CLINICAL TRIAL PROTOCOL

Study Title: Nucleos(t)ide withdrawal in HBeAg negative hepatitis B virus infection to promote HBsAg clearance. (Nuc-B)

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This protocol describes the NUC-B trial and provides information about procedures for entering participants. The protocol should not be used as a guide for the treatment of other participants; every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study, but centres entering participants for the first time are advised to contact the trials centre to confirm they have the most recent version.

Problems relating to this trial should be referred, in the first instance, to the study coordination centre.

This trial will adhere to the principles outlined in the Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031), amended regulations (SI 2006/1928) and the International Conference on Harmonisation Good Clinical Practice (ICH GCP) guidelines. It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

ABBREVIATIONS

AE	Adverse Event
Anti-HBe	Antibody to hepatitis B ‘e’ antigen
cccDNA	Covalent closed circular DNA
CI	Chief Investigator
CRF	Case Report Form
DMEC	Data Monitoring and Ethical Committee
DNA	Deoxyribonucleic Acid
eCRF	Electronic Case Report Form
EMA	European Medicines Agency
FDA	Food and Drug Administration
GMP	Good Manufacturing Practice
HBcAg	Hepatitis B core antigen (nucleocapsid)
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HBV DNA	Hepatitis B virus deoxyribonucleic acid
HBeAg	Hepatitis B ‘e’ antigen
HCV	Hepatitis C virus
HDV	Hepatitis D virus
HIV	Human Immunodeficiency Virus
ICMJE	International Committee of Medical Journal Editors
ICTU	Imperial Clinical Trials Unit
IFN	Interferon
IMP	Investigational Medicinal Product
IND	Investigational New Drug
MA	Marketing Authorisation
MHRA	Medicines and Healthcare Products Regulatory Agency
o.w.	Once weekly
QA	Quality Assurance
qHBsAg	Quantitative measurement of hepatitis B surface antigen
REC	Research Ethics Committee
SAE	Serious Adverse Event

SAP	Statistical Analysis Plan
s.c.	Subcutaneous
SOP	Standard Operating Procedure
SSAR	Suspected Serious Adverse Reaction
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMG	Trial Management Group
TSC	Trial Steering Committee

Table of Contents

TRIAL SUMMARY

TITLE Nucleos(t)ide withdrawal in HBeAg negative hepatitis B virus infection to promote HBsAg clearance. (NUC-B)

OBJECTIVES The aim of this trial is to explore whether finite treatment with nucleos(t)ide analogues is feasible in patients with HBeAg (Hepatitis B 'e' antigen) negative chronic HBV (Hepatitis B Virus) infection. This aim encompasses an exploration of therapeutic strategies to achieve HBsAg (Hepatitis B surface antigen) loss; specifically the trial is designed to evaluate whether a short course of pegylated interferon after nucleos(t)ide analogue withdrawal stimulates antiviral immunity sufficiently to increase the rate of HBsAg loss.
Loss of HBsAg, the most important serological marker of HBV infection, is widely accepted (including by the Food and Drug Administration (FDA) & European Medicines Agency (EMA)) as an indication of cure in chronic HBV infection and is associated with absence of long term sequelae in non-cirrhotic patients. HBsAg is a cheap and universally available serum marker. Loss of HBsAg allows patients to be discharged from follow-up with consequent health-economic benefits.

DESIGN NUC-B will be a multicentre, randomised, open label trial comparing two management strategies to promote HBsAg loss in patients with HBeAg negative chronic HBV infection.

SAMPLE SIZE A trial with 90% power to detect a difference in HBsAg loss between these groups at the $P < 0.05$ threshold would require 109 subjects in each arm. Allowing for ~10% withdrawal / dropout in each arm we will aim to recruit 120 subjects to each arm; 240 patients in total.

ELIGIBILITY CRITERIA Adult patients aged 18 and above with chronic HBeAg negative hepatitis B virus infection on nucleos(t)ide analogues treatment for 3 years or more.

TREATMENT/MAIN STUDY PROCEDURES

Registered patients will be followed for 4 – 12 weeks between initial screening and randomisation in order to collect data on quantitative HBsAg levels and anti-viral immune responses. During this period a sub-set of consenting patients will receive a liver biopsy for analysis of fibrosis and necroinflammatory scores, measurement of cccDNA and analysis of T cell and NK cell infiltrates.

If the patient remains eligible, electronic randomisation will then allocate subjects to two arms:

1. Control arm
Patients randomised to the Treatment Withdrawal arm will discontinue their nucleos(t)ide analogue treatment at baseline.

2. Interferon arm
Patients randomised to switch from nucleoside analogues to Pegylated interferon will discontinue their current therapy at baseline. No treatment will be given for 4 weeks and then they will commence pegylated interferon 180 mcg s.c. o.w. α2a and will continue taking weekly interferon for a total of 16 weeks. They will subsequently discontinue all antiviral medications.

PRIMARY ENDPOINT
HBsAg loss 3 years after randomisation

SECONDARY ENDPOINTS

Secondary endpoints will look at 1) Efficacy endpoints and 2) Safety endpoints. Efficacy endpoints will look at levels of HBsAg, HBV DNA, antiviral T cells response and changes in NK cells response at various timepoints after randomisation. Safety endpoints will look at proportions of patients with exaggerated hepatitis flare, those resuming nucleos(t)ide analogue therapy and adherence to pegylated interferon therapy.

1 INTRODUCTION

Over 250 million people worldwide (an estimated 300,000 in the UK) are chronically infected with hepatitis B virus (HBV) leading to a million deaths per year from hepatocellular carcinoma or complications of cirrhosis (1). Four phases of disease are recognised in patients with chronic HBV infection; these are defined according to the presence or absence of the hepatitis B 'e' antigen (HBeAg), ALT values and the level of HBV DNA in serum (2). Elimination of infection, which requires clearance of the HBV minichromosome (cccDNA) from hepatocytes, indicated by loss of the HBV surface antigen (HBsAg) is the ultimate goal of management but this is rarely achieved. Treatment is required to arrest progression of disease during the HBeAg +ve hepatitis phase and during the HBeAg –ve hepatitis phase. Over 80% of patients requiring treatment in European countries (including the UK) are in the HBeAg –ve hepatitis phase (3). Treatment options for HBeAg –ve hepatitis are either 12 months of pegylated interferon or indefinite therapy with nucleos(t)ide analogues such as Entecavir or Tenofovir. Interferon therapy is considered successful if the patient achieves a sustained virological response (HBV DNA < 2000 IU/ml and normal ALT) 24 weeks after treatment which occurs in fewer than 30%. High rates of relapse and the side effect profile of interferon make this an unpopular option and no more than 12% of patients may clear infection (HBsAg loss) over 5 years (4,5). The majority of patients and clinicians therefore opt for treatment with nucleos(t)ide analogues which suppress viral replication resulting in resolution of fibrosis, reversal of cirrhosis and reduction in the incidence of hepatocellular carcinoma (HCC). Side effects of treatment are rare although renal tubular toxicity is described with Tenofovir. Viral resistance to Tenofovir is not reported but low rates of resistance have been described with Entecavir. However, nucleoside analogues rarely result in HBsAg loss and treatment must be maintained and monitored (HBV viral load, LFTs and ultrasounds) every 6 months indefinitely leading to massive health care costs – the drugs alone cost £3500 per patient per year with monitoring costs and patient productivity losses increasing the burden (6).

Two recent papers have addressed the issue of finite therapy in patients with HBeAg negative chronic HBV infection (7,8). The original paper by Hadziyiannis and colleagues assessed nucleos(t)ide withdrawal after a minimum of four years' treatment in a group of patients infected with HBV genotype D. These patients were allowed to undergo a virological flare and were followed up for 6 years. Eventually, 30% lost HBsAg. In contrast, Seto assessed the impact of nucleos(t)ide withdrawal in 184 patients infected with HBV genotypes B or C after two years of treatment. Hepatitic flares or resumption of high level viral replication was not permitted so the majority of patients returned to drug treatment. Interim analysis of a similar study presented by Berg et al at EASL 2015 confirmed the findings of Hadziyiannis up to one year after nucleos(t)ide cessation.

High level viral replication is associated with suppression of HBV-specific adaptive immune responses leading to viral persistence (9). Recent data suggest that there is partial restoration of immune responses in patients on long term treatment with antiviral drugs that suppress viral replication (10).

Interferon (standard or pegylated) has been used in the treatment of HBV for 20 years due to its antiviral and immunostimulatory effects. Combining interferon with nucleos(t)ide analogues as initial treatment for HBV infection does not improve therapeutic efficacy (4). However, a number of authorities now believe that sequential therapy (with or without a short break) may amplify immune responses due to the complementary immunological effects of IFN and nucleos(t)ide analogues on innate and adaptive arms respectively and result in increased rates of HBsAg clearance. Based on

the above, we argue that it is timely to evaluate management options which may result in a cure of HBV, release patients from indefinite drug treatment and increase cost effective care.

1.1 CLINICAL SETTING

The trial will be conducted in an outpatient setting in specialist Hepatology or Infectious Diseases clinics treating chronic viral hepatitis patients. The patient population to be addressed have chronic hepatitis B virus infection in the HBeAg negative hepatitis phase of the disease. The patients will already be on treatment with nucleoside or nucleotide analogues for viral suppression. In routine standard of care this group of patients would remain on treatment indefinitely with reviews in clinic every 3 – 6 months.

1.2 Comparison of treatment strategies

The trial will compare the efficacy of two treatment strategies to achieve loss of HBsAg

A. Control Arm

Patients randomised to the Treatment Withdrawal arm will discontinue their nucleoside analogue treatment at baseline.

B. Interferon Arm

Patients randomised to switch from nucleoside analogues to Pegylated interferon will discontinue their current therapy at baseline. No treatment will be given for 4 weeks and then they will commence pegylated interferon 180 mcg s.c. o.w α2a. and will continue taking weekly interferon for a total of 16 weeks. They will subsequently discontinue all antiviral medications.

1.2.1 Immunology studies

Elimination of HBV is dependent on robust innate (NK cell) and adaptive (CD4 / CD8 T cells) immune responses which are defective in chronic infection (9). It is postulated that HBsAg loss is most likely where the number of infected hepatocytes is low and the cellular immune response is amplified by the management strategy.

Suppression of viral replication has been shown to partially restore HBV-specific T cell responses. By contrast NK cells but not T cell responses are amplified by interferon therapy (11). The purpose of the mechanistic studies is therefore to determine whether sequential therapy can harness both arms of the immune response to optimise cure rates. Peripheral blood mononuclear cells will be isolated at several timepoints during the study and stored in liquid nitrogen. Enumeration of interferon gamma producing T cells will be performed using ELISPOT assays after incubation of peripheral blood mononuclear cells for 48 hours with HBV peptides. NK cell phenotype and function will be analysed using multiparameter flow cytometry to quantify activating and inhibitory receptors, intracellular cytokine production and degranulation.

In a subset of ~20 consenting patients liver biopsy or fine needle liver aspirates will be obtained to allow quantification of the intrahepatic & cccDNA and to characterise intrahepatic T and NK cells using FACS Dynamic changes in the serum HBsAg concentration will be correlated with peripheral blood cellular immune responses and with cccDNA levels.

1.2.2 Virology studies

HBsAg loss is associated with clearance of HBV-infected hepatocytes and elimination of cccDNA. Earlier studies suggest that the change in HBsAg concentrations in serum reflect the changes in cccDNA concentration in hepatocytes and therefore may represent an ideal biomarker for identifying patients who are moving towards HBsAg loss.
Samples will be taken at various timepoints to track the dynamics of HBsAg concentration changes and to enable an analysis of the predictive value of this biomarker. HBsAg concentrations will be performed using the Abbott Architect qHBsAg system in Dr Nastouli's laboratory.

There are 7 different genotypes and a number of variants in the pre-C/C and S open reading frames of the hepatitis B virus. Viral genotypes have been associated with variation in the natural history of infection and with the response to treatment. At baseline all patients will have undetectable virus in their serum but samples taken 4 weeks after withdrawal should have sufficient circulating virus to allow genotype and sequencing of the virus. Genotyping with be performed in Dr Nastouli's laboratory.
In patients undergoing liver biopsy prior to baseline a sample will be used to measure the cccDNA level quantitatively using an in-house real-time PCR assay in Dr Dorner's laboratory

1.3 Rationale for the study

There are estimated to be 300,000 patients with chronic HBV infection in the UK and 250 million worldwide (1, 2). Approximately 10% of these individuals require treatment which therefore imposes a significant treatment cost which is perpetuated due to the need for constant viral suppression to derive clinical benefit. Nucleos(t)ide analogue therapy is effective in suppressing the virus and we know that successful suppression reduces the risk of progression to decompensation or from hepatitis to cirrhosis. However, the risk of liver cancer is not completely eliminated. Frequent and careful monitoring is required to ensure the success of nucleos(t)ide analogues which depends on patient adherence, avoidance of viral resistance and a low rate of adverse events. Treatment therefore imposes a burden of medical monitoring visits and expensive virological assays. Finite therapy which results in viral elimination (HBsAg loss) would be more convenient to the patients and financially benefit the health service. Furthermore the risk of liver cancer amongst patients who have lost HBsAg is substantially reduced.

1.4 Risk / Benefit Assessment

Three papers have addressed the issue of finite therapy in patients with HBeAg negative chronic HBV infection over the last three years. The first looked at nucleos(t)ide withdrawal after a minimum of 4 years treatment in a group of patients infected with HBV genotype D(7). These patients were allowed to undergo a virological flare and were followed up for 6 years. Eventually 30% lost HBsAg. In contrast Seto looked at the impact of nucleos(t)ide withdrawal in 184 patients after two years of treatment(8). Significant differences in trial design affected the outcome: hepatitic flares or resumption of high level viral replication was not permitted so the majority of cases were returned to drug treatment.

High level viral replication is associated with suppression of HBV-specific adaptive immune responses leading to viral persistence (9). Recent studies suggest that there is partial restoration of T cell responses during long term treatment with nucleos(t)ide analogues (10). Interferon therapy appears to amplify NK cell but not T cell responses whilst treatment withdrawal also amplifies innate immune responses (11, 12). Interferon treatment after a short period of nucleos(t)ide withdrawal should therefore maximise immune responses and allow clearance of infected hepatocytes leading to HBsAg loss.

Interferon (standard or pegylated) has been used in the treatment of HBV for 20 years due to its antiviral and immunostimulatory effects (4, 13). Combining interferon with nucleos(t)ide analogues as initial treatment for HBV infection does not improve off-treatment virological control(4). However, adding pegylated interferon after a prolonged course of nucleos(t)ide analogue treatment increases therapeutic efficacy in HBeAg positive patients (14). It is now an ideal time to evaluate management options which result in a cure for HBV and release patients from indefinite drug treatment and the associated NHS cost.

2 OBJECTIVES AND ENDPOINTS

The aim of this trial is to explore whether finite treatment with nucleos(t)ide analogues is feasible in patients with HBeAg negative chronic HBV infection. This aim encompasses an exploration of therapeutic strategies to achieve HBsAg loss; specifically the trial is designed to evaluate whether

a short course of pegylated interferon after nucleos(t)ide analogue withdrawal stimulates antiviral immunity sufficiently to increase the rate of HBsAg loss.

2.1 Primary Objective

The primary objective is to determine the rate of HBsAg loss three years after randomisation in patients with HBeAg-negative chronic HBV infection. The rate of HBsAg loss will be compared in patients with simple treatment discontinuation against patients who are given a short course of pegylated interferon after nucleos(t)ide withdrawal.

Loss of HBsAg, the most important serological marker of HBV infection, is widely accepted (including by FDA & EMA) as an indication of cure in chronic HBV infection and is associated with absence of long term sequelae in non-cirrhotic patients. HBsAg is a cheap and universally available serum marker. Loss of HBsAg allows patients to be discharged from follow-up with consequent health-economic benefits.

2.2 Secondary Objectives

1. To explore the safety of nucleoside withdrawal
2. To explore whether using interferon therapy in conjunction with treatment cessation is safe
3. To compare the number of patients in each treatment arm who become inactive HBV carriers.
4. Identification of virological and immunological biomarkers which predict a group of patients in which the loss of HBsAg is feasible
5. Correlate changes in HBsAg quantification and/or HBsAg status with innate and adaptive immune responses to HBV
6. To identify changes in immunological responses to HBV which occur after treatment withdrawal and after sequential NUC/IFN treatment

2.3 Primary Endpoint

The primary endpoint will be the proportion of patients who lose HBsAg in each treatment arm 3 years after randomisation. Qualitative assays of HBsAg will be conducted on patients every 6 months using standard laboratory ELISA assays. Loss of HBsAg will be confirmed by repeating the test at least one month after the initial negative result. Patients in whom both tests are negative will be deemed to have lost HBsAg.

2.4 Secondary Endpoints

Efficacy endpoints

- The proportion of patients who achieve HBsAg loss who also have undetectable HBV DNA
- The proportion of patients in each group who become inactive HBV carriers; ie achieve a sustained virological response (HBV DNA < 2000 IU/ml & normal ALT values) at 3 years after randomisation
- Magnitude of reduction in quantitative HBsAg levels at 1, 6, 12, 24 & 36 months after randomisation
- Magnitude of changes in antiviral T cells response at 1, 5, 6, 12, 24 & 36 months after randomisation
- Magnitude of changes in NK cells response at 1, 5, 6, 12, 24 & 36 months after randomisation

Safety endpoints

- Proportion of patients in each group with exaggerated hepatitis flares

- Proportion of patients resuming nucleos(t)ide analogue therapy
- Adherence to pegylated interferon therapy

2.5 Development of predictive biomarkers

Baseline and early dynamic changes in qHBsAg, ELISPOT and NK cell parameters will be used alongside clinical and laboratory analyses to construct prognostic models for the prediction of HBsAg loss.

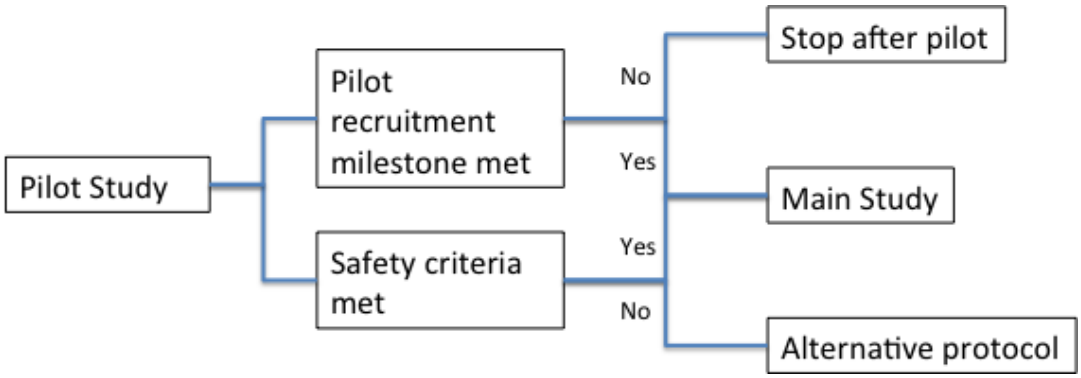
3 STUDY DESIGN

3.1 Pilot Phase

A pilot phase will be conducted at the beginning of the trial. This will consist of 50 patients recruited across 4 sites. Milestones within the pilot are based on numbers of patients recruited as well as safety aspects/numbers of exaggerated flares as shown in the flow diagram below. The pilot phase will be conducted according to the same protocol as the main trial and the pilot phase patients will count towards the overall trial sample size. Exaggerated flares are defined in Section 6.4 (i).

The milestones for the pilot phase are as follows:

- If the pilot phase fails to recruit 40 patients in 4 months then NIHR-EME will discontinue the study.
- If more than 40% of patients in either arm of the trial experience exaggerated flares by the end of the pilot phase then that arm will be discontinued. If this occurs in the interferon arm then the interferon arm will be modified to a control and combined interferon treatment protocol in which pegylated interferon is added to the nucleos(t)ide analogue for 16 weeks prior to cessation of therapy rather than leaving a gap between nucleos(t)ide analogues withdrawal and pegylated interferon treatment (and compared to the control arm still) – see Figure 2. If this occurs in the control arm then the control arm will be switched to the interferon and combined interferon treatment protocol (and compared against the standard interferon arm) – see Figure 3.
- If more than 60% of patients in the pilot experience exaggerated flares the trial will be discontinued.
- If more than 40% of patients in both arms experience exaggerated flare by the end of the pilot phase then the trial will be terminated if the DMEC and TSC recommend so.



A safety assessment will be made by the TSC and DMEC when all pilot patients will have completed initial screening, therapy gap and two months of interferon therapy. The trial will be assessed against the above milestones and a decision on the continuation of the trial made.

3.2 Design

The main trial for NUC-B will be performed in at least 10 sites in England. It is a randomised, open-label, multicentre trial comparing nucleoside / nucleotide analogue withdrawal against nucleoside/ nucleotide analogue withdrawal followed by a 16 week course of pegylated interferon in HBeAg-negative chronic hepatitis B virus infected patients. Patients will be followed for 3 years after randomisation.

3.3 Treatment regimens

Table 1: Summary of treatment groups

Treatment Strategy	Number of subjects	Month 1	Month 2 - 5	Month 6 – 36
A: Withdrawal	120 (25 from pilot)	Withdraw nucleos(t)ide analogues	Follow up	Follow up
B: Interferon	120 (25 from pilot)	Withdraw nucleos(t)ide analogues	Pegylated interferon α2a 180 mcg s.c. o.w.	Follow up
Total number of subjects	240			

Figure 1: Study flow chart

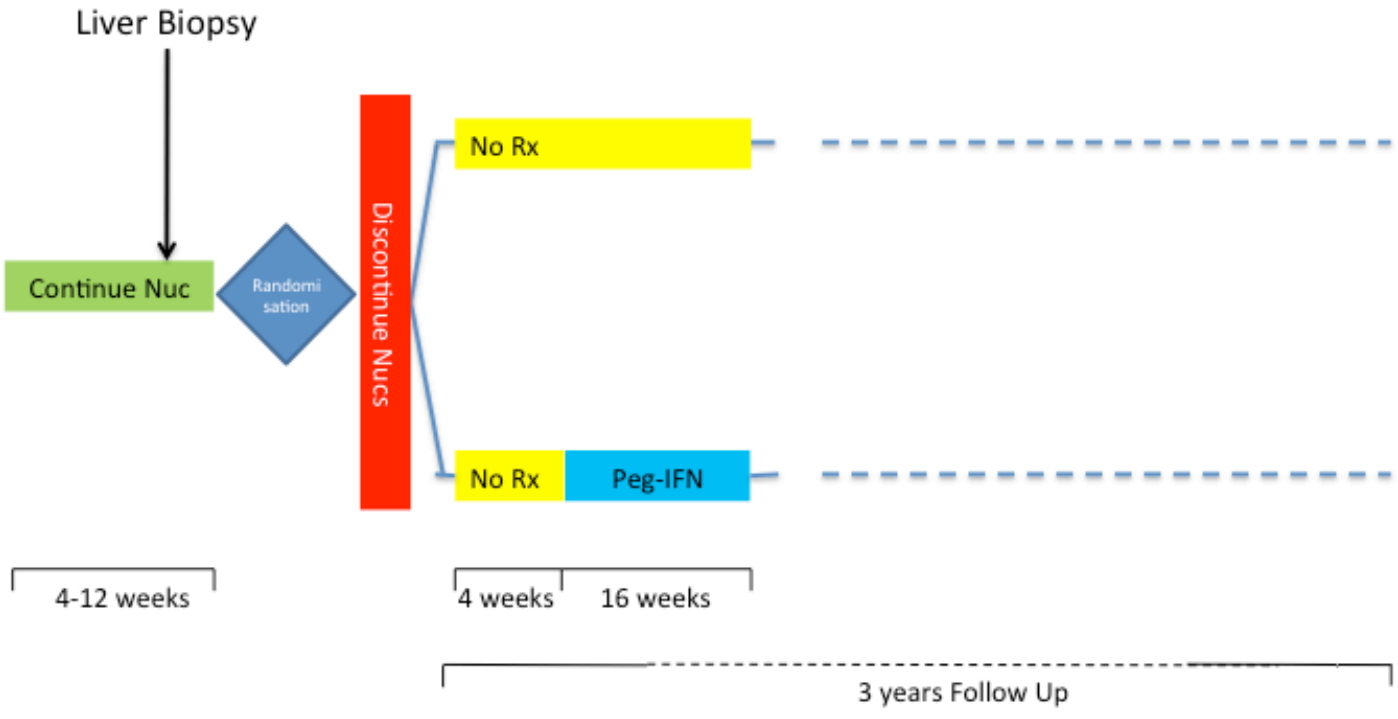


Figure 2: Control and combined interferon treatment Protocol (if more than 40% of patients in the original interferon arm of the pilot phase experience exaggerated flares by the end of the pilot phase)

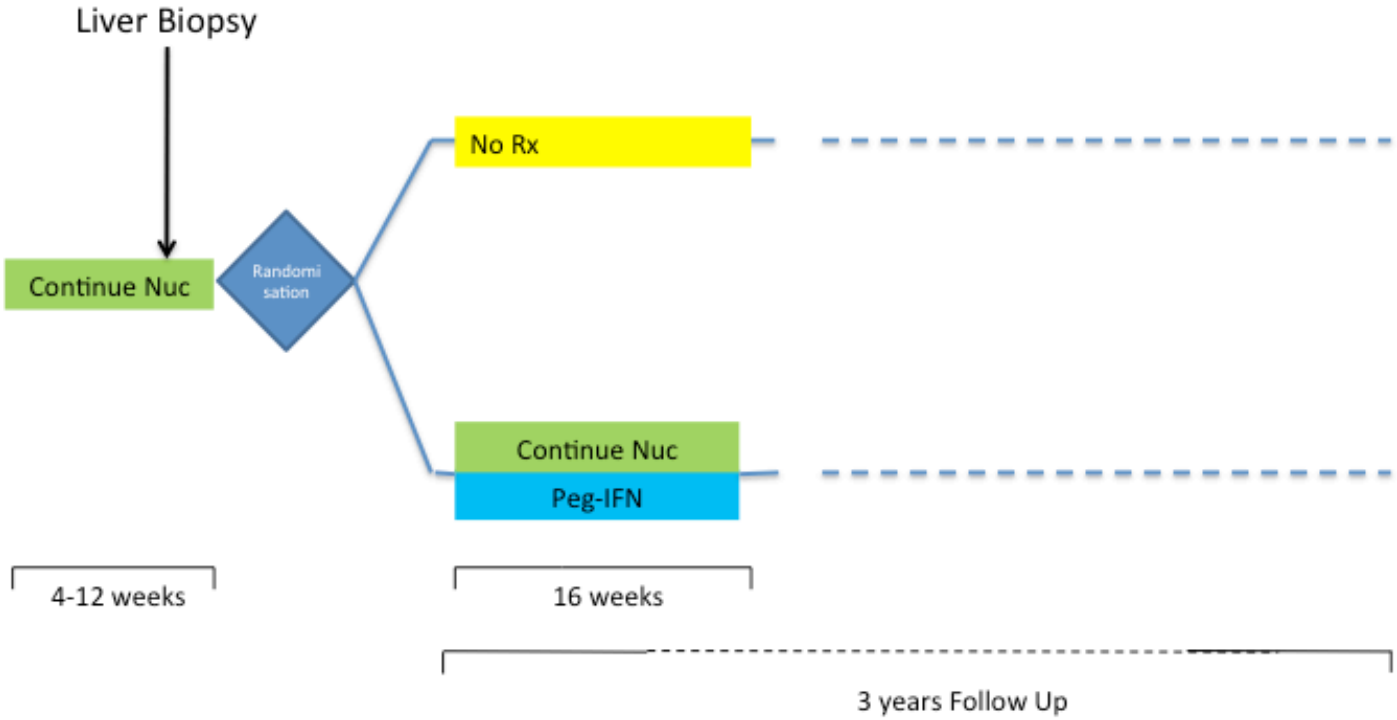
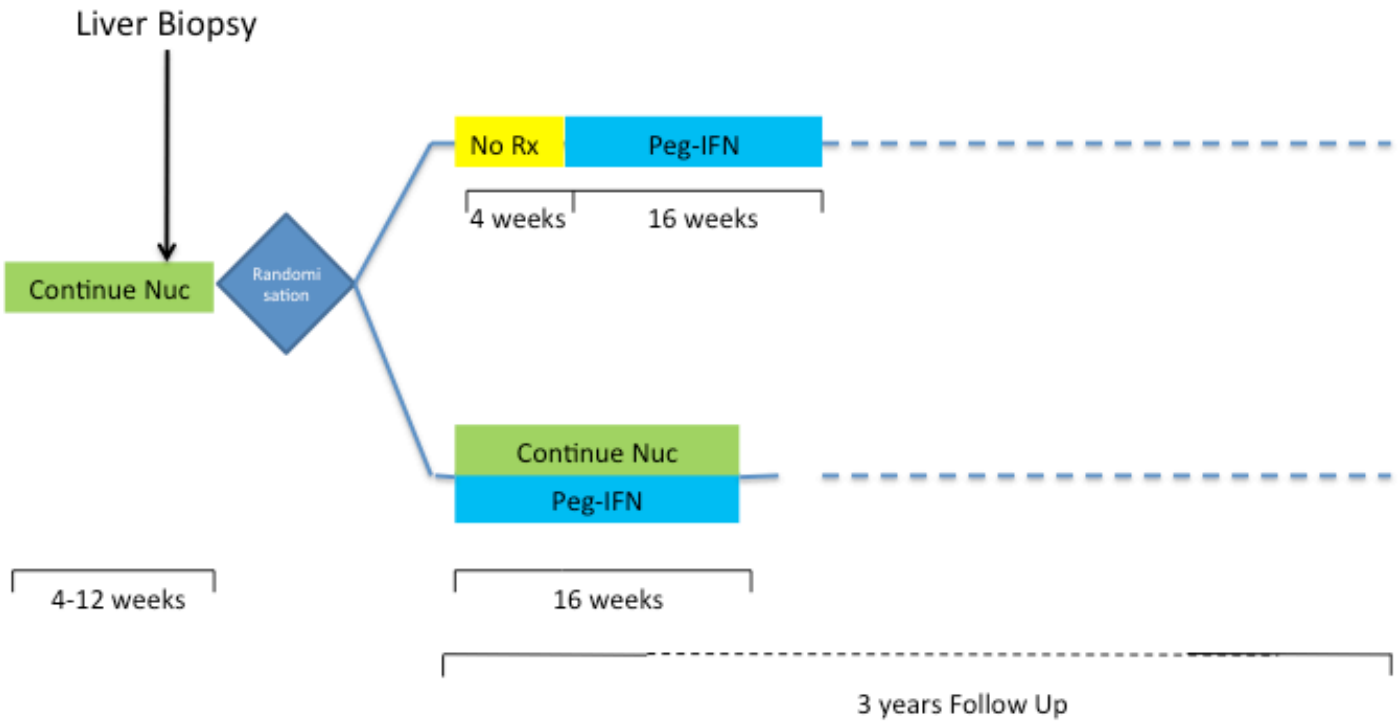


Figure 3: Interferon and combined interferon treatment Protocol (if more than 40% of patients in the original control/treatment withdrawal arm of the pilot phase experience exaggerated flares by the end of the pilot phase)



4 PARTICIPANT ENTRY

4.1 Study population

Subjects for the trial will be recruited from hepatology or hepatitis specialist clinics where appropriate patients are already being monitored on treatment with nucleos(t)ide analogues. By definition these patients are strongly adherent with clinic appointments and antiviral drugs. Patients will be given a patient information sheet and invited to an appointment at least one week later.

The trial will be open to patients with HBeAg negative chronic HBV infection who have been treated with nucleos(t)ide analogues for a minimum of three years with HBV DNA < 400 IU/ml for a minimum of two years. After screening all patients will be monitored for 4 – 12 weeks to confirm they continue to meet the inclusion criteria as listed below.

(i) Inclusion criteria

- Age ≥ 18
- Chronic HBV infection
- HBeAg negative
- Nucleos(t)ide analogues treatment for ≥3 years
- HBV DNA < 400 IU/ml ≥2 years
- Informed consent

(ii) Exclusion criteria

- Cirrhosis at any time
- HBeAg to anti-HBe seroconversion within the last 3 years
- Interferon use in the last 3 years
- Contraindications to interferon use
- Participation in HBV-specific therapeutic vaccine studies within 12 months
- HCV, HDV or HIV co-infection
- Immunosuppressant use
- Contraindications to interferon use including:

  - The presence of, or history of severe psychiatric condition, particularly severe depression, suicidal ideation or suicidal attempt
  - Presence of or history of psoriasis
  - Platelet count < 90000/mm^3
  - Absolute Neutrophil count < 1500/mm^3
  - Uncontrolled thyroid function
- Pregnancy or breastfeeding
- Women of child-bearing potential not using an effective method of contraception
- Men not using an effective method of contraception
- Clinically significant comorbidities that, in the opinion of the investigator, render the patient unsuitable.

5 PROCEDURES AND MEASUREMENTS

The trial will be open label meaning that both patients and their medical staff will know to which arm of the trial they are randomised. All patients in the trial will discontinue nucleos(t)ide analogues at baseline with the exception of the interferon arm in the alternative protocol (see Figures 2 and 3).

Occurrence of flares which require recommencement of nucleos(t)ide analogues will be an important measure of safety and efficacy in this trial and will therefore not be counted as a withdrawal from the study. Based on the previous studies of treatment cessation it is reasonable to expect up to 45% of patients to require retreatment.

A. Control arm

Patients randomised to the control arm will discontinue their nucleos(t)ide analogue treatment at baseline. They will be followed up until 3 years after randomisation.

B. Interferon arm

Patients randomised to the Interferon arm will discontinue their nucleos(t)ide analogue treatment at randomisation. No treatment will be given for 4 weeks and then they will commence pegylated interferon 180 mcg s.c. o.w α2a and will continue taking weekly interferon for a total of 16 weeks. They will subsequently discontinue all antiviral medications. They will be followed up until 3 years after randomisation.

Although pegylated interferon has a long list of side effects which may be both unpleasant for the patients and potentially harmful, poor adherence to treatment is not anticipated. All the centres in this study are experienced in the use of pegylated interferon for the treatment of both HBV and HCV infection and invariably achieve adherence rates over 95%.

<u>Control and combined interferon treatment Protocol – see Figure 2</u>

By the end of the pilot phase, if more than 40% of patients in the interferon arm experience exaggerated flares then the arm will be modified to a control and combined interferon treatment protocol in which pegylated interferon is added to the nucleos(t)ide analogue for 16 weeks from randomisation and prior to cessation of therapy rather than leaving a gap between nucleos(t)ide analogues withdrawal and pegylated interferon treatment (and compared to the control arm still). They will subsequently discontinue all antiviral medications after the 16 weeks. They will be followed up until 3 years after randomisation.

<u>Interferon and combined interferon treatment Protocol – see Figure 3</u>

By the end of the pilot phase, if more than 40% of patients in the control arm experience exaggerated flares then the control arm will be modified to the interferon and combined interferon treatment protocol in which pegylated interferon is added to the nucleos(t)ide analogue for 16 weeks from randomisation and prior to cessation of therapy. This will be compared against the standard interferon arm (used in the pilot phase). They will subsequently discontinue all antiviral medications. They will be followed up until 3 years after randomisation.

5.1 Screening and pre-randomisation evaluations

Written informed consent will be obtained before the subject undergoes any screening procedures as described in section 9.9.

All patients will have blood samples taken which will be processed and stored for later measurement of qHBsAg, ELISPOT assays and NK cell function.

Patients who consent to liver biopsy (estimated to be around 20 in number) will have a liver biopsy between screening and baseline with histological measurement of fibrosis, necroinflammatory scores and number of infected cells using immunohistochemistry for HBcAg and HBsAg. Biopsy tissue will be used to measure the level of cccDNA and to extract lymphoid cells for immunological phenotyping.

A Fibroscan (or ARFI) measurement will be made at baseline to confirm that cirrhosis is not present.

An ultrasound of the liver will be performed at baseline to exclude the presence of hepatocellular carcinoma.

5.2 Randomisation

Patients will be electronically randomised using the InForm eCRF online custom built database to the two management arms in equal proportions using variable block sizes. Randomisation will be stratified by gender and by HBsAg titre at baseline (< or ≥ 2000 IU/ml) as this is the strongest predictor of clinical outcome in interferon based treatment and was found to be a predictor of outcome in the Hadziyannis study.

5.3 Visit Schedules

Control Arm

Visit number	1	2	3	4	5	6	7	8	9	10	11	12	13
Week	-4	0	4	8	12	16	24	32	52	78	104	130	156
Control Group	Screening[2]	Baseline	Post week 4	Post week 8	Post week 12	Post week 16	Post week 24	Post month 8	Post month 12	Post Month 18	Post month 24	Post month 30	Post month 36
Consent	X												
Demographic data	X												
Inclusion / Exclusion		X											
Randomisation		X											
Medical history	X												
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination	X								X		X		X
Liver biopsy[1]	X												
USS/Fibroscan	X												
HBV Genotyping			X										
qHBsAg	X												
HBsAg		X	X				X		X		X		X
HBV DNA	X	X	X	X	X	X	X	X	X	X	X	X	X
LFTs	X	X	X	X	X	X	X	X	X	X	X	X	X
Thyroid function	X												
Electrolytes & creatinine	X												
Prothrombin time	X	X	X	X	X	X	X	X	X	X	X	X	X
FBC	X		X				X	X	X	X	X	X	X
PBMC collection (for immunology assays)		X	X				X		X		X		X
Plasma/serum storage (for virology)		X	X				X		X		X		X
Adverse events		X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy test		X											

1. Liver biopsy to be performed between screening and baseline in a sub-set of ~20 patients, 2. Baseline to occurs 4 – 12 weeks after initial screening

Interferon arm

Visit number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Week	-4	0	4	6	8	12	16	20	24	32	42	52	78	104	130	156
Interferon Group	Screening[2]	Baseline	Gap week 4	IFN week 2	IFN week 4	IFN week 8	IFN week 12	IFN week 16	Post IFN week 4	Post IFN week 12	Post IFN week 22	Post randomisation month 12	Post randomisation Month 18	Post randomisation month 24	Post randomisation month 30	Post randomisation month 36
Consent	X															
Demographic data	X															
Inclusion / Exclusion		X														
Randomisation		X														
Medical history	X															
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination	X											X		X		X
Liver biopsy[1]	X															
Ultrasound/Fibroscan	X															
HBV Genotyping			X													
qHBsAg	X															
HBsAg		X	X						X			X		X		X
HBV DNA	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
LFTs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Thyroid function	X				X			X		X						
Electrolytes & creatinine	X		X			X		X		X		X				
Prothrombin time	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FBC	X		X	X	X	X	X	X	X		X	X		X		X
PBMC collection (for immunology assays)		X	X					X	X			X		X		X
Plasma/serum storage (for virology)		X	X					X	X			X		X		X
Adverse events		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy test		X	X													

1. Liver biopsy to be performed between screening and baseline in a sub-set of ~20 patients, 2. Baseline to occurs 4 – 12 weeks after initial screening

Alternative Protocol Interferon Arm

Visit number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Week	-4	0	2	4	8	12	16	20	24	28	40	52	78	104	130	156
Interferon Group	Screening[2]	Baseline	IFN week 2	IFN week 4	IFN week 8	IFN week 12	IFN week 16	Post IFN week 4	Post IFN week 8	Post IFN week 12	Post IFN week 24	Post randomisation month 12	Post randomisation Month 18	Post randomisation month 24	Post randomisation month 30	Post randomisation month 36
Consent	X															
Demographic data	X															
Inclusion / Exclusion		X														
Randomisation		X														
Medical history	X															
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination	X											X		X		X
Liver biopsy[1]	X															
Ultrasound/Fibroscan	X															
HBV Genotyping								X								
qHBsAg	X															
HBsAg		X		X				X				X		X		X
HBV DNA	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
LFTs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Thyroid function	X			X			X				X					
Electrolytes & creatinine	X				X		X			X		X				
Prothrombin time	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
FBC	X		X	X	X	X	X	X			X	X		X		X
PBMC collection (for immunology assays)		X						X		X		X		X		X
Plasma/serum storage (for virology)		X						X		X		X		X		X
Adverse events		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy test		X														

1. Liver biopsy to be performed between screening and baseline in a sub-set of ~20 patients, 2. Baseline to occurs 4 – 12 weeks after initial screening

Additional visits may be made during interferon therapy to support or instruct patients but these will not be considered as study visits and will not be recorded in the study CRF.

Additional visits may be made at the discretion of the investigator for safety monitoring if hepatitis flares occur or for other safety reasons. These will be recorded in the unscheduled visit form.

Visit windows/tolerance:

Arm	Visit	Tolerance
Interferon Arm (including alternative interferon arms)	2	2 weeks – 3 months
	3-12	+/- 3 days
	13-16	+/- 10 days
Withdrawal (control arm)	2	2 weeks – 3 months
	3-8	+/- 3 days
	9 - 13	+/- 10 days

5.4 Treatment

In the interferon arm patients will commence treatment four weeks after nucleos(t)ide analogue withdrawal with pegylated interferon α2a 180 mcg s.c once weekly for 16 weeks. The first injection will be administered in the clinic supervised by an experienced research nurse or clinical nurse specialist.

5.5 Follow-up

In the control arm visits 3-6 will be used to monitor safety. In the Interferon arm visits 4 – 8 will be used to monitor and manage the side effects of interferon therapy.

Biological samples will be taken according to the relevant visit schedule in Section 5.3.

5.6 Laboratory Evaluations

(i) Haematology

Full blood counts and prothrombin times will be measured using standard methods in the routine haematology department at each participating site.

(ii) Biochemistry

Liver function tests (bilirubin, albumin, ALT, alkaline phosphatase), thyroid function tests (TSH & T4), glucose, electrolytes and creatinine will be measured using standard methods in the routine haematology department at each participating site.

(iii) HBV DNA

HBV DNA will be measured quantitatively using real-time PCR based assays in the routine virology departments at each participating site. Results will be reported back on the eCRF.

(iv) HBV genotype

Plasma from an EDTA specimen taken at week 4 will be collected and processed at each participating site and stored at -80^{o}C until transported to Virology at UCLH. Genotyping will be performed by sequence analysis of the HBV DNA.

(v) HBsAg quantification

A quantitative HBsAg measure will be performed at each local hospital on a screening visit blood sample. The result will be used for stratification in the randomisation system.

HBsAg concentrations will be performed using the Abbott Architect qHBsAg system in Dr Nastouli's laboratory. Serum samples will be collected in the virology department at each participating site and stored at -80^{o}C until transported, frozen, in batches to Virology at UCLH.

(vi) Liver Biopsy

In patients who consent to have a liver biopsy as part of the study the procedure will be performed by a trained hepatologist or radiologist and two passes will be made with the biopsy needle to obtain 2 liver specimens of 1.5 – 2 cm in length. One of these will be placed in to 10% formaldehyde and sent to the histology laboratory for paraffin embedding and sectioning. The first 2 sections will be used for standard H&E and pico-sirius red staining to assess necroinflammatory and fibrosis scores. Two sections will be used for immunohistochemistry staining for HBsAg and HBV core antigens. Two sections will be sent to Dr Dorner's laboratory for measurement of cccDNA.

The second liver specimen will be collected in sterile PBS and sent by courier to Prof Maini's laboratory.

(vii) ELISPOT

30-40 ml of peripheral blood will be collected into heparinised tubes. Samples will be transported by courier to Dr Chokshi's laboratory at the Institute of Hepatology. Peripheral blood mononuclear cells (PBMC) will be extracted using standard laboratory techniques and then frozen in liquid nitrogen until used for analysis in batches. Immunology samples will also be collected at unscheduled visits if the patient experiences a hepatitis flare.

(viii) NK Cells

PBMCs from Dr Chokshi's laboratory will be transferred to the Maini laboratory for analysis of NK cell numbers and activation status using fluorescence activated cell sorting techniques (FACS) after staining with monoclonal antibodies.

(ix) Sample storage

10 ml serum samples will be taken at several timepoints (denoted on the visit schedule), processed at each participating site and stored at -80^{o}C until transferred to Imperial College. These will be stored in the Imperial Biobank and used in future research to explore virological, metabolic or immunological determinants of clinical response.

5.7 Clinical investigations

Physical examination

A brief clinical examination will be conducted to eliminate features associated with decompensated cirrhosis and to measure vital signs. Vital signs will be measured at every visit, including the following: systolic and diastolic blood pressure, heart rate and temperature.

Ophthalmology examination

All patients in the interferon arm will have a baseline eye examination.

Concomitant medications
Records of all prescribed and over the counter medications will be collected at each visit.

Medical History
A history of significant previous illnesses and ongoing co-morbidities will be collected.

Fibroscan (or ARFI)

A measurement will be made at the screening visit to confirm that cirrhosis is not present.

Ultrasound

An ultrasound of the liver will be performed at the screening visit to exclude the presence of hepatocellular carcinoma.

Pregnancy test
A pregnancy test will be performed at the baseline visit for all females who are not considered to be using adequate contraception. In the interferon arm an additional pregnancy test will be performed at the visit before interferon treatment commences to ensure that patients are not pregnant before commencing treatment. Patients will be asked to use adequate contraception whilst on interferon to prevent pregnancy or for male participants to prevent pregnancy in a female partner.

Adequate birth control methods are defined as those that can achieve a failure rate of less than 1% per year when used consistently and correctly. Examples include hormonal contraception, intra uterine device, bilateral tubal occlusion, vasectomised partner, sexual abstinence. Abstinence is only acceptable if it is true abstinence in line with the preferred and usual lifestyle of the patient. Periodic abstinence such as calendar, ovulation, post-ovulation, and declaration of abstinence for the duration of exposure to IMP and withdrawal are not acceptable methods of contraception.

6 TREATMENTS

Patients in the Interferon arm (and alternative protocol interferon arms) will receive Pegylated interferon α2a 180 mcg once weekly by subcutaneous injection. This is an open label study using a licensed medication in a licensed indication. Therefore the Peg-IFN will be issued on a standard hospital prescription and dispensed from standard pharmacy stock. No special labelling or packaging will be required. Storage and dispensing will be according to normal pharmacy protocols.

6.1 Adherence

Subjects in the Interferon arm will be monitored at visits 4,5,6,7 and 8 during the Peg-IFN treatment phase (or visits 2,4,8,12 and 16 in the alternative protocols). Adherence will be monitored by patient reporting and any dose reductions will be reported in the CRF.

6.2 Drug administration

The patients will self administer peg-IFN injections subcutaneously. They will be taught how to perform the injection by research nurses or clinical nurse specialists on their first dose. Nurses will also explain where it is suitable to inject.

6.3 Dose Modifications for Toxicity

If neutrophil counts fall below 1 x 10^9/ml or platelets fall below 100 x 10^9/ml the dose of Peg-IFN will be reduced to 90 mcg / week. If the neutrophil count falls below 0.5 10^9/ml or the platelet count falls below 50 10^9/ml then Peg-IFN will be discontinued until the counts recover.

6.4 Hepatitis Flare

A hepatitis flare is defined as an ALT greater than twice the upper limit of normal (ALT> 2 X ULN). If a patient reaches this level at any visit they will be asked to return for an unscheduled visit at which further blood tests will be taken. If the patient continues to meet the criteria for a flare they will continue to be seen at unscheduled visits until the flare has resolved (at which point they will return to the routine visit schedule) or if the flare worsens and meets the definition of an exaggerated flare they will recommence nucleos(t)ide analogues as described below.

6.5 Discontinuation of Study Treatment and Withdrawal from Study

(i) Recommencement of nucleos(t)ide analogues

Retreatment with nucleos(t)ide analogue therapy will be recommenced according to the following criteria for exaggerated flares:

1. Disturbance of liver synthetic function
    a. Bilirubin > 35 uMol/l
    b. INR > 1.3
2. ALT > 20 x upper limit of normal
3. If HBV DNA and/or ALT remain persistently elevated for more than 6 months
4. If, in the opinion of investigator or patient there is a risk to health

It is possible that there may be variation in the criteria used to determine whether a patient with hepatitis flare post cessation would need to be restarted on treatment according to criteria number 4. Data will be collected on patients considered for retreatment to determine whether any systematic differences appear between the two groups.

Recommencement will not be considered as a withdrawal from the study. A form will be completed in the eCRF to document the date and reason for recommencement. Patients who resume nucleos(t)ide analogue therapy will be monitored at intervals at unscheduled visits until ALT, Bilirubin and INR test results begin to normalise and once they have returned to normal then the patients will return to the study visit schedule until the end of the study.

(ii) Permanent discontinuation of study treatment

Subjects may discontinue study medication for the following reasons:

- At the request of the subject.
- Adverse event/ Serious Adverse Event
- Allergic reaction to IMP
- In the event of pregnancy
- If the investigator considers that a subject's health will be compromised due to adverse events or concomitant illness that develops after entering the study.

(iii) Withdrawal from Study

Withdrawal from the study refers to discontinuation of study treatment and study procedures and can occur for the following reasons:

- Subject decision
- Loss to follow-up

In this situation the patient will be encouraged, where possible, to resume the nucleos(t)ide analogue treatment which they were taking before enrolling in the study.

7 PHARMACOVIGILANCE

7.1 Adverse Event (AE)

An AE is any untoward medical occurrence in a patient or clinical trial subject administered a medicinal 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 the trial medication, whether or not considered related to the IMP.

7.2 Adverse Event recording

For the purposes of the study, AEs will be followed up according to local practice until the event has stabilised or resolved, or the Follow-up Visit, whichever is the sooner. SAEs will be recorded throughout the study.

Non serious AR/AEs

All such toxicities, whether expected or not, should be recorded in the adverse event section of the relevant case record form within one month of the form being due.

(i) Severity of Adverse Events

Definitions for assessment of severity:

Mild: Awareness of event but easily tolerated
Moderate: Discomfort enough to cause some interference with usual activity
Severe: Inability to carry out usual activity

(ii) Causality of Adverse Events

The assignment of the causality should be made by the investigator responsible for the care of the participant using the definitions listed below.

If any doubt about the causality exists the local investigator should inform the study coordination centre who will notify the Chief Investigator. The pharmaceutical companies and/or other clinicians may be asked to advise in some cases.

In the case of discrepant views on causality between the investigator and others, all parties will discuss the case. In the event that no agreement is made, the MHRA will be informed of both points of view.

Definitions for assessment of causality:

Unrelated: No evidence of any causal relationship

Unlikely: There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant treatment).

Possible: There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition, other concomitant treatments).

Probable: There is evidence to suggest a causal relationship and the influence of other factors is unlikely.

Definite: There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.

7.3 Abnormal Laboratory Test Results

Abnormalities in liver function tests and HBV DNA will not be recorded as adverse events. All other clinically important abnormal laboratory test results occurring during the study will be recorded as adverse events. The clinically important abnormal laboratory tests will be repeated at appropriate intervals until they return either to baseline or to a level deemed acceptable by the investigator or until a diagnosis that explains them is made.

7.4 Serious Adverse Events (SAE)

i) Definition of SAE

An SAE is defined as any event that

- Results in death;
- Is life-threatening*;
- Requires hospitalisation or prolongation of existing inpatient's hospitalisation**;
- Results in persistent or significant disability or incapacity;
- Is a congenital abnormality or birth defect;

* "Life-threatening" in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

** "Hospitalisation" means any unexpected admission to a hospital department. It does not usually apply to scheduled admissions that were planned before study inclusion or visits to casualty (without admission).

Medical judgement should be exercised in deciding whether an adverse event/reaction is serious in other situations. Important adverse events/reactions that are not immediately life-threatening, or do not result in death or hospitalisation but may jeopardise a subject, or may require intervention to prevent one of the other outcomes listed in the definition above should also be considered serious.

(ii) Reporting of SAEs

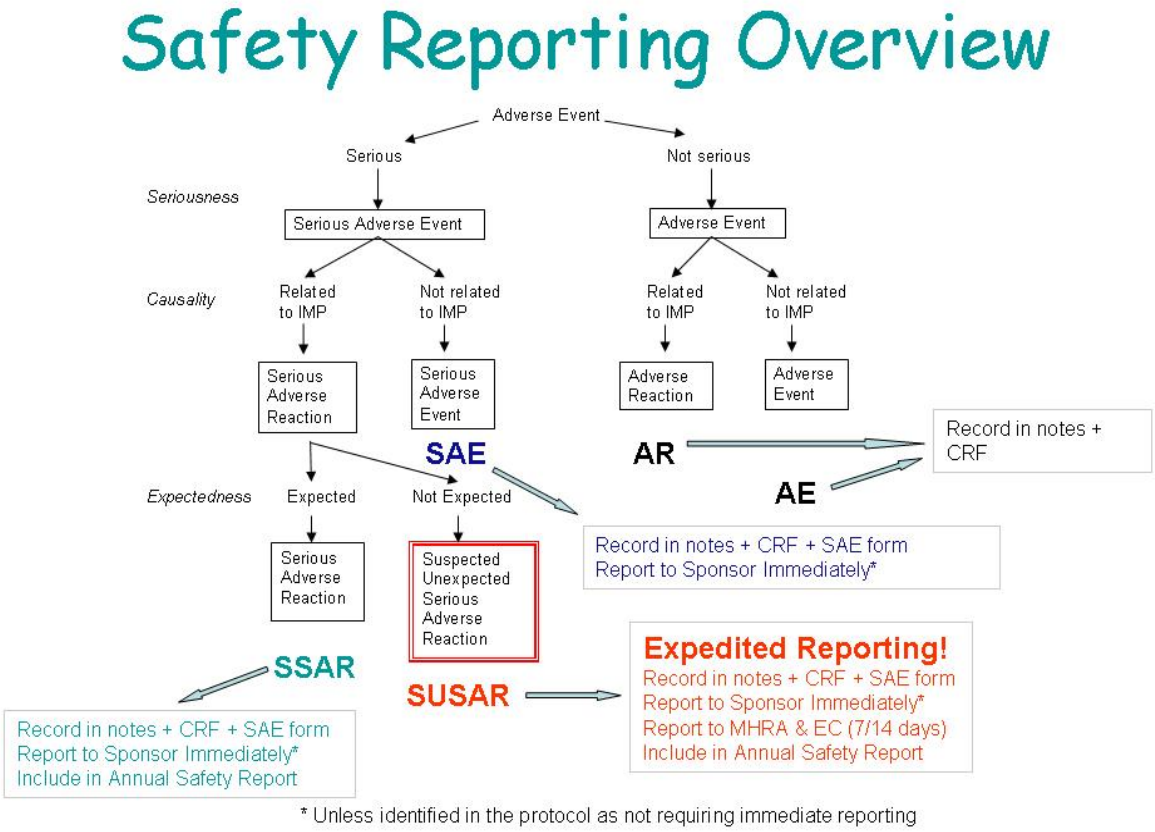
Rapid reporting of all SAEs i.e. within 24 hours, occurring during the study must be performed as detailed in the study specific SAE reporting instructions. An overview of the Safety Reporting Process is detailed in the diagram below. If the investigator becomes aware of safety information that appears to be drug related, involving a subject who participated in the study, even after an individual subject has completed the study, this should be reported to the Sponsor.

All SAEs will be reviewed by the Chief Investigator or a designated medically qualified representative to confirm expectedness and causality.

Reporting of SAEs and review by the CI will be via the trial data collection system (InForm eCRF).

Fatal or life threatening SAEs should be reported on the day that the local site is aware of the event. The SAE form asks for nature of event, date of onset, severity, corrective therapies given, outcome and causality (i.e. unrelated, unlikely, possible, probably, definitely). The responsible investigator should assign the causality of the event. Additional information should be sent within 5 days if the reaction has not resolved at the time of reporting.

An SAE form should be completed and entered into the eCRF for all SAEs within 24 hours. This will automatically send alert emails to the Chief Investigator, the Project Manager and the Sponsor.



Contact details for reporting SAEs and SUSARs

Follow the study specific procedure for reporting SAEs and SUSARs and complete an SAE form & submit to the Study Coordination Centre as soon as possible (within 24 hrs). SAE forms are available on the InForm eCRF system.

E-mail: nucbtrial@imperial.ac.uk

(iii) Definition of an Adverse Reaction (AR)

All untoward and unintended responses to an IMP related to any dose administered or relating to the withdrawal of the patient from nucleos(t)ides. All AEs judged by either the reporting investigator or the sponsor as having reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship.

Exaggerated hepatitis flares are defined according to the following criteria:

1. Disturbance of liver synthetic function
    a. Bilirubin > 35 uMol/l
    b. INR > 1.3
2. ALT > 20 x upper limit of normal

3. If HBV DNA and/or ALT remain persistently elevated for more than 6 months
4. If, in the opinion of investigator or patient there is a risk to health

These are an expected reaction to nucleos(t)ide withdrawal and will not be recorded or reported as an AR/AE. Exaggerated flares will be recorded in the Case Report Form to monitor patient safety throughout the trial as described elsewhere in the protocol.

Expected non-serious side effects of interferon (as documented in the SMPC) will not be recorded as adverse reactions.

(iv) Definition of a Serious Adverse Reaction (SAR)

A SAR is defined as a SAE that is judged to be related to any dose of study drug administered to the subject or withdrawn from the subject.

(v) Definition of Suspected Unexpected Serious Adverse Reaction (SUSAR)

Any SAR that is NOT consistent with the applicable product information as set out in the Investigator Brochure (IB) or Summary of Product Characteristics (SPC) ie is unexpected.

An unexpected reaction to nucleos(t)ide withdrawal is defined as one which has temporal or biological relationship to the intervention but does not include a hepatitis flare.

(vi) Reporting of SUSARs

In the case of suspected unexpected serious adverse reactions, the staff at the site should:

Complete the SAE eCRF (within 24 hours). The study coordination centre will be notified by email and may contact the local site for further information and may require anonymised copies of all relevant investigations and documents.

Or

Contact the study coordination centre by phone and complete the SAE form within the following 24 hours as above.

The study coordination centre will notify the MHRA, REC and the Sponsor of all SUSARs occurring during the study according to the following timelines; fatal and life-threatening within 7 days of notification and non-life threatening within 15 days. All investigators will be informed of all SUSARs occurring throughout the study.

Local investigators should report any SUSARs and / or SAEs as required by the protocol and study specific SOPs. Follow up of patients who have experienced a SUSAR should continue until recovery is complete or the condition has stabilised.

(vii) Annual reporting of Serious Adverse Events

Annual Safety reports will be submitted to the Sponsor, the Ethics Committee and Regulatory Authority in accordance with regulatory requirements.

(viii) Pregnancy

If a patient becomes pregnant during the study then they will be withdrawn from interferon treatment and encouraged to resume nucleos(t)ide analogues. The outcome of the pregnancy will be reported.

8 STATISTICAL ANALYSES

8.1 Sample Size and power considerations

HBsAg loss is the primary endpoint; the trial will compare the rate of HBsAg loss in patients three years after cessation of nucleos(t)ide analogues with the rate of HBsAg loss in patients treated with pegylated interferon after cessation of nucleos(t)ide analogues. Only one published study provides an estimate of the rate of HBsAg loss in patients who stopped treatment after 4 years of nucleos(t)ide analogues which was 10% at 1 year,18% at 2 years and 24% at 3 years. A similar study reported by Berg (EASL 2015) confirmed the 10% HBsAg loss reported at 1 year. In the present study we will recruit some patients who have had treatment with nucleos(t)ide analogues for only 3 years and we will therefore use a conservative assumption for the rate of HBsAg loss in this group at 15% at 2 years and 20% at 3 years.

There is no data on the rate of HBsAg loss in patients who have been treated with nucleos(t)ide analogues and subsequently been treated with pegylated interferon. In a trial of pegylated interferon for naïve HBeAg negative hepatitis infection 42.4% had lost HBsAg at the end of follow up if the level of HBV DNA had been <70 IU/ml at the end of treatment. As this is an exploratory study we have assumed that the rate of HBsAg loss in this group will be 30% at 2 years and 40% at 3 years. This increment would represent the very significant clinical benefit which would justify the use of interferon (with associated side effects) in this group of patients.

A trial with 90% power to detect a difference in HBsAg loss between these groups (40% vs 20%) at the $P < 0.05$ threshold would require 109 subjects in each arm. Therefore allowing for ~10% withdrawal / dropout in each arm we will aim to recruit 120 subjects to each arm; a total of 240 patients in total. If either arm is discontinued after the pilot, then there will still be at least 80% power in comparison with an alternative trial arm in the post pilot participants.

8.2 Data Analysis

(i) Primary Endpoint Analysis

This analysis will be performed primarily on an intention to treat (ITT) basis. The ITT population will be all subjects who have been randomized. Secondarily, a per protocol analysis will be performed. The per protocol population will be those confirmed to be eligible who have not resumed nucleos(t)ide analogues for patient/ physician preference and those, in the interferon arm, who have completed at least 8 weeks of interferon therapy. Rates of HBsAg loss will be compared between the two groups using logistic regression adjusting for the stratification factors HBsAg titre and centre. Results will be presented as odds ratios and their 95% confidence intervals.

(ii) Secondary outcomes

Secondary outcomes to be compared between the two groups will include the primary outcome assessed at one year and at two years from baseline. Kaplan-Meier plots will be produced to show the persistence of HBsAg. The plots will be supported by formal secondary analyses using Cox proportional hazards model methods

Achievement of the inactive carrier state (defined above) will be analysed using the same procedures as the primary outcome. A statistical analysis plan will be developed and approved by the Trial Steering committee prior to any regular analysis for reports to the Data Monitoring Committee. This plan will include details of the statistical methods to be used to analyse the outcomes, and describe the methods to undertake sensitivity analyses for handling missing data. The plan will contain conform with the Clinical Trials Unit standard operating procedures

(iii) Predictors of HBsAg Loss

Predictive factors for HBsAg loss will be explored using univariate and multivariate logistic regression analysis. Separate models will be fitted for the following variables: gender, age, duration of antiviral therapy, viral genotype, viral load pre-treatment, HBsAg titre, centre, peak ALT value post withdrawal, peak viral load post withdrawal, hepatic cccDNA, number of infected hepatocytes and ELISPOT counts. Odds ratios and their 95% confidence intervals produced from each of the models will be presented. Following this, a multivariate logistic regression analysis will be performed will include the variables that were statistically significant in the univariate analysis plus a variable for treatment group.

(iv) Safety analysis

The occurrence of exaggerated hepatitis flares, defined in Section 6.4, will be monitored in each arm of the trial and presented as part of the regular reporting to the Data Monitoring Committee.

9 REGULATORY, ETHICAL AND LEGAL ISSUES

9.1 Treatment

9.1.1 Investigational Medicinal Product Details

Drug Name	Dosage	Description
Pegylated Interferon	180 mcg o.w. s.c. α2a	Standard pharmacy supplies of this licensed medication will be issued on regular prescriptions with no specific trial labelling.

9.1.2 Labelling, storage and dispensing

Labelling, storage and dispensing will be as per routine pharmacy clinical procedures using standard pharmacy supplies. Trial-specific labelling is not required as the IMP does not require any particular manufacturing or packaging processes, it has an MA, is used within its MA, is prescribed by a healthcare professional and is labelled according to The Medicines for Human Use Regulations 1994.

9.1.3 Accountability

There are no requirements for accountability or shipping and destruction records for the trial as the IMP is used within its authorisation and prescribed from routine stock in the NHS. We will monitor, and record on the eCRF, patient adherence to pegylated interferon therapy as described in section 6.1 as a safety endpoint.

9.2 Declaration of Helsinki

The investigator will ensure that this study is conducted in full conformity with the 7th revision of the 1964 Declaration of Helsinki.

9.3 Good Clinical Practice

The study will be conducted in accordance with the guidelines laid down by the International Conference on Harmonisation for Good Clinical Practice (ICH GCP E6 guidelines).

9.4 Independent Ethics Committee Approval

(i) Initial Approval

Prior to the enrolment of subjects, the REC must provide written approval of the conduct of the study at named sites, the protocol and any amendments, the Subject Information Sheet and Consent Form, any other written information that will be provided to the subjects, any advertisements that will be used and details of any subject compensation.

(ii) Approval of Amendments

Proposed amendments to the protocol and aforementioned documents must be submitted to the REC for approval as instructed by the Sponsor. Amendments requiring REC approval may be implemented only after a copy of the REC's approval letter has been obtained.

Amendments that are intended to eliminate an apparent immediate hazard to subjects may be implemented prior to receiving Sponsor or REC approval. However, in this case, approval must be obtained as soon as possible after implementation.

(iii) Annual Progress Reports

The REC will be sent annual progress reports in accordance with national requirements.

(iv) Annual Safety Reports and End of Trial Notification

The REC will be sent annual safety updates in order to facilitate their continuing review of the study (reference. ICH GCP E6 Section 3.1.4) and will also be informed about the end of the trial, within the required timelines.

9.5 Regulatory Authority Approval

The study will be performed in compliance with UK regulatory requirements. Clinical Trial Authorisation from the Medicines and Healthcare Products Regulatory Agency (MHRA) will be obtained prior to the start of the study. In addition, the MHRA will approve amendments prior to their implementation (as instructed by the Sponsor), receive SUSAR reports and annual safety updates, and be notified of the end of the trial.

9.6 R&D Approvals

R&D approval will be obtained from each participating site before any trial procedures commence at that site.

9.7 Insurance and Indemnity

Imperial College London, the Sponsor of the trial has civil liability insurance, which covers this study in all participating centres. Imperial College London also holds negligent harm and non-negligent harm insurance policies which apply to this study.

9.8 Trial Registration

The study will be registered on a trial database (clinicaltrials.gov and/or ISRCTN) in accordance with requirements of the International Committee of Medical Journal Editors (ICMJE) regulations.

9.9 Informed Consent

Informed consent will be obtained from all participants. Patient information sheets and consent forms will be provided in English, Urdu and Mandarin. Translation services provided by the NHS will be used to ensure that patients' questions or concerns are appropriately addressed.

The patient will be informed about the trial by the responsible clinician or a member of the research team and given a copy of the Patient Information Sheet (PIS). Informed patients will be given an adequate amount of time to consider their participation in the trial. If the patient decides to participate in the trial they will be asked to sign the Patient Consent Form which will then be countersigned by the responsible clinician / researcher. The patient will retain one copy of the signed Consent Form. Another copy will be placed in the patient's medical records whilst the original will be retained in the research record for the patient at sites.

The right of the participant to refuse to participate without giving reasons must be respected. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

9.10 Contact with General Practitioner

It is the investigator's responsibility to inform the subject's General Practitioner by letter that the subject is taking part in the study provided the subject agrees to this, and information to this effect is included in the Patient Information Sheet and Informed Consent. A copy of the letter should be filed in research record for the patient at sites.

9.11 Subject Confidentiality

The investigator must ensure that the subject's confidentiality is maintained. On the CRF or other documents submitted to the Sponsors, subjects will be identified by a subject ID number only. Documents that are not submitted to the Sponsor (e.g., signed informed consent form) should be kept in a strictly confidential file by the investigator.

The investigator shall permit direct access to subjects' records and source document for the purposes of monitoring, auditing, or inspection by the Sponsor, authorised representatives of the Sponsor, Regulatory Authorities and RECs.

9.12 Data Protection

All personnel involved in the study will observe or work within the confines of the local data protection guidelines.

9.13 End of Trial

The end of the trial will be last patient last visit.

9.14 Study Documentation and Data Storage

The investigator will retain essential documents until notified by the Sponsor, and for at least ten years after study completion. Patient files and other source data (including copies of protocols, CRFs, original reports of test results, IMP dispensing logs, correspondence, records of informed consent, and other documents pertaining to the conduct of the study) will be retained. Documents will be stored in such a way that they can be accessed/data retrieved at a later date.

No study document will be destroyed without prior written agreement between the Sponsor and the investigator. Should the investigator wish to assign the study records to another party or move them to another location, written agreement must be obtained from the Sponsor.

10 DATA MANAGEMENT

10.1 Source Data

Source documents include original documents related to the trial, to medical treatment and to the history of the participant, and adequate source documentation must be maintained to allow reliable verification and validation of the trial data. What constitutes the source data for this trial will be outlined in the study Monitoring Plan.

10.2 Language

CRFs will be in English. Generic names for concomitant medications should be recorded in the CRF wherever possible. All written material to be used by subjects must use vocabulary that is clearly understood, and be in the language appropriate for the study site. Patient information sheets and consent forms will be provided in English, Urdu and Mandarin.

10.3 Database

Electronic CRF: the principal means of data collection from participant visits will be Electronic Data Capture (EDC) via the internet using the InForm database. Data is entered into the EDC system via site personnel. All source data recorded in the CRF will be signed by the Investigator or his/her appropriate designee. All changes made following the electronic signing will have an electronic audit trail with a signature and date. Specific instructions and further details will be outlined in the CRF manual.

10.4 Data Collection

Details of procedures for eCRF completion will be provided in a study manual.

11 STUDY MANAGEMENT STRUCTURE

11.1 Trial Steering Committee

A Trial Steering Committee (TSC) will be convened including as a minimum an independent Chair, 2 independent clinicians, a lay representative, the Chief Investigator and Trial Manager. The role of the TSC is to provide overall supervision of trial conduct and progress. A TSC Charter will be devised to list the roles and responsibilities of the TSC members. There will be 6-monthly meetings of the TSC.

11.2 Trial Management Group

A Trial Management Group (TMG) will be convened including the Chief Investigator, co-investigators and key collaborators, trial statistician and trial manager. The TMG will be responsible for day-to-day conduct of the trial and operational issues including recruitment and other practical aspects of the trial.

The day-to-day management of the trial will be co-ordinated through the Imperial Clinical Trials Unit and the Chief Investigator.

11.3 Data Monitoring Committee

An independent Data Monitoring and Ethical Committee (DMEC) will be set up to monitor progress, patient safety and any ethical issues involved in this trial. They will review trial progress, recruitment rates, event rates and safety data. A separate charter will be drawn up defining their exact remit and criteria for reporting to the trial steering committee. There will be 6-monthly meetings of the independent DMEC.

11.4 Early Discontinuation of the Study

See pilot phase above for a description of the criteria for discontinuing the trial during the pilot phase.

In the main study, if, in the opinion of the Chief Investigator, clinical events indicate that it is not justifiable to continue the trial, the Trial Steering Committee may terminate the trial following consultation with the Sponsor.

11.5 Risk Assessment

A study-specific risk assessment will be performed prior to the start of the study to assign a risk category of 'low', 'medium' or 'high' to the trial. Risk assessment will be carried out by the ICTU QA Manager in collaboration with the Study Manager/Operations Manager and the result will be used to guide the monitoring plan. The risk assessment will consider all aspects of the study and will be updated as required during the course of the study.

11.6 Monitoring

The study will be monitored periodically by a trial monitor to assess the progress of the study, verify adherence to the protocol, ICH GCP E6 guidelines and other national/international requirements and to review the completeness, accuracy and consistency of the data.

A monitoring plan will be devised based on risk analysis and described in detail in the monitoring manual. A Trial Monitor will visit all sites and facilities where the trial will take place to ensure compliance with the protocol, GCP and local regulatory compliance.

Initiation visits will be completed at all trial sites prior to the recruitment of participants, and will consist of review of protocol and trial documents, training with respect to trial procedures (informed consent, SAE reporting, inclusion and exclusion criteria), review of recruitment strategy, review of site facilities and equipment, essential document receipt, collection and filing, and archiving and inspection.

The investigators will allow the monitors to:

- inspect the site, the facilities, IMP management and materials used for the trial
- meet all members of the team involved in the trial, and ensure all staff working on the trial are experienced and appropriately trained, and have access to review all of the documents relevant to the trial
- have access to the electronic case record forms and source data
- discuss with the investigator and site staff trial progress and any issues on a regular basis

The monitor will ensure that;

- all participant records will be inspected for confirmation of existence, eligibility and informed consent
- there is adherence to the protocol, including consistency with inclusion/exclusion criteria
- there is GCP and regulatory compliance
- Trial Documentation is complete and up to date (e.g. correct versions of documents being used, source data captured) and relevant documents are collected for the Trial Master File (TMF)
- The eCRFs have been completed correctly and accurately, and all entries correspond to data captured in source documents

At the end of the trial, close out visits will be performed by the monitor after the final participant visit has been completed.

Each investigator will also be notified that an audit or inspection may be carried out - by the sponsor, sponsor's representatives or the host institution, or regulatory authorities - at any time, before, during or after the end of the trial. The investigator must allow the representatives of the audit or inspection team:

- to inspect the site, facilities and material used for the trial,
- to meet all members of his/her team involved in the trial,

to have direct access to trial data and source documents, to consult all of the documents relevant to the trial. If an Investigator is informed of an impending audit or inspection, the trial coordination centre should be notified immediately.

11.7 Quality Control and Quality Assurance

Quality Control will be performed according to Imperial Clinical Trials Unit internal procedures. The study may be audited by a Quality Assurance representative of the Sponsor and/or ICTU. All necessary data and documents will be made available for inspection.

The study may be subject to inspection and audit by Imperial College London under their remit as Sponsor, the Study Coordination Centre and other regulatory bodies to ensure adherence to GCP.

11.8 Disclosure of Data and Publication

Verbal or written discussion of results prior to study completion and full reporting should only be undertaken with written consent from the Sponsor.

Therefore all information obtained as a result of the study will be regarded as CONFIDENTIAL, at least until appropriate analysis and review by the investigator(s) are completed.

The results may be published or presented by the investigator(s), but the Sponsor will be given the opportunity to review and comment on any such results before any presentations or publications are produced.

All publications and presentations relating to the study will be authorised by the Trial Management Group. Authorship will be determined according to the internationally

agreed criteria for authorship (www.icmje.org). Authorship of parallel studies initiated outside of the Trial Management Group will be according to the individuals involved in the project but must acknowledge the contribution of the Trial Management Group and the Study Coordination Centre.

11.9 Patient and Public Involvement (PPI)

At least one PPI representative will sit on the Trial Steering Committee and will provide input from a patient perspective at trial meetings. Representatives have reviewed and provided feedback on all of the project documents prior to the ethics and regulatory submissions and their comments have been incorporated.

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SIGNATURE PAGE 1 (CHIEF INVESTIGATOR)

The signature below constitutes approval of this protocol by the signatory and provides the necessary assurances that this study will be conducted according to all stipulations of the protocol including all statements regarding confidentiality.

Study Title: Nucleos(t)ide withdrawal in HBeAg negative hepatitis B virus infection to promote HBsAg clearance. (Nuc-B)

Protocol Number: Protocol number: 16SM3217

Signed: _____

Mark Thursz
Professor of Hepatology

Date: _____

SIGNATURE PAGE 2 (SPONSOR)

The signatures below constitute approval of this protocol by the signatory.

Study Title: Nucleos(t)ide withdrawal in HBeAg negative hepatitis B virus infection to promote HBsAg clearance. (**Nuc-B**)

Protocol Number: Protocol number: 16SM3217

Signed: _____

Gary Roper
Head of Regulatory Compliance
Imperial College, London

Date: _____

SIGNATURE PAGE 3 (STATISTICIAN)

The signatures below constitute approval of this protocol by the signatory.

Study Title: Nucleos(t)ide withdrawal in HBeAg negative hepatitis B virus infection to promote HBsAg clearance. (**Nuc-B**)

Protocol Number: Protocol number: 16SM3217

Signed: _____

Name of Statistician
Title
Organisation/Company

Date: _____

SIGNATURE PAGE 4 (PRINCIPAL INVESTIGATOR)

The signature of the below constitutes agreement of this protocol by the signatory and provides the necessary assurance that this study will be conducted at his/her investigational site according to all stipulations of the protocol including all statements regarding confidentiality.

Study Title: Study title

Protocol Number: Protocol number: 16SM3217

Address of Institution: _____

_____

_____

Signed: _____

Print Name and Title: _____

Date: _____