interferon} \neq HBsAgRR_{Control}$

HBsAgRR denotes HBs antigen response rates. The subscripts show the respective treatment group.

The response rates will be compared between the treatment groups by Fisher's exact test. The primary analysis population is the modified Intention to treat population comprising all randomised patients for which the primary endpoint can be assessed.

The following supportive analyses are planned:
The primary analysis (Fisher's exact test) will be repeated for the Per Protocol Population.
Furthermore, a logistic regression model including covariates assumed to predict treatment response (HBV genotype, age, sex, duration of antiviral therapy before the study) will be applied.

In the primary analysis subjects with missing HBsAg measurements at week 48 will be analysed according to the last observation carried forward principle. This approach is considered slightly conservative but allows all randomised patients to be included into the confirmatory modified Intention to treat analysis.

7.3.2 Analysis of secondary endpoints

The primary analysis population for the secondary endpoints is the modified Intention to treat population.
Secondary endpoints will be analysed using appropriate methods depending on the scale of the respective parameter. Analyses of secondary endpoints will be interpreted descriptively.

7.3.3 Analysis of Subgroups

Age, sex, HBV-Genotype, level of quantitative HBsAg, nucleos(t)ide regimen (substances, combination, duration) will be subject to subgroup analyses.

7.3.4 Interim analyses

There will be no interim analysis in the sense that the primary endpoint is analysed prematurely and a decision about the continuation of the study is derived.

However, it is planned to compare the rates of patients with at least 10% HBsAg loss 24 weeks after the start of study treatment compared to baseline between the treatment groups as soon as these data are available. The analysis will be interpreted in an explorative manner. Therefore no adjustment of the significance level is required.

The influence of this premature analysis on the primary analysis and the predictability of the results of the primary analysis based on the results of the premature analysis are expected to be limited for the following reasons:

- The recruitment phase will be terminated at the time point of the premature analysis.
- According to the expected accrual time, the analysis will be performed only around 16 weeks before the end of treatment of the last patient.
- The loss rates in the early response analysis (at least 10% HBsAg loss) and the primary analysis (reduction of at least $1\log_{10}$ HBsAg) are defined differently.

7.4 Analysis of adverse events

All summaries and listings of safety data will be performed for the safety population.

Frequencies of subjects experiencing at least one adverse event (AE) will be displayed by body system and preferred term according to MedDRA terminology. Detailed information collected for each AE will include: A description of the event, duration, whether the AE was serious, intensity,

relationship to trial drug, action taken, clinical outcome. Summary tables will present the number of subjects observed with AEs and corresponding percentages. Additional subcategories will be based on event intensity and relationship to trial drug.

A subject listing of all AEs will be prepared.

7.5 Analysis of clinical laboratory findings

Listings will be prepared for each laboratory measure and will be structured to permit review of the data per subject as they progress on treatment.

Summary tables will be prepared to examine the changes of laboratory measures over time. Additionally, shift tables will be provided to examine the changes of laboratory data from normal baseline to values outside the corresponding reference range during/after treatment.

Changes in vital signs (blood pressure, heart rate and temperature) and, cardiologic evaluation by ECG will be analysed descriptively by distributional parameters (such as mean and standard deviation) or absolute and relative frequencies.

8 QUALITY CONTROL AND QUALITY ASSURANCE

8.1 Requirements for investigational sites and staff

The investigator should be able to demonstrate (e.g. based on retrospective data) a potential for recruiting the required number of suitable subjects within the agreed recruitment period.

The investigator should have sufficient time to properly conduct and complete the trial within the agreed trial period.

The investigator should have available an adequate number of qualified staff and adequate facilities for the foreseen duration of the trial to conduct the trial properly and safely.

The investigator should ensure that all persons assisting with the trial are adequately qualified, informed about the protocol, any amendments to the protocol, the trials treatments, and their trial-related duties and functions.

8.2 Direct entries

Data entries be entered in the CRF as Direct, are listed in the Monitor Manual in the section source data control.

8.3 Direct access to source data/documents

The investigator/institution must permit trial-related monitoring and auditing by representatives of the sponsor, as well as inspections by the appropriate competent authorities and Ethics committees, providing direct access to source data/documents (Confidentiality see 10.3).

The subjects will be informed that representatives of the sponsor, independent ethics committee (IEC) or competent authorities may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws.

8.4 Investigator site file and archiving

The investigator will be provided with an investigator site file (ISF) at the start of the trial. The investigator will archive all trial data and relevant correspondence in the ISF. The ISF, all source data and all documents will be kept filed according to the requirements of the German Drug Law and ICH-GCP guidelines after termination of the trial.

It is the responsibility of the investigator to ensure that the subject identification lists are stored for at least 15 years beyond the end of the clinical trial. All original subject files must be stored for the longest possible time permitted by the regulations at the hospital, research institute, or practice in

question. If archiving can no longer be maintained at the site, the investigator will notify the sponsor.

8.5 Monitoring

Monitoring will be done by personal visits from a clinical monitor according to SOPs of the IZKS Mainz.

To initiate the trial, the monitor will visit all participating local trial centers. The monitor shall ensure that the investigators and their staff understand all requirements of the protocol and their regulatory responsibilities.

The monitor will ensure that the investigator will maintain a list of subinvestigators and other appropriately qualified persons to whom he or she has delegated significant trial-related duties (personnel log).

Each site will be visited by the monitor at regular intervals to ensure compliance with the trial protocol, GCP and legal aspects. The monitor will review the entries into the CRFs for completeness and correctness and verify the entries on the basis of the source documents. The presence of correct informed consents will be checked for every subject.

To close the trial, the monitor will visit all trial centres.
Details will be specified in the monitoring manual for this trial.

The investigator must allow the monitor to look at all relevant documents and must provide support at all times to the monitor.

8.6 Inspection by authorities and audits

Competent authorities and by the sponsor authorised persons (auditor) may request access to all source documents, CRF, and other trial documentation in case of an inspection or audit. Direct access to these documents must be guaranteed by the investigator who must provide support at all times for these activities. Source data documents can be copied during inspection or audit in case the identity of the subject have been made unrecognizable.

8.7 Audits

No audits are planned for this trial.

9 DATA MANAGEMENT

9.1 Responsibilities

Clinical Data Management is conducted by IZKS Mainz according to SOPs.
In case of discrepancies the data management team is authorised to directly contact the responsible person at the trial site. The queries will be implemented into the MACRO database. This will allow the trial sites to conduct data corrections more easily and it will also guarantee that the queries are filed to the corresponding variables in the database. The investigator has to agree the contact per e-mail or phone.

A detailed methodology for the data management in this trial will be documented in a data management plan that will be dated and maintained by IZKS Mainz. This plan has to be signed by the sponsor, the head of the data management team and the responsible data manager. The document may modify the plans outlined in this protocol; however any major modifications of the data handling will also be reflected in a protocol amendment.

9.2 Data collection

This trial will be performed using an electronic case report form (eCRF). The investigator and the trial site staff will receive system documentation, training and support for the use of the eCRF. In case of new trial site staff the training can be performed by personnel of the trial site.

For data entry support the IZKS Mainz can be contacted. Each trial site has one responsible person who supports IZKS Mainz in the implementation of technical and organisational processes. A list of these persons is enclosed in the DMP.

All protocol-required information collected during the trial must be documented in the eCRF by the investigator, or a designated representative. All data entry, modification or deletion will be recorded automatically in an electronic audit trail indicating the individual subject, the original value, the new value, the reason for change, who made the change and time and date of the change. All data changes will be clearly indicated. Former values can be viewed in the audit trail. All electronic data will be entered by the site (including an electronic audit trail) in compliance with applicable record retention regulations.

The system will be secured to prevent unauthorized access to the data or the system. Only people provided with a user ID and a password will be able to enter or change data. The investigator will maintain a list of individuals who are authorized to enter or correct data.

Computer hardware and software (for accessing the data) will be maintained at or made available for the site in compliance with applicable regulations. All technical preconditions for each trial site are recorded in the DMP.

The system is capable of making exact copies of data in legible paper form for inspections and audits. The investigator or a designated subinvestigator, following review of the data in the eCRF, will confirm the validity of each subject's data by electronic signature or by signing a paper printout of a listing of all subjects enrolled in the trial.

The architecture of the computer system will be described in the data management plan.

9.3 Data handling

During data entry integrity checks help to minimize entry failures. These data entry checks are based on the data validation plan, signed by the LKP. The data entry system allows the trial monitors to control the entry process with the help of the built-in review functions. Comments and requests can be promptly processed by the trial site.

After completion of data entry the database access rights will be taken away and the database will be exported into the data transformation system as mother database.
Final checks for plausibility, consistency and completeness of the data will be performed. Based on these checks, queries will be produced. Any missing data or inconsistencies will be reported back to the respective site and clarified by the responsible investigator. If no further corrections are to be made in the database it will be declared closed and used for statistical analysis.

All data management activities will be done according to the current Standard Operating Procedures (SOPs) of IZKS Mainz.

9.4 Storage and archiving of data

According to GCP, the investigator will archive all trial data (subject identification list, source data) and relevant correspondence in the Investigator Site File (ISF). The ISF, all source data and all documents itemized in section 8 of the ICH Consolidated Guideline on GCP will be archived after finalization of the trial according to the legal regulations.

The principal investigator will be responsible for storage and archiving of the trial data (source data and CRFs). Storage and archiving of the electronic data during the trial will be assured by IZKS Mainz. After completion of the trial all electronic data will be handed over to the sponsor.

10 ETHICAL AND LEGAL ASPECTS

10.1 Good clinical practice

The procedures set out in this trial protocol, pertaining to the conduct, evaluation, and documentation of this trial, are designed to ensure that all persons involved in the trial abide by good clinical practice (GCP) and the ethical principles described in the Declaration of Helsinki (1996, 2008). The trial will be carried out in keeping with local legal and regulatory requirements.

The requirements of the AMG, the GCP regulation, and the Federal Data Protection Law (BDSG) will be kept.

10.2 Patient information and informed consent

Before being admitted to the clinical trial, the subject must consent to participate after being fully informed about the nature, scope, and possible consequences of the clinical trial.

The documents must be in a language understandable to the subject and must specify who informed the subject.

A copy of the signed informed consent document must be given to the subject. The original signed consent document will be retained by the investigator.

The investigator will not undertake any measures specifically required only for the clinical trial until valid consent has been obtained.

If the subject has a primary physician the investigator should inform the subject's primary physician about the subject's participation in the trial and if the subject agrees to the primary physician being informed.

After reading the informed consent document and after having been fully informed by the investigator (informed consent discussion), the subject must give consent in writing. The subject's consent must be confirmed by the personally dated signature of the subject and by the personally dated signature of the person conducting the informed consent discussion.

If the subject is unable to read, oral presentation and explanation of the written informed consent form and information to be supplied to subjects must take place in the presence of an impartial witness. Consent must be confirmed orally and by the personally dated signature of the subject or by a local legally recognized alternative (e.g., the subject's thumbprint or mark). The witness and the person conducting the informed consent discussions must also sign and personally date the consent document.

10.3 Confidentiality

The name of the subjects and other confidential information will not supplied to the sponsor.

The name of the subjects and other confidential information are subject to medical professional secrecy and the regulations of the German law on data protection (Bundesdatenschutzgesetz). During the clinical trial, subjects will be identified solely by means of an individual identification code (e.g. patient screening number, randomisation number). Trial findings stored on a computer will be stored in accordance with local data protection law and will be handled in strictest confidence. For protection of these data, organizational procedures are implemented to prevent distribution of data to unauthorized persons. The appropriate regulations of data legislation will be fulfilled in its entirety.

The subject will declare in the written consent to release the investigator from the medical professional secrecy to allow identification of subject's name and/or inspection of original data for monitoring purposes by health authorities and authorized persons (monitors).

The investigator will maintain a personal subject identification list (subject numbers with the corresponding subject names) to enable records to be identified.

10.4 Responsibilities of investigator

The investigator will ensure that all persons assisting with the trial are adequately informed about the protocol, any amendments to the protocol, the trial treatments, and their trial-related duties and functions.

The investigator will maintain a list of subinvestigators and other appropriately qualified persons to whom he or she has delegated significant trial-related duties.
Any changes of the authorized trial personnel are to be communicated without delay to the clinical monitor.

10.5 Approval of trial protocol and substantial amendments

Before the start of the trial, the trial protocol, informed consent document, and any other appropriate documents will be submitted to the independent ethics committee (IEC)/institutional review board (IRB). The approval by the IEC should preferably mention the title of the trial, the trial code, if applicable the trial site, and the documents they reviewed. It must mention the date on which the decision was made and must be officially signed by a committee member. This documentation must also include a list of members of the IEC present on the applicable EC meeting.

If applicable, the documents will also be submitted to the competent authority in Germany (BfArM) in accordance with the respective local legal requirements.

Investigational products can only be supplied to the investigator after documentation on all ethical and legal requirements for starting the clinical trial has been received by the sponsor ("regulatory greenlight"). Before the first subject is enrolled in the trial, all ethical and legal requirements must be met.

Neither the investigator nor the sponsor will alter this trial protocol without obtaining the written agreement of the other. The IEC and, if applicable, the competent authorities must be informed of all subsequent protocol amendments and administrative changes, in accordance with the respective local legal requirements.

Amendments must be evaluated to determine whether formal approval must be sought and whether the informed consent document should also be revised.

The investigator must keep a record of all communications with the IEC and the competent authorities.

10.6 Continuous information to independent ethics committee/ institutional review board

The EC must be informed by the sponsor of all subsequent protocol amendments which require formal approval in accordance with the legal requirements.

According to the current legal requirements in Germany (GCP-V) the independent EC must be informed by the sponsor of SUSARs and of serious AEs which occur during the trial and might affect the safety of subjects or the conduct of the trial if not otherwise stated in the vote.

The EC must be informed by the sponsor of trial process regularly if not otherwise stated in the vote. The EC must be informed of the end of the trial in accordance with legal requirements (within 90 days or within 15 days in case of premature closure of the clinical trial).

10.7 Submission to local regulatory/competent authorities

Before the start of trial, the sponsor is responsible for submission of all documents necessary to the competent authority for approval. The local regulatory authority responsible for the investigator will be informed of the trial.

10.8 Independent data monitoring committee (IDMC)

Not applicable.

10.9 Insurance

According to § 40 AMG, the sponsor has subscribed to an insurance policy covering, in its terms and provisions, its legal liability for injuries caused to participating persons and arising out of this research performed strictly in accordance with the scientific protocol as well as with applicable law and professional standards. The insurance was taken out at HDI-Gerling Versicherung AG (insurance number: 5701030703010, maximum limit: € 500.000,- per participating person).

Any impairment of health which might occur in consequence of trial participation must be notified to the insurance company. The subject is responsible for notification. The insured person will be

agreed to all appropriate measures serving for clarification of the cause and the extent of damage as well as the reduction of damage.

During the conduct of the trial, the subject must not undergo other clinical treatment except for cases of emergency. The subject is bound to inform the investigator immediately about any adverse events and additionally drugs taken. The terms and conditions of the insurance should be delivered to the subject.

10.10 Agreements

10.10.1 Financing of the trial

The trial is funded by Roche Pharma AG. The general conditions of financing for this trial are given in separate agreements.

10.10.2 Report

After conclusion of the trial, a report shall be written by the sponsor, in cooperation with the coordinating investigator, according to ICH-GCP. The report will include a statistical analysis and an appraisal of the results from a medical viewpoint. It will be based on the items listed in this trial protocol.

10.10.3 Publication policy

Any publication of the results, either in part or in total (articles in journals or newspapers, oral presentation, etc.) by the investigators, their representatives, or by the sponsor, shall require the approval of the principal investigator. It is planned to publish the results of the trial as an original article in an appropriate medical journal as well as presentation at congresses. The principal investigator is first author of the article and will present the data at the major congresses. The choice of the journal for the publication will be made by principal investigator in agreement with the co-authors. Besides the Principal Investigator, further authors of this article have to meet the following points:

- Substantial contribution to the recruitment of subjects, i.e. one of the five best recruiting centers within the trial.
- Substantial contribution to interpretation of the data.
- Substantial contribution to drafting the article or revising it critically for important intellectual content.

11 SIGNATURES

The present trial protocol was subject to critical review and has been approved in the present version by the persons undersigned. The information contained is consistent with:

- The current risk-benefit assessment of the investigational medicinal product.
- The moral, ethical, and scientific principles governing clinical research as set out in the Declaration of Helsinki and the principles of GCP.

Sponsor / Operating Institution of the Sponsor

Name Univ.-Prof. Dr. med. P. R. Galle

22. April 2013 — S. U.

Date — Signature

Coordinating/Principal Investigator

Name Univ.-Prof. Dr. med. P. R. Galle

22. April 2013 — [signature]

Date — Signature

Biometrician

Name

24.04.13 — [signature]

Date — Signature

12 DECLARATION OF INVESTIGATOR

I have read the above trial protocol and I confirm that it contains all information to accordingly conduct the clinical trial. I pledge to conduct the clinical trial according to the protocol.

I will enroll the first subject only after all ethical and regulatory requirements are fulfilled. I pledge to obtain written consent for trial participation from all subjects.

I know the requirements for accurate notification of serious adverse events and I pledge to document and notify such events as described in the protocol.

I pledge to retain all trial-related documents and source data as described. I will provide a Curriculum Vitae (CV) before trial start. I agree that the CV may be submitted to the responsible competent authorities.

I will conduct the trial in compliance with the protocol, GCP and the applicable regulatory requirements.

Investigator

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22. April 2013
Date

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22.4.13
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Signature

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