Clinical Study Protocol

LONG TITLE OF THE TRIAL

A phase I, randomised, double-blind, placebo-controlled, multi-centre, ascending-dose trial to evaluate the safety, tolerability and immunogenicity of Vaccine FP-02.2 in HBeAg-negative hepatitis B patients as an add-on treatment to entecavir or tenofovir.

SHORT STUDY TITLE

Phase I safety and immunogenicity of FP-02.2 in chronic hepatitis B

PROTOCOL VERSION NUMBER AND DATE

Version 6 – 11 August 2016

TRIAL REGISTRY NUMBER

EudraCT number: 2015-000880-15

OTHER RESEARCH REFERENCE NUMBERS

FP02.2_CS_01

SPONSOR / CO-SPONSORS / JOINT-SPONSORS

Altimmune UK Ltd / Altimmune Inc

SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the trial in compliance with the approved protocol and will adhere to the principles of Good Clinical Practice, the principles of the Declaration of Helsinki, applicable local regulations, the Sponsor's SOPs, and other regulatory requirements as amended.

I agree to ensure that the confidential information contained in this document will not be used for any purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor.

I also confirm that I will make the findings of the study publically available through publication or other dissemination tools without any unnecessary delay and that an honest, accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

For and on behalf of the Study Sponsor:

Signature: Sybil A. Tasker Date: 11 AUG 2016

Name (please print): Sybil A. Tasker M.D.

Position: Sr V.P Clinical R+D

Chief Investigator:

Signature: Mark Thurszt Date: 15 AUG 2016

Name: (please print): Mark Thursz

Position

Statistician:

Signature: M. C Date: 17 AUG 2016

Name: (please print): MATTHEW GIBB

Position: Biostatistician

Sponsor's medical advisor:

Signature: Geoff Kitson Date: 11 AUG 2016

Name: (please print): GEOFF KITSON

Position: Medical Advisor

Signature of the Site Principal Investigator:

I agree to the terms of this Protocol and all amendments. I will conduct the study according to the procedures specified herein, and according to the principles of Good Clinical Practice (GCP) and local regulations.

Site No.: .....

Signature: Date:
.....

Name, Title (please print):

.....

Address:

.....

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.....

KEY TRIAL CONTACTS

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TRIAL SUMMARY

Trial Title	A phase I, randomised, double-blind, placebo-controlled, multi-centre, ascending-dose trial to evaluate the safety, tolerability and immunogenicity of Vaccine FP-02.2 in HBeAg-negative hepatitis B patients as an add-on treatment to entecavir or tenofovir.	
Internal ref. no.	FP-02.2_CS_01	
Clinical Phase	Phase I	
Trial Design	Randomised, double-blind, placebo-controlled, multi-centre, ascending-dose	
Trial Participants	Hepatitis B-infected, HBeAg-negative patients, aged 18-65 years, receiving on-going treatment with entecavir or tenofovir for at least 2 years	
Planned Sample Size	Approximately 60 subjects equally randomised between 6 treatment arms with 10 subjects per arm.	
Treatment duration	Subjects will receive 2 months active treatment (3 injections administered 28 days apart, i.e. over a period of 56 days). Subjects will be followed up 6 months after the last injection.	
	Objectives	**Outcome Measures**
Primary	To assess the safety and tolerability of Vaccine FP-02.2 at Day 85 following vaccinations on Days 1, 29 and 57.	Adverse events, clinical laboratory abnormalities, local tolerability, vital signs and electrocardiogram (ECG)
Secondary	To measure immunological response of Vaccine FP-02.2 at Day 85 following vaccinations on Days 1, 29 and 57.	FP-02.2-specific T cell immunity
Investigational Medicinal Product(s)	Vaccine FP-02.2, a potential therapeutic Hepatitis B vaccine, is a freeze-dried product containing nine different synthetic peptides of 32-40 amino acids in length and covalently linked to a fluorocarbon delivery vector. Peptides have been selected from the natural sequence of Core (4 peptides), Polymerase (4 peptides) and Surface (1 peptide) antigens from the HBV proteome.	
Formulation, Dose, Route of Administration	Subjects will receive one of the following formulations by an intramuscular injection of 1mL of: • Low-dose FP-02.2 (150µg/peptide), • High-dose FP-02.2 (500µg/peptide) • Low-dose FP-02.2 (150µg/peptide) + IC31® (500/20nmol KLK/ODN1a) • High-dose FP-02.2 (500µg/peptide) + IC31® (500/20nmol KLK/ODN1a) • Placebo • IC31 alone on three occasions, 28 days apart.	
Study schedule	<u>Screening</u> Screening should be performed to confirm study eligibility 28 to 1 (±2) days before the first injection.	

<table>
<tr><td></td><td>
<u>Study period</u>

Up to 3 days prior to first injection eligible subjects will be randomised into one of 6 treatment arms distributed across three cohorts as shown below:

Study cohorts/treatment arms
<table>
<tr><th>Cohort</th><th>Treatment arm</th><th>Approximate number of subjects</th></tr>
<tr><td rowspan="2">1</td><td>Placebo</td><td>5</td></tr>
<tr><td>FP-02.2 Low dose</td><td>10</td></tr>
<tr><td rowspan="4">2</td><td>Placebo</td><td>5</td></tr>
<tr><td>IC31® adjuvant only</td><td>5</td></tr>
<tr><td>FP-02.2 Low dose + IC31® adjuvant</td><td>10</td></tr>
<tr><td>FP-02.2 High dose</td><td>10</td></tr>
<tr><td rowspan="2">3</td><td>IC31® adjuvant only</td><td>5</td></tr>
<tr><td>FP-02.2 High dose + IC31® adjuvant</td><td>10</td></tr>
</table>

The subjects will receive 3 injections at intervals of 28 days (Day 1, Day 29, and Day 57). On the days of the injections the subjects will stay in the clinic for 4 hours post the first injection and 2 hours post injections 2 and 3, and be monitored closely including an assessment of local reactions at the injection site. Approximately 24 hours after the first injection, the subjects will be asked to attend the clinic for safety assessments. For subsequent injections, subjects will receive a telephone call approximately 24 hours after each injection as a safety follow-up. The subjects will be asked to return to the clinic 7 days after each injection for further assessments as well as 28 days after the last injection (Day 85). A follow-up visit will be performed 6 months after the final injection.

For each cohort, dosing will start with a sentinel group consisting of one subject receiving each active and control treatment (FP-02.2 (±IC31®), IC31® or placebo), e.g. for Cohort 1, the sentinel group will consist of 1 subject receiving placebo and 1 subject receiving Low Dose Vaccine FP-02.2. After the assessments on Day 8, the Safety Review Committee (SRC) (defined as the country CIs, if applicable, and the study medical monitors) will review the safety data from the sentinel group and recommend whether or not the remaining subjects of a cohort should be dosed. Any PI with patients in the sentinel group will provide input to the SRC. The outcome of this review will be communicated to the sponsor.

In order to progress to the next cohort, the SRC with input from the Investigators contributing patients to the dosing cohort will meet to review safety data up to Day 36 from Cohort 1. Following this review subjects may be dosed in Cohort 2 assuming the SRC review identifies no safety concerns. Similarly, subjects may be dosed in Cohort 3 after review of safety data up to and including Day 36 from subjects in Cohort 2.
</td></tr>
<tr><td>Criteria for evaluation</td><td><u>Safety and Tolerability</u>

The safety evaluation will include adverse events (AEs), clinical laboratory safety</td></tr>
</table>

	tests (serum biochemistry and haematology), physical examinations (for diagnoses only), vital signs (blood pressure, pulse rate and body temperature), 12-lead ECG parameters and local injection site reactions. Immunogenicity IFNγ ELISpot assay (ex vivo and cultured) specific for Vaccine FP-02.2 peptides using cryopreserved peripheral blood mononuclear cells (PBMC). Exploratory: Other immunological assays such as T cell proliferation assays, intracellular cytokine assays, ELISA-based assays to measure Vaccine FP-02.2-specific antibodies, or other suitable assays to measure the Vaccine FP-02.2-specific immune response from PBMC or serum. The frequency of regulatory cells, expression of co-stimulatory and inhibitory markers measured by flow cytometry. In consenting subjects, gene expression may be studied, which has the potential for identifying genetic markers that predict vaccine immunogenicity, or successful clinical responses, as well as elucidating biological mechanisms of vaccine efficacy. Virological HBsAg (quantitative), HBsAb
Sample size estimation	No formal sample size calculation has been performed. A convenience sample of 10 subjects will be enrolled into each of the six study arms (approximately 60 subjects overall) which is considered an appropriate study size at this phase of development of Vaccine FP-02.2.
Statistical considerations	There are six treatment arms dosed in 3 cohorts: 1. Low-dose FP-02.2 (Cohort 1) 2. High-dose FP-02.2 (Cohort 2) 3. Low-dose FP-02.2 + adjuvant (IC31®) (Cohort 2) 4. High-dose FP-02.2 + adjuvant (IC31®) (Cohort 3) 5. Placebo (pooled Placebo subjects from Cohorts 1 and 2) 6. IC31® only (pooled IC31® subjects from Cohorts 2 and 3) The standard summary statistics for continuous baseline and outcome variables are: number of subjects (n), mean, standard deviation, co-efficient of variation (CV), median, quartiles, minimum and maximum. The standard summary statistics for categorical baseline and outcome variables are: count and proportion (expressed as percentage). Adverse events shall be coded according to the current version of the Medical Dictionary for Regulatory Activities. The subject incidence, severity, duration, relatedness and outcome of all treatment emergent AEs will be tabulated by treatment arm, system organ class and preferred term. Injection site tolerability will be tabulated by treatment arm, time point, reaction, and grade of severity. Safety data in general will be listed and summarised by treatment arm and time point using the standard summary statistics over the safety analysis set.

	Shift tables will be presented for safety laboratory results and vital signs. Listings of safety laboratory results and vital signs will have assessments below and above the reference range highlighted, together with an indicator for clinical significance. Immunological outcome variables will be summarised by treatment arm and by time point tabulated using the standard summary statistics. A detailed description of the planned analysis of each immunogenicity and virological endpoint will be presented in a separate statistical analytical plan.

FUNDING AND SUPPORT IN KIND

FUNDER(S) (Names and contact details of ALL organisations providing funding and/or support in kind for this trial)	**FINANCIAL AND NON FINANCIAL SUPPORT GIVEN**
Altimmune	**100% financial support**

ROLE OF STUDY SPONSOR AND FUNDER

Altimmune (formerly known as Vaxin) is the Sponsor of the study.

The Sponsor will be responsible for the IMP supply management, the design and overall performance of the study, data analysis, clinical study report, archiving of the Trial Master File (TMF) after the end of the study and the publication of the results.

The Sponsor may transfer all or parts of the responsibilities in regard to the performance of the study and data analysis, interpretation and publication to a Contract Research Organisation (CRO). The Sponsor will control the final decision in any of these aspects.

LIST OF CONTENTS

LIST OF ABBREVIATIONS

AE	Adverse Event
AESI	AE of special interest
ALT	Alanine aminotransferase
AR	Adverse Reaction
AST	Aspartate transaminase
CA	Competent Authority
CHB	Chronic hepatitis B
CI	Chief Investigator
CMI	Cell-mediated immunity
CRO	Contract Research Organisation
CTA	Clinical Trial Authorisation
DNA	Deoxyribonucleic acid
DSUR	Development Safety Update Report
EC	Ethics Committee
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
ELISA	Enzyme-linked immunosorbent assay
ELISpot Assay	Enzyme-Linked Immuno Spot Assay
FAS	Full analysis set
GCP	Good Clinical Practice
GLP	Good laboratory practice
HBeAg	Hepatitis B e antigen
HBsAb	Antibody to hepatitis B surface antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HDV	Hepatitis D virus
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
IB	Investigator Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use.
IFN-γ	Interferon gamma
IM	Intramuscular

IMP	Investigational Medicinal Product
INR	International normalised ratio
IRT	Interactive Response Technology
IUD	Intrauterine device
IUS	Intrauterine hormone-releasing system
MedDRA	Medical Dictionary for Regulatory Activities
NA	Nucleoside analogue
NUC	nucleos(t)ide HBV polymerase inhibitor
OECD	Organisation for Economic Co-operation and Development
PBMC	Peripheral blood mononuclear cells
PI	Principal Investigator
PT	Prothrombin time
SAE	Serious Adverse Event
SAP	Statistical Analytical Plan
SAR	Serious Adverse Reaction
SD	Standard deviation
SmPC	Summary of Product Characteristics
SRC	Safety Review Committee
SUSAR	Suspected Unexpected Serious Adverse Reaction
ULN	Upper limit of normal

TRIAL FLOW CHART

Table 1 Flow chart

	Screening	1st			2nd			3rd				Follow- up
Visit Number	**-[6] (SV)**	**1[6] (SV)**	**2[6] (SV)**	**3[6] (SV)**	**4[6] (SV)**	**- (TC)**	**5[6] (SV)**	**6 (SV)**	**- (TC)**	**7[6] (SV)**	**8[6] (SV)**	**9[6, 9] (SV)**
Study Day	**-28 to -1**	**1**	**2**	**8**	**29**	**30**	**36**	**57**	**58**	**64**	**85**	**225**
Visit Windows	**±2 days**			**±1 day of Day 8**	**28 (±7 days) days after Visit 1**		**7 (±1 day) days after Visit 4**	**28 (±7 days) days after Visit 4**		**7 (±1 day) after Visit 6**	**28 (±1 day) after Visit 6**	**24 (±4 weeks) weeks after Visit 6**
Procedure												
Informed consent	X											
Review eligibility	X	X			X			X				
Randomisation	X											
Vaccination		X			X			X				
Phone call						X			X			X[5]
HBsAg, HBsAb	X	X			X			X			X	X
HBV DNA	X											X
Vital signs	X	X	X	X	X		X	X		X	X	X
Medical history	X											
Dispense Diary		X[2]			X[2]			X[2]				
Injection Site Tolerability		X[1]	X	X	X[1]	X	X	X[1]	X	X		
Pregnancy test[4]	X	X[3]			X[3]			X[3]			X	
Safety lab tests	X			X	X[3]		X	X[3]		X	X	X
Physical examination[5]	X	X	X	X	X		X	X		X	X	X
Fibroscan	X											
Alcohol, drugs of abuse, HIV, HDV and HCV serology	X											
Concomitant medication	X	X	X		X			X			X	
Blood for PBMC[7]	X	X[3]		X	X[3]		X	X[3]		X	X	X
12-lead ECG	X											X
Documentation of	X	X	X	X	X	X	X	X	X	X	X	X[8]

	Screening	1st			2nd			3rd				Follow- up
Visit Number	**-[6] (SV)**	**1[6] (SV)**	**2[6] (SV)**	**3[6] (SV)**	**4[6] (SV)**	**- (TC)**	**5[6] (SV)**	**6 (SV)**	**- (TC)**	**7[6] (SV)**	**8[6] (SV)**	**9[6, 9] (SV)**
Study Day	**-28 to -1**	**1**	**2**	**8**	**29**	**30**	**36**	**57**	**58**	**64**	**85**	**225**
Visit Windows	**±2 days**			**±1 day of Day 8**	**28 (±7 days) days after Visit 1**		**7 (±1 day) days after Visit 4**	**28 (±7 days) days after Visit 4**		**7 (±1 day) after Visit 6**	**28 (±1 day) after Visit 6**	**24 (±4 weeks) weeks after Visit 6**
AEs												

AE: adverse event; CMI: cell-mediated immunity; DNA: deoxyribonucleic acid; ECG: electrocardiogram HBsAg/Ab: Hepatitis B surface Antigen/Antibody; HBV: Hepatitis B virus; HCV: Hepatitis C virus; HDV: Hepatitis D virus; HIV: Human Immunodeficiency Virus; SV: Site Visit; TC: Telephone Call

[1] On the days of the vaccination, injection site tolerability will be assessed by the Investigator up to a minimum of 4 hours after the first injection and approximately 24 hours and 7 days after the first injection. For subsequent injections, injection site tolerability will be assessed by the Investigator up to 2 hours on the day of injection by the study site. Further assessment of injection site tolerability will be recorded using diary cards from 2 hours to 7 days after injection. Data from the diary cards will be transcribed into the appropriate eCRF.
[2.] The injection site diary is given to patients after the 1st, 4th and 6th visit, but can only be reviewed post drug, i.e. at the next sequential visit.
[3] Prior to injection of IMP.
[4] Women of childbearing potential only. Serum pregnancy test will be performed at screening; a urine pregnancy test will be performed at other scheduled time points.
[5] A full physical examination will be conducted at screening and a brief physical examination will be performed at other time points indicated and will include examination of general appearance, injection site, respiratory and cardiovascular systems and the abdomen, particularly the upper right quadrant.
[6] Visit intervals are as follows:

- screening and randomisation: subjects must be screened -1 to 28 days (±2 days) and can be randomised to treatment up to 3 days prior to the first injection.
- first dose: Visits 1 and 2 must be done on Days 1 and 2, respectively; Visit 3 may be done within ± 1 day of Day 8.
- second dose: Visit 4 may be done 28 days (± 7 days) after Visit 1; subjects must be contacted the day after Visit 4; Visits 5 should be done 7 (± 1 day) days after Visit 4.
- third dose: Visit 6 may be done 28 days (± 7 days) after Visit 4; subjects must be contacted the day after Visit 6; Visits 7 and 8 may be done 7 (± 1 day) and 28 (±1 day) days after Visit 6.
- follow-up: Visit 9 must be done at 24 (± 28 days) weeks after the last dose (Visit 6).

The scheduled assessments may shift in instances where a visit window is used; subsequent visit dates should be recalculated from the date of the new visit. If a second dose visit is missed, the dose can be given 6 weeks after the first dose and noted as out of window. If the dose is later than 6 weeks, the second dose visit can be skipped and given at Visit 3 at 8 weeks as per schedule to avoid a delay in the schedule.
[7] PBMC samples for CMI; in consenting subjects HLA typing and other genomic analyses may also be performed.
[8] Only SAEs and AESIs need to be documented during Follow-up (Visit 9).
[9] Subjects will be contacted at approximately 4 week intervals during the follow-up phase.

1 BACKGROUND

Hepatitis B virus (HBV) has infected >2 billion people worldwide, of whom more than 360 million people (5% of the world's population) remain chronically infected. HBV is the tenth leading cause of death worldwide with over 600,000 HBV-related deaths per year. Life threatening liver disease (cirrhosis, liver failure and hepatocellular carcinoma) occurs in as many as 40% of patients with chronic hepatitis B (CHB). Even though prophylactic vaccination programmes have led to declines in de novo HBV infections in many countries, the burden of disease in adult patients remains high. The treatment of CHB has improved dramatically in the last 10 years, owing to the development of new antiviral compounds including polymerase inhibitors or pegylated alpha-interferon. Despite significant retardation in HBV-related disease progression and reduction in mortality, those treatments rarely achieve clinical cure [13], manifested by surface antigen (HBsAg) loss, notably due to their inability to promote an effective anti-HBV immunologic control. Treatment guidelines recommend antiviral therapy with nucleos(t)ide HBV polymerase inhibitors (NUCs) for all patients with biochemical or histological disease. Antiviral treatment can rarely be stopped and lifelong therapies are usually required, as NUCs only suppress HBV replication without inducing a clearance of infected liver cells. Long term safety of NUC therapy is uncertain, while long term cost implications are unsustainable. Therapies are urgently needed to enable timely cessation of NUC treatment and deliver efficient and effective healthcare. Therapeutic vaccination is a promising approach to induce immune control over the disease. CD4+ and CD8+ T cell responses have been shown to be critical for clearance of acute HBV infection and immune control can be linked to the strength and quality of HBV-specific T cell responses. CHB patients are known to have profound defects in anti-HBV-specific T cell immune responses, thereby compromising critical protective and disease control mechanisms. So far, two large trials evaluating therapeutic vaccines (with or without approved treatments) as a means to potentially promote an anti-HBV immunity in CHB patients have reported disappointing results with no evidence of HBsAg clearance (or increased rate of hepatitis B e antigen [HBeAg] seroconversion) [8, 15]. A limitation of these two trials was the use of HBsAg, which was primarily designed to preferentially boost antibody-mediated immune responses as opposed to induction of CD4+ and CD8+ T cell responses, as observed in individuals with spontaneously resolved infections [6]. Altimmune's novel therapeutic vaccine, FP-02.2, is designed to restore effective T cell immunity with potential for disease modification and potentially serological cure.

This study evaluating the safety and immunogenicity of FP-02.2 is a double blind, randomised and placebo controlled clinical trial in HBeAg-negative CHB patients receiving entecavir or tenofovir. The study will assess two doses of the vaccine given as single intramuscular (IM) injections on three occasions four weeks apart. While it is anticipated that FP-02.2 will be immunogenic in the absence of an adjuvant as demonstrated with a similar vaccine relying on the same vaccine platform [4], a co-formulation with an adjuvant (IC31®) will be also tested to potentially achieve a higher level of immune response and/or counteract immune tolerance mechanisms. Seventy-two evaluable patients will be randomised into six treatment arms 1. Low-dose FP-02.2, 2. High-dose FP-02.2, 3. Low-dose FP-02.2 + adjuvant (IC31®), 4. High-dose FP-02.2 + adjuvant (IC31®), 5. Placebo, 6. IC31® only, distributed over three cohorts according to the scheme set out in **Error! Reference source not found.**. This group size is not powered to measure efficacy but is of standard size to assess differences in vaccine treatments. The CHB patient population to be recruited in the study are HBeAg-negative, representing the majority of cases in many areas. The vaccine will be tested as an add-on to entecavir or tenofovir, two antiviral drugs known to be highly effective in their ability to inhibit HBV replication but also to promote a partial restoration of HBV-specific T cell functionality [2]. The study intends to recruit male

and female subjects, aged 18-65 years who have received nucleotide/nucleoside treatment for at least 2 years.

FP-02.2 is a freeze-dried vaccine containing 9 synthetic peptides (each 32 to 40 amino-acid residues) and mannitol. Each of the 9 peptides is modified by a fluorocarbon moiety providing immune enhancing properties [5]. FP-02.2 peptides are derived from three HBV antigens with four peptides derived from HBV polymerase, four from HBV core protein and one from HBV surface protein representing collectively 18.7% of the HBV proteome. These peptides are derived from the most conserved portions of the HBV proteome, presumably bearing higher viral fitness costs and resulting in a high level of T cell cross-reactivity irrespective of the HBV genotype. The peptides, derived immunodominant antigen regions, have the ability to promote dual CD4+ and CD8+ T cell responses and support broad coverage across ethnically diverse populations. The manufacturing of the peptides relies on a well-established solid phase synthesis using Fmoc chemistry and conventional Reversed-Phase High-Performance Liquid Chromatography purification systems for the production of a fully characterisable active pharmaceutical ingredient with low cost of goods. Vaccine FP-02.2/IC31® is an adjuvanted formulation of FP-02.2, in which FP-02.2 is combined with the clinically-tested vaccine adjuvant IC31®. IC31® is composed of synthetic TLR9 agonist (ODN1a) complexed with a cationic peptide (KLK). The positively-charged KLK peptide provides a delivery system and stabilising function for ODN1a, which cannot activate the immune system by itself, likely due to its short half-life. KLK also contributes to the depot effect of IC31®, prolonging antigen exposure to professional antigen presenting cells at the site of injection. IC31® has been assessed extensively in pre-clinical and clinical studies demonstrating an acceptable safety profile and excellent adjuvanticity in combination with various vaccines [9, 14, 10, 12].

The sequence of each of the 9 peptides contained within FP-02.2 has been identified from within the HBV proteome using a bioinformatics platform so that each peptide contains a high density cluster of human major histocompatibility complex class I and class II binding epitopes that can be recognised by a broad population irrespective of their genetic background. In addition these peptides have been selected to direct the immune system to target conserved regions of the virus thereby being able to induce immune responses against the prevalent HBV genotypes, A, B, C and D. These peptides were demonstrated to promote poly-functional CD4+ and CD8+ T cell responses in peripheral blood mononuclear cells (PBMC) isolated from CHB donors, irrespective of infecting HBV genotypes or patient ethnicities. In mice, FP-02.2 induces a strong T cell response that is significantly enhanced in the presence of IC31®. FP-02.2 promotes a robust T cell response either in the spleen or in the liver either in naïve mice or mice infected with an AAV-HBV replicon. Two good laboratory practice (GLP)-compliant toxicology studies have been completed with Vaccine FP-02.2 ± IC31®, one evaluating repeated doses toxicology in mice and the other evaluating local injection site tolerability in rabbits. In the repeat-dose toxicity study performed in mice, IM immunisation of FP-02.2 ± IC31® on four occasions over 6 weeks was well tolerated, with no treatment related deaths, minor changes in clinical pathology and several histopathological changes observed at injection sites. Observed changes were consistent with expected local and systemic responses to a potent immunostimulatory vaccine product. In the local tolerability study performed in rabbits, IM immunisation of FP-02.2 ± IC31® was well tolerated with no evidence of systemic toxicity or local dermal intolerance. Changes to muscle tissue observed with histopathological examination on Day 5 were considered almost totally reversible following a second assessment on Day 22.

There is no available clinical research data to date on the investigational product.

2 RATIONALE

Limited treatment options are available for CHB, the leading cause of end-stage liver disease and hepatocellular carcinoma worldwide. Only interferon-alpha has been able to induce HBsAg loss, which is considered to be a clinical “cure”, in a relatively low proportion of patients (<10%) and therefore establishing a clinical precedent for predicting efficacy. Interferons have a high cost, a poor tolerability (particularly in combination with ribavirin) and some HBV genotypes remain poorly responsive to treatment. Consequently, NUCs remain the main treatment strategies with five NUCs being approved in Europe to treat CHB. The most potent and preferred drugs, entecavir (brand name Baraclude®, Bristol-Myers Squibb) or tenofovir disoproxil furamate (tenofovir, brand name Viread®; Gilead), have a favourable side-effect profile and are able to induce HBV deoxyribonucleic acid (DNA) suppression in almost all patients and have a high barrier to resistance. However, these treatments suppress but do not eradicate HBV and rarely achieve clinical “cure" through HBsAg loss or seroconversion. Therefore, patients will require long durations if not life-long treatment to maintain virus suppression and to derive continued clinical benefit. Furthermore, the long-term safety of NUC therapy is currently unknown and costs for entecavir and tenofovir treatment are frequently reimbursed for only 2-3 years in many countries. Therefore, novel therapies that enable a timely stopping of NUC therapy are urgently needed.

Therapeutic vaccination is a very promising intervention for hepatitis B as a way to induce immune control over the disease. T cell responses have been shown to be critical for clearance of acute HBV infection. Cellular immune responses differ between the different phases of CHB and immune control of HBV can clearly be linked to the strength and quality of HBV-specific T cell responses [3, 2]. Therapeutic HBV vaccines based exclusively on HBsAg have failed to show benefit due to pre-existing immune tolerance from high levels of circulating HBsAg, even under effective concomitant antiviral treatment. Novel vaccine strategies based on combinations of HBsAg with other HBV antigens have emerged[1] but have not yet shown efficacy in a clinical study. The limited clinical investigations to date have been completed to address T cell correlates of protection with limitations related to the genetic variability of the virus.

The design of FP-02.2 takes advantage of a vaccine platform developed by Altimmune (formerly Vaxin, formerly Immune Targeting Systems) that is dedicated to the development of synthetic peptide-based vaccines aimed at promoting T cell responses against mutating viruses. This platform comprises two complementary arms i) a bioinformatics methodology to select broadly immunogenic long peptide sequences against viral regions conserved across genotypes and ii) a novel vaccine formulation using multiple fluorocarbon-linked peptides to produce a depot following IM administration that prolongs vaccine half-life and enhances immunogenicity in situ.

The nine peptides included in FP-02.2 have been selected based on an extensive screening process relying on bioinformatics, preclinical screening and key manufacturing aspects. FP-02.2 has been designed to deliver the most conserved domains of HBV surface, core and polymerase antigens (corresponding cumulatively to 18.7% of the HBV proteome) to achieve multi-epitopic CD4+ and CD8+ T cell responses, irrespective of ethnicity, human leukocyte antigen (HLA) background or infecting HBV genotype. This approach is required to optimise antiviral activity by exerting broad immune pressure on the virus and consequently minimising viral escape mechanisms. This approach has a significant advantage over other vaccine approaches in development, such as DNA or viral vaccines, by allowing for multi-use booster capability.

In two study arms, FP-02.2 will be used in conjunction with the vaccine adjuvant IC31® to potentially increase immune responses to the vaccine and overcome immune tolerance and the suppression mechanism observed in CHB. A fixed dose of IC31® will be used (500nmol KLK/ 20nmol ODN1a) that has been previously tested in 8 Phase I/IIa clinical trials in combination with TB or seasonal influenza vaccines.

This study will be the first in man study for Vaccine FP-02.2 and will assess the safety and tolerability of three IM injections of a therapeutic hepatitis B fluoropeptide vaccine in HBeAg-negative hepatitis B patients as an add-on treatment to entecavir or tenofovir. Immunogenicity analysis of the specific T cell responses induced by Vaccine FP-02.2 or Vaccine FP-02.2/IC31® will be undertaken to aid selection of the optimal dose to be used for future studies.

Clinical evaluation of Vaccine FP-02.2 will seek to address these key issues:

1. At estimated effective dose(s), and in the presence or absence of an estimated effective dose of a compatible adjuvant, is the vaccine safe and tolerated in nucleoside analogue (NA)-treated HBV patients with stable disease?
2. Is there evidence of induction of an immune response to the vaccine and what impact does an adjuvant (IC31®) have on the immune response – i.e. magnitude and anti-viral phenotype of T cell response.
3. To what extent is the immune response robust and durable?
4. Is there evidence of a pharmaco-dynamic effect of the vaccine indicative of potential efficacy i.e. reduction in serum HBsAg and/or increase in serum antibody to HBsAg; if so is the response dose dependent and is the response modified by inclusion of IC31®?

2.1 Assessment and management of risk

2.1.1 Potential benefits

It is anticipated that the results of the Phase I study with Vaccine FP-02.2 will establish the safety profile and potential immunogenicity profile of the vaccine in patients with stable HBV infections. The incorporation of various immunogenicity readouts in the study protocol will provide information on the immune response to the vaccine over a range of dosages and formulations, so as to enable selection of a suitable dosage and formulation for use in larger scale clinical trials, including a Phase II study powered to establish significance for efficacy readouts. Volunteers taking part in the study may obtain some benefit, in terms of attaining an immune response that may be therapeutic to current HBV infection but this should not be expected.

2.1.2 Potential Risks

The potential risks associated with Vaccine FP-02.2 administration are anticipated to be:

Unintended Immune Stimulation

FP-02.2 is a vaccine formulation that is intended to induce a specific cellular immune response towards targeted HBV epitopes. Primary pharmacodynamic studies in animals, and data generated utilising human PBMC indicate that the fluorocarbon conjugation of the peptides in the Vaccine FP-02.2 formulation enhances the specific cellular immune response. Clinical trials conducted with another peptide-based vaccine product [11] found no increase in the incidence of auto-antibodies amongst treated subjects, compared to those receiving placebo. Moreover, clinical trials conducted

with a similar peptide based vaccine product targeting influenza A [4] found no immune stimulation issues in adults (young and old).

The risk for Vaccine FP-02.2 to induce an unintended or autoimmune response in humans is considered to be low as evidenced by:

- The presence of pre-existing immunity to Vaccine FP-02.2 antigens in HBV patients chronically exposed to HBV
- The absence of significant sequence homology with human proteins

The vaccine was designed to recover HBV specific T cells and bring their frequencies to protective levels. We have demonstrated in an in vitro PBMC assay, low frequency CD4+ and CD8+ T cells specific for the nine FP-02.2 peptides can be expanded in many, if not all, HBV patients. This indicates that during infection, similar viral peptides are presented during the course of the infection to the immune system.

In clinical studies conducted in patients with other therapeutic HBV vaccines, no notable adverse effects or acute flares of hepatitis were detected. For example, in an open-label, controlled, randomised study, 195 patients with HBeAg-positive CHB received 12 doses of HBsAg with AS02B adjuvant candidate vaccine plus lamivudine daily for 52 weeks or lamivudine daily alone[15]. The combined administration of vaccine and lamivudine was safe and well tolerated in the context of a vigorous HBsAg-specific lymphoproliferative response, cytokine production and anti-HBs antibodies. Also a phase III clinical study conducted in patients with chronic HBV evaluating Nasvac, a therapeutic HBV vaccine administered on 5 occasions, 7 reported no notable adverse events (AEs) or acute flares of hepatitis. Nevertheless, the theoretical risk of Vaccine FP-02.2 inducing an immune response, resulting in the death of hepatocytes infected with HBV, which could result in fulminant hepatic failure, remains and will be monitored for by regularly assessing liver function tests.

In conclusion, this information indicates that the nine long peptides selected for inclusion into a novel HBV vaccine are at low risk of inducing cross-reactive immunity that may lead to auto-reactive T cell responses. In addition, the risk of generalised, non-specific immune stimulation following Vaccine FP-02.2 administration is considered to be low.

Local and Systemic Reactions

In a repeat-dose toxicity study performed in mice, intramuscular immunisation of FP-02.2 ± IC31® on four occasions over 6 weeks was well tolerated, with no treatment-related deaths, minor changes in clinical pathology and several histopathological changes observed at injection sites. Observed changes were consistent with expected local and systemic responses to a potent immunostimulatory vaccine product. A local tolerability study performed in rabbits, intramuscular immunisation of FP-02.2 ± IC31® was well tolerated with no evidence of systemic toxicity or local dermal intolerance. Changes to muscle tissue observed with histopathological examination on Day 5 were considered almost totally reversible following a second assessment on Day 22.

Local injection site and systemic reactions to vaccinations are common with all vaccinations and pain, erythema and induration of mild intensity can be expected at the injection site and fever, malaise or body aches may occur.

Reproductive Effects

No reproductive toxicity studies have been performed. There is no evidence from examination of the reproductive tract tissues in the repeated dose toxicity study in mice, of an effect of Vaccine FP-02.2 on the female or male reproductive organs. A number of contraceptive requirements are included in the protocol. The risk of adverse effects on reproduction is considered to be very low.

Other Risk Factors and Precautions

There may be other effects or risk factors that are currently unknown.

To assess unknown risks clinical studies will include assessment of a full panel of haematology, clinical chemistry and liver function parameters, physical examination, electrocardiogram (ECG), vital signs, as well as local tolerability.

Until reproductive toxicity data are available it is important that women participating in trials and female partners of male subjects do not become pregnant. Contraceptive requirements will be mandated for each trial, and described in the Investigator's Brochure (IB).

Other Toxicity

No other toxicity is anticipated for Vaccine FP-02.2 to that discussed in the previous sections.

2.1.3 Subject selection

The study is designed as a first in man study in patients with stable HBeAg-negative CHB who are otherwise healthy with no compromise in their liver function. The rationale for moving directly into patients with CHB, and not administering Vaccine FP-02.2 to healthy volunteers first, was based on the following points:

- Evaluating the safety of Vaccine FP-02.2, in the absence of HBV exposure i.e. in healthy volunteers will not adequately assess safety of the immune response generated to FP-02.2 in the context of existing HBV infection.
- Patients with CHB have, unlike 90% of the population, failed to clear the initial infection and the chronic exposure to HBV has a significant impact on the immune responsiveness to HBV antigens, compared to healthy non-infected subjects; therefore, the immune response to Vaccine FP-02.2 in patients is likely to be very different to the immune response to Vaccine FP-02.2 in healthy volunteers.

From these considerations, it was determined that assessing safety and immunogenicity of Vaccine FP-02.2 in healthy volunteers would be of limited value with the results likely to show a greater immune response generated to Vaccine FP-02.2 and not being able to assess the key safety variable i.e. the acute immune-toxicity in the presence of HBV infected liver cells. Therefore, it was determined that it is most appropriate to conduct this first in man study in stable patients chronically infected with HBV.

2.1.4 Study site

Having made the decision to initiate the first in man study in patients chronically infected with HBV, consideration was given to where the study should be conducted. The safety aspects of the study

were the most important consideration. Sites need to be able to manage both acute systemic reactions (immediately after injection) and the longer term safety of patients, have access to a facility which is able to manage an acute flare and has expertise, experience and ability to care for patients with CHB. An effective immunotherapeutic product will induce an immune response against the HBV, which will result in the death of hepatocytes infected with HBV; if sufficient hepatocytes are killed in this process, an acute flare with hepatic decompensation could theoretically occur.

To mitigate the risks identified above the following procedures will be undertaken:

- A sentinel group will be dosed prior to the dosing of each of the study cohorts.
- All subjects will be kept in clinic for 4 hours after the first injection and 2 hours post dose following injection 2 and 3, for observation and assessment for any acute reaction. It is not possible to measure pharmacokinetic parameters for a vaccine and, therefore, no serial blood samples will be taken. The observation period will only assess acute safety. Following the four-hour review after the first dose, or the two-hour review period following the second and third doses, it is considered unlikely that any acute reactions will develop and it is appropriate for the subject to return home.
- After discharge, the patient should call the Investigator at their site on the contact number provided, for any non-urgent medical advice. If the patient becomes very unwell, they should seek emergency care and the evaluating physician should call the Investigator for advice and information.
- Subjects will return to the clinic for a safety check 24±2 hours after the first administration of investigational medicinal product (IMP). The assessment will include a general assessment, vital signs, discussion regarding AEs and assessment of the injection site.
- Bloods for assessing liver function will be taken at 7 days after each injection and prior to the subsequent injection. These results will be reviewed by the Principal Investigator (PI) for each site. Any subject experiencing an increase in alanine aminotransferase (ALT) to 10 times the upper limit of normal (ULN), a prothrombin time, reported as the international normalised ratio (INR) of more than 1.3 or a total bilirubin greater than 3 times the ULN, will be called back to study site for assessment and will not receive any more doses of Vaccine FP-02.2. Subjects with an ALT above 5 times the ULN will be closely monitored and dosed at the discretion of the Investigator.
- Any subject experiencing a flare with decreased liver function will be managed by the PI for the site, as per local clinical protocols.
- A safety review committee (SRC) (defined as the country CIs, if applicable, and the study medical monitors) will review and assess all safety data for Cohorts 1 and 2 after at least 15 or 30 subjects, respectively, have completed Day 36 assessments.

Communication between the sites to discuss subject safety is of critical importance and, in addition to the SRC. Sites are encouraged to contact the Sponsor's medical monitor or regional CIs for any questions or concerns about the study, especially if related to patient safety.

2.1.5 Dose selection

Dosages for early human studies with vaccines are often based on optimal immunogenic doses in animal models (based on amount of antigen, without correction for bodyweight or body surface area), and demonstration that this optimal immunogenic dose (in terms of µg of antigen) does not cause overt toxicity when injected into a suitable pre-clinical toxicity species, such as mice, rats or rabbits. Injection of the optimal immunogenic dose into the toxicity study species will provide a safety margin

(of at least 10-fold, and often greater) when compared to the same dose injected into humans, when the dosage is corrected for bodyweight differences between species. Pre-clinical results in mice demonstrate that the highest immunogenicity was observed using a dose of 100µg/peptide. Safety and immunogenicity of the vaccine platform (using multiple fluoropeptides) have previously been evaluated across three phase I clinical studies in the context of a universal influenza A vaccine. The first study (NCT01265914) was a dose escalation study in healthy young adults, where the 6 fluoropeptide 'flu vaccine was given at days 1, 29 and 99 and tested at doses of 50, 150 and 500µg/peptide [4]. The second study (NCT01677676) was a dose optimisation study testing doses of 150 and 250µg/peptide administered at days 1 and 29, with and without an adjuvant (Advax - a novel polysaccharide adjuvant), in healthy young adults. The third study (NCT02071329) assessed the flu vaccine at 250µg/peptide with or without Advax and/or concomitant TIV in healthy older adults (65-74 years) administered on days 1 and 29. Across all studies, a robust T Cell immune response to the vaccine was measured in subjects receiving doses between 150 and 500µg/peptide and after two injections.

Taking this previous clinical experience with the vaccine platform together with immunogenicity in mice, it was reasonable to propose 150µg/peptide as the starting dose for the first-in-man study for FP-02.2. Moreover, this dosage was within in the range of effective dosages that have been studied in clinical trials of similar peptide vaccine approaches such as lipopeptide-based vaccines that reported acceptable safety profiles. For example, a dosage range of 50-500µg/peptide (250µg – 2500µg total peptide) was examined in a healthy volunteer trial of a human immunodeficiency virus (HIV) lipopeptide vaccine, with good tolerability and immunogenicity [11].

The range of human dosages in the planned phase I study will be 150µg and 500µg per peptide (i.e. up to ~10µg/kg per peptide for a 50kg subject). In the repeat dose toxicity study, the dosage administered to mice (0.05mL of human formulation, containing 25µg per peptide = 1000µg/kg per peptide for a 25 g mouse) provides a 100-fold safety margin over the planned maximum human dosage, which equates to an 8-fold margin allowing for allometric scaling. For a 70 kg subject the safety margin will be approximately 11. For IC31®, the human dosage in the planned phase I study will be 500 nmol/20 nmol KLK/ODN1a, in a volume of 1mL for human administration (i.e. up to 10 nmol/0.4 nmol/kg for a 50 kg subject). The dosage administered to mice (0.05mL of human formulation, containing 25 nmol/1 nmol = 1000 nmol/40 nmol/kg for a 25 g mouse), therefore provides a 100-fold safety margin over the planned maximum human dosage, which equates to an 8-fold safety margin allowing for allometric scaling.

2.1.6 Dose escalation

For reasons of participants' safety, the clinical trial design will be performed in a stepwise fashion introducing each component based on the determined risk attributed to that cohort's treatment. Table 2 outlines the dosing regimen.

Table 2 Dosing regimen.

Cohort	Treatment arm	Approximate number of subjects
1	Placebo	5
	FP-02.2 Low-dose	10
2	Placebo	5
	IC31® adjuvant only	5
	FP-02.2 Low-dose + IC31® adjuvant	10
	FP-02.2 High-dose	10
3	IC31® adjuvant only	5
	FP-02.2 High-dose + IC31® adjuvant	10

For each cohort, the first subject from each treatment arm will form a sentinel group, e.g. for Cohort 1, the sentinel group will consist of 1 subject receiving placebo and 1 subject receiving Low-dose Vaccine FP-02.2. The CI and any PI(s) involved in dosing sentinel subjects will review safety data collected up to and including Day 8 visit as detailed in Section 4 before recommending whether or not the remaining subjects of a cohort should be dosed.

In order to escalate between doses, the SRC will review safety and tolerability data up to and including the Day 36 visit from subjects in Cohort 1 and verify no safety concerns have been detected before enrolling subjects into Cohort 2. Similarly, subjects may be enrolled into Cohort 3 after review of safety and tolerability data up to and including Day 36 from subjects from Cohort 2. If safety and tolerability data are not acceptable the SRC may recommend lowering the dose of FP-02.2 or omitting the use of IC31®.

All data used to support dose escalation will be quality checked. Dose decisions between cohorts will be documented.

3 OBJECTIVES AND OUTCOME MEASURES/ENDPOINTS

3.1 Primary objective

To assess the safety and tolerability of Vaccine FP-02.2 at Day 85 following vaccinations on Days 1, 29 and 57.

3.2 Secondary objectives

To measure immunological response of Vaccine FP-02.2 at Day 85 following vaccinations on Days 1, 29 and 57.

3.3 Outcome measures/endpoints

3.3.1 Primary endpoints/outcomes

- Solicited AEs related to injection site tolerability during the week following each vaccine administration
- Unsolicited AEs through day 85

- Serious adverse events (SAEs) and AEs of special interest (AESIs) through 6 months after last vaccine dose
- Vital signs
- ECG
- Safety Laboratory including laboratory assessment of liver inflammation and function

3.3.2 Secondary endpoints/outcomes

Immunological parameters:

The immunogenicity of Vaccine FP-02.2 will be determined using an IFNγ ELISPOT assay. The output for the ELISPOT assay will be number of cells producing interferon gamma (IFN-γ) in response to exposure to native peptides derived from FP-02.2 using an ex vivo and cultured assay. Negative assay controls will contain no peptide and positive controls will use a T cell mitogen.

Additional exploratory immunological assays such as T Cell proliferation assays, intracellular cytokine staining assays, ELISA-based assays to measure FP-02.2-specific antibodies or other suitable assays may be used to further characterise the immune responses to Vaccine FP-02.2. The frequency of regulatory cells, expression of co-stimulatory and inhibitory markers may be measured by flow cytometry.

Immunological data from samples collected within the follow-up phase will be regarded as exploratory, including IFNγ ELISPOT data.

Virological parameters:

The following will be measured by means of ELISA-based assays:

- Quantitative HBsAg
- Anti HBsAg antibody
- A validated HBV DNA TaqMan assay will be used to quantitatively measure HBV DNA.

3.4 Exploratory endpoints/outcomes

- HBV genotype characterisation (limited to genotype A, B C or D) by means of ELISA to understand if there is a relationship between immunological responses and infecting HBV genotype will be performed on Day 1.
- In consenting subjects, gene expression may be studied which has the potential for identifying genetic markers that predict vaccine immunogenicity, or successful clinical responses, as well as elucidating biological mechanisms of vaccine efficacy. HLA genotyping will be performed to understand if the vaccine specific immune responses are independent of the subject's HLA background.

4 TRIAL DESIGN

This is a double-blind, randomised, placebo-controlled, dose-ascending study to evaluate the safety, tolerability and immunogenicity of Vaccine FP-02.2 in HBeAg-negative hepatitis B patients as an add-on treatment to entecavir or tenofovir.

Actively treated subjects will receive either a Low or High dose of Vaccine FP-02.2 with or without IC31®.

A placebo arm is included in the study. Safety and immunogenicity data generated from the placebo arm will allow comparison to data generated from the Vaccine FP-02.2 High and Low dose study arms. The placebo group is also necessary to understand the rate of natural HBsAg seroconversion in an untreated study arm. An IC31® alone arm is included in the study which will additionally allow comparison of safety and immunogenicity data with data generated from the Vaccine FP-02.2 High and Low dose/IC31® study arms.

Approximately of 60 subjects (excluding replacements for subjects deemed ineligible after dosing) will complete this trial and will be assigned equally to 6 study arms (approximately 10/study arm).

Screening will occur up to 28 days prior to the first injection.

Up to 3 days before the first injection, eligible subjects will be randomised to treatment arm as shown in Table 2, depending on the cohort.

Subjects will receive 3 injections at intervals of 28 days (Day 1, Day 29, and Day 57). On the days of the injections the subjects will stay in the clinic for at least 4 hours after the first injection and 2 hours after the second and third injections and be monitored closely including an assessment of local reactions at the injection site. Approximately 24 hours after the first injection, the subjects will be asked to attend the clinic for safety assessments. For subsequent injections, subjects will receive a telephone call approximately 24 hours after each injection for safety follow-up. The subjects will be asked to return to the clinic 7 days after each injection for further assessments as well as 28 days after the last injection (Day 85). A follow-up visit will be performed 6 months after the end of the treatment period (i.e. 6 months after the last dose).

The first cohort starts with a low dose of Vaccine FP-02.2, 150µg/peptide (1350µg total peptide or placebo). The next cohort receives a higher dose of Vaccine FP-02.2, 500µg/peptide (4500µg total peptide) or a low dose of Vaccine FP-02.2, 150µg/peptide, in conjunction with 500nmol/20nmol KLK/ODN1a IC31® or placebo or 500nmol/20nmol KLK/ODN1a IC31® alone. The final cohort will test the high dose of Vaccine FP-02.2, in conjunction with 500 nmol/20 nmol KLK/ODN1a IC31® or 500nmol/20nmol KLK/ODN1a IC31® alone. See Table 2.

For each cohort, dosing will start with a sentinel group consisting of one subject from each treatment arm, receiving active or control (FP-02.2 (IC31®), IC31® or placebo), e.g. for Cohort 1, the sentinel group will consist of 1 subject receiving placebo and 1 subject receiving Low-dose Vaccine FP-02.2. After the assessments on Day 8, the SRC will meet and review the safety data from the sentinel group and recommend whether or not the remaining subjects of a cohort should be dosed. Any PI with patients in the sentinel group will provide input to the SRC meeting.

In order to progress to the next cohort, the SRC with input from the Investigators contributing patients will meet to review safety data up to Day 36 from Cohort 1. Following this review subjects may be dosed in Cohort 2 assuming the SRC has identified no safety concerns. Similarly, subjects may be dosed in Cohort 3 after review of safety data up to and including Day 36 from subjects in Cohort 2.

5 STUDY SETTING

This is a multi-national, multi-centre clinical trial and each site is responsible for recruitment, treatment, follow-up and continuing care for subjects enrolled in each site.

The study sites were selected based on their large cohorts of patients with HBeAg-negative chronic HBV infection and appropriate experience in clinical trials.

6 ELIGIBILITY CRITERIA

6.1 Inclusion criteria

1. Male and female subjects aged 18-65 years.
2. Diagnosed with CHB defined as HBsAg positive for at least 24 months.
3. Subject has received entecavir or tenofovir for at least 2 years with a stable dose for at least 6 months prior to screening.
4. HBeAg negative for at least 2 years prior to inclusion in the study.
5. HBV DNA <50 IU/mL for ≥ 12 months
6. ALT or AST ≤ 1.5 x the ULN via the local laboratory at the Screening Visit
7. Able to give written informed consent to participate
8. Females should fulfil one of the following criteria:
    a. At least one year menopausal
    b. Surgically sterile
    c. Same-sex relationship
    d. Women of childbearing potential not surgically sterilised or with laboratory confirmed menopausal status are required to use a highly effective contraceptive measure with low used dependency such as:
        - Combined (oestrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation
        - Progestogen-only hormonal contraception implants associated with inhibition of ovulation
        - Intrauterine device (IUD)
        - Intrauterine hormone-releasing system (IUS)
        - Bilateral tubal occlusion
        - Vasectomised partner – must have had medical assessment of successful surgery.

        From screening until one menstrual cycle after the last dose of IMP (Day 57).

        Subjects who practice true abstinence or who exclusively have same sex partners need not use contraception, provided it is in line with their preferred and usual lifestyle. Periodic abstinence (eg, calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception. Should any such subject stop practicing true abstinence, they must use contraception as described above.

Males should fulfil one of the following criteria:

- Surgically sterile
- Willing to abstain from sexual intercourse or use a reliable form of contraception (e.g. condom with spermicide), if having sex with a pregnant or non-pregnant woman of childbearing potential, from screening until 3 months after the final dose of IMP.
- Surgically sterilised or post-menopausal female partner or same-sex relationship

6.2 Exclusion criteria

1. Liver disease other than CHB (a diagnosis of steatosis is permitted providing inclusion criterion 6 is met).
2. Evidence of Liver cirrhosis on Fibroscan at screening (Liver cirrhosis is defined as a Fibroscan measurement of >11.5 KPa), or previous history or evidence of cirrhosis on radiological imaging, Fibroscan or liver biopsy.
3. Positive serology for HIV-1 or HIV-2 or HCV or HDV antibodies.
4. Immunodeficient or autoimmune conditions due to disease or medication e.g. systemic steroids within previous 12 weeks. (Topical or inhaled steroids are permissible).
5. Clinically relevant co-morbidity, e.g. autoimmune disease.
6. Clinically relevant anaemia or leukopenia in the opinion of the investigator.
7. Cancer or treatment for cancer within 3 years prior to screening excluding basal cell carcinoma of the skin, which is allowed.
8. Known or suspected intolerance or hypersensitivity to the IMP or closely related compounds or any of the stated ingredients.
9. Receipt of any IMP within 90 days prior to screening or currently receiving IMP or intent to receive IMP.
10. Current substance or alcohol abuse that in the opinion of the Investigator would interfere with compliance or with interpretation of study results.
11. Any condition that in the opinion of the Investigator might interfere with study objectives.
12. Pregnant or breastfeeding.
13. Subjects should not have received, during the 6 month period prior to screening, any medications or other treatments that may adversely affect the immune system such as allergy injections, immunoglobulins, interferons, cytotoxic drugs or other drugs known to be frequently associated with significant major organ toxicity, or systemic corticosteroids (oral or injectable). Immunosuppressive treatment such as azathioprine or mercaptopurine is not permitted in the 6 months prior to screening.
14. Administration of live vaccines (such as live influenza vaccinations or live travel vaccinations) from 10 days prior to the screening visit until Day 85.

7 TRIAL PROCEDURES

7.1 Recruitment

7.1.1 Patient identification

Patients who may be eligible for the study will be identified by the Investigator from the site's records of patients with CHB. If a network of hospitals/clinics exists, other hospitals/clinics may identify suitable patients and refer them to the investigator's site.

7.2 Consent

Written informed consent will be obtained from all subjects at the screening visit, prior to entry into the study. The Investigator will explain to each subject orally and in writing the nature, significance, risks and implications of the trial before inclusion. In particular, the subjects will be informed about the following:

- the possibility of withdrawing from the clinical trial at any time by revoking the consent and without any resulting disadvantage.
- how personal and health-related data will be collected and used during the study.

All subjects will receive a copy of the subject information sheet and a copy of their signed and dated informed consent form (ICF).

7.2.1 Screening

The following screening assessments will be conducted between days -28 and -1:

- Sign informed consent
- Obtain medical history and demographic data
- Review of eligibility criteria
- Laboratory studies:
    - HBsAg monitoring
    - HBsAb monitoring
    - HBV DNA monitoring
- Vital signs
- Physical examination including measurement of weight and height
- Fibroscan
- Serum pregnancy test in women of childbearing potential
- Safety laboratory tests
- Urine dipstick test for drug abuse and alcohol screen
- Test for HIV, HDV and HCV
- Documentations of AEs
- Blood for cell-mediated immunity (CMI)
- 12-lead ECG

Patients who pass screening but are not dosed within 28 days may be re-screened once.

Subjects who have given their written informed consent will be allocated unique subject identification numbers in sequential order according to their admission to the study within each site by Interactive Response Technology (IRT). This number will be used for identification throughout the study and will not be used for any other participant.

7.3 The randomisation scheme

Subjects will be randomly assigned to 1 of 6 blinded treatment groups by the IRT into 3 cohorts as described in Table 2.

Following completion of the Screening activities, subjects who meet the all the inclusion and none of the exclusion criteria will be registered by the IRT.

The first subjects in each treatment arm of a cohort (i.e. active alone, active +IC31®, placebo and IC31® alone, as applicable) will form the sentinel group and, where possible, all sentinel subjects per treatment will be dosed at the same clinical site. Subsequently subjects in each cohort will be randomised to the applicable active or control treatment, to meet the required number of subjects to be dosed in each arm.

The randomisation list will be drawn up by an independent statistician.

7.3.1 Method of implementing the allocation sequence

After successfully completing the screening visit, subjects will be assigned a unique randomisation number in the order in which they are randomised. Randomisation numbers are provided in Table 3, these numbers are different to the subject identification numbers allocated at admission to the study.

Table 3 Randomisation Numbers

Cohort	Randomisation numbers	Sentinel randomisation numbers
1	1001–1018	1001–1002
2	2001–2036	2001–2004
3	3001–3018	3001–3002

7.4 Blinding

The Pharmacy staff will be unblinded for the purpose of final drug preparation. The pharmacist will consult the IRT for dose allocation and the Pharmacy staff will prepare each dose in compliance with the randomisation list.

Blinding of treatment assignment is critical to the integrity of this clinical study. Methods used to ensure blinding are described in the following paragraphs.

Due to the fact that formulations cannot be made to look identical, a double-observer technique will be used to maintain blind at the clinical site. Study staff either preparing the syringes and/or vaccinating the participants will be independent and will not take part in any other activity of the study (e.g. clinical assessments).

Injection materials and their labels should not be shown to subjects and subjects should be instructed to look away during the vaccination.

Knowledge of the randomisation list will be limited to the persons responsible for creation of the randomisation list, pharmacy staff who prepare the study treatments, and any unblinded study monitors or auditors, until all data has been entered in the electronic Case Report Form (eCRF), quality control and verification of the eCRF and assignment of subjects to the analysis populations has been completed, the database has been locked and the study formally unblinded.

Data provided to the SRC will be blinded.

7.5 Unblinding

If unblinding is required in the interest of the safety of a subject, an Investigator will discuss the matter with the Sponsor before using the IRT to unblind. In a medical emergency, the Investigator or delegate may unblind via the IRT for that subject without prior consultation with the Sponsor. In that event, the Investigator or delegate will notify the Sponsor as soon as possible that the randomisation code has been broken for the subject. If the blind is broken, the date, time, and reason must be recorded. For new patients, the method of unblinding for emergency purposes will be via the IRT.

7.6 Trial assessments

7.6.1 Safety assessments

7.6.1.1 Medical history

A complete medical history will be obtained at the screening visit and relevant medical history will be recorded in the eCRF. Enquiries will be made regarding all body systems including allergies/drug sensitivities, past surgeries, substance abuse and any other diseases or disorders.

7.6.1.2 Safety laboratory tests

Safety laboratory tests will be performed at the times indicated in the flow chart **Error! Reference source not found.Error! Reference source not found.Error! Reference source not found.Error! Reference source not found.**(see **Error! Reference source not found.**Table 1 Flow chart. At the screening visit the subjects will be screened for alcohol and drugs of abuse and infection with HIV, HDV and HCV. Further details are provided in Section 7.11.1.

The Investigator must review the laboratory report and document this review by signing the laboratory report form. Any clinically relevant values or changes in laboratory test values, as assessed against the normal reference ranges, must be recorded in the AE section of the subject's eCRF.

7.6.1.3 Vital signs

Vital signs will be measured at the times indicated in the flow chart (see Table 1 Flow chart). Vital signs include sitting blood pressure, pulse rate (after at least 5 min rest), and body temperature.

7.6.1.4 Electrocardiogram

A 12-lead ECG will be performed by the local site at the times indicated in the flow chart (see **Error! Reference source not found.**).

7.6.1.5 Physical examination

A physical examination will be performed by a physician at the times indicated in the flow chart (see **Error! Reference source not found.**). A full physical examination will be conducted at screening

including examination of weight and height, general appearance, eyes, ears, nose, throat, chest/respiratory system, heart/cardiovascular system, gastrointestinal system/liver, musculoskeletal system/extremities, dermatological system/skin, thyroid/neck, lymph nodes, and neurological system/psychiatric system.

A brief physical examination will be conducted at all other times including examination of general appearance, respiratory and cardiovascular systems and the abdomen, particularly the upper right quadrant. Any clinically relevant change in physical examination should be recorded as an AE.

7.6.1.6 Documentation of adverse events

See Section 9.

7.6.1.7 Documentation of local tolerability

Injection site tolerability will be assessed by the Investigator up to a minimum of 4 hours after the first injection and approximately 24 hours and 7 days after the first injection. For subsequent injections, injection site tolerability will be assessed by the Investigator up to 2 hours on the day of injection, 24 hours by means of a phone call and 7 days post injection.

Additionally, a diary card will be provided to the subjects to record injection site reactions from 4 hours (first injection) or 2 hours (subsequent injections) post injection to 7 days post injection. Data from the diary card will be transcribed into the appropriate eCRF.

The following solicited local injection site reactions will be assessed:

- Erythema/redness
- Swelling
- Induration/hardening
- Pain/tenderness (pain on pressure)
- Pain (spontaneous; pain without pressure)
- Pruritus/itching or burning sensation
- Wheal(s)

The symptoms will be graded as described in Table 4 Grading of solicited local injection site reactions.

Table 4 Grading of solicited local injection site reactions

	Grade 0	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Erythema/redness	0-2.4 cm	2.5-5 cm	5.1-10 cm	>10 cm	Necrosis or exfoliative dermatitis
Swelling	0-2.4 cm	2.5-5 cm and does not interfere with activity	5.1-10 cm or interferes with activity	>10 cm or prevents daily activity	Necrosis
Induration/hardening	0-2.4 cm	2.5-5 cm and does not interfere with activity	5.1-10 cm or interferes with activity	>10 cm or prevents daily activity	Necrosis
Pain/tenderness (pain on pressure)	Absent	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	Emergency room visit or hospitalisation
Pain (spontaneous; pain without pressure)	Absent	Does not interfere with activity	Repeated use of non-opioid pain reliever > 24 hours or interferes with activity	Any use of opioid pain reliever or prevents daily activity	Emergency room visit or hospitalisation
Pruritus/itching	Absent	Mild	Moderate	Severe	Not applicable
Wheal(s)	0-2.4 cm	2.5-5 cm	5.1-10 cm	>10 cm	Not applicable
Burning sensation	Absent	Mild	Moderate	Severe	Not applicable

Erythema, swelling, induration, tenderness, and pain are graded according to the FDA Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (2007).

7.6.1.8 Additional safety examinations

If there are any unclear symptoms or observations the responsible physician in charge may perform further medical examinations, other than outlined in this protocol, including further clinical laboratory tests, in order to clarify the relevance or to diagnose symptoms.

7.6.2 Immunological and virological assessments

7.6.2.1 Determination of immunological parameters

Blood samples will be collected at the times indicated in the flow chart to evaluate CMI responses.

- IFN-γ ELISpot assay (ex vivo and cultured assay): to determine Vaccine FP-02.2-specific IFN-γ production from cryopreserved PBMC

- Other immunological assays may be performed such as T cell proliferation assays, intracellular cytokine assays, ELISA-based assays to measure Vaccine FP-02.2-specific antibodies, or other suitable assays to measure the immune response from PBMC or serum.
- The frequency of regulatory cells, expression of co-stimulatory and inhibitory markers may be measured by flow cytometry from cryopreserved PBMC.
- HLA genotyping will be performed on cryopreserved PBMC samples from consenting subjects to build an understanding of how the immune response to Vaccine FP-02.2 may or may not be independent of HLA genotype. Further genotyping may be performed from consenting subjects to assess the relationship between immune responses to the vaccine or HBV and associated gene profiles.

7.6.2.2 Determination of virological parameters

- Quantitative HBV DNA levels (PCR)
- Quantitative HBsAg levels (ELISA)
- Quantitative HBsAb levels (ELISA)

Immunological (IFNγ ELISpot assay) and virological assessments will follow the OECD Principles of GLP.

7.6.3 Assessment schedule

Screening Visit

The screening assessment is described in Section7.2.1.

Randomisation will be performed by IRT up to 3 days prior to first injection to allow data from screening to be available for confirmation of eligibility criteria and for the central pharmacy to supply medication to site.

Visit 1:

The following assessments and procedures will be performed on Day 1:

Pre-vaccination

- Review of eligibility criteria
- Urine pregnancy test in women of childbearing potential
- Blood for CMI
- HBsAg and HBsAb monitoring
- Vital signs
- Physical examination
- Documentation of concomitant medication
- Documentation of AEs
- Vaccination
- Contact IRT for drug supply
- Dispense diary card

Post-vaccination

- Injection site tolerability

- Documentation of concomitant medication
- Documentation of AEs

The subject will be asked to stay in the clinic for at least 4 hours following the first injection.

Visit 2

The following assessments and procedures will be performed on Day 2:

- Vital signs
- Physical examination
- Injection site tolerability
- Documentation of AEs and concomitant medications
- Review diary card for local tolerability

Visit 3

The following assessments will be performed on Day 8:

- Vital signs
- Physical examination
- Injection site tolerability
- Safety laboratory tests
- Documentation of concomitant medication
- Blood for CMI
- Documentation of AEs
- Review diary card for local tolerability
- Dispense new diary cards

Visits 4 and 6 (administration of booster vaccinations)

The following assessments will be performed on Days 29 and 57:

Pre-vaccination

- Urine pregnancy test in women of childbearing potential (pre-vaccination)
- Safety laboratory tests (pre-vaccination)
- Vaccination
- Vital signs
- HBsAg and HBsAb monitoring
- Physical examination
- Documentation of concomitant medication
- Documentation of AEs
- Blood for CMI

Post-vaccination

- Injection site tolerability (post-vaccination)
- Documentation of concomitant medication
- Documentation of AEs
- Dispense diary card

The subject will be asked to stay in the clinic for 2 hours after each booster vaccination.

Telephone Contact 24 hours after booster vaccinations

Telephone contact enquiring about AEs and injection site tolerability (assessed by subject) on days 30 and 58.

Visits 5 and 7

The following assessments will be performed on Days 36 and 64:

- Vital signs
- Physical examination
- Injection site tolerability
- Safety laboratory tests
- Documentation of concomitant medication
- Blood for CMI
- Documentation of AEs
- Review diary card for local tolerability

Visit 8:

The following assessments will be performed on Day 85:

- HBsAg and HBsAb monitoring
- Vital signs
- Physical examination
- Urine pregnancy test in women of childbearing potential
- Safety laboratory tests
- Documentation of concomitant medication
- Blood for CMI
- Documentation of AEs

7.7 Follow-up assessments

Subjects will be contacted (telephone call) by clinic staff at approximately 4-weekly intervals between Day 85 and Day 225 to check subject health.

Visit 9:

A follow-up visit will be performed at Day 225. The following assessments will be performed:

- HBsAg and HBsAb monitoring
- HBV DNA monitoring
- Vital signs
- Physical examination
- Safety laboratory tests
- Documentation of concomitant medication
- Blood for CMI
- 12-lead ECG
- Documentation of SAEs and AESIs (autoimmune disease and liver flares)

7.8 Qualitative assessments – Nested studies

Not applicable.

7.9 Allowed visit intervals

Allowed visit intervals are as follows:

- ***screening and randomisation:*** subjects must be screened 7 to 28 days (±2 days) and can be randomised to treatment up to 3 days prior to the first injection.
- ***first dose:*** Visits 1 and 2 must be done on Days 1 and 2, respectively; Visit 3 may be done within ± 1 day of Day 8
- ***second dose:*** Visit 4 may be done within 28 days (± 7 days) of Visit 1; subjects must be phoned the day after Visit 4; Visit 5 should be done 7 (± 1 day) days after Visit 4
- ***third dose:*** Visit 6 may be done 28 days (± 7 days) after Visit 4; subjects must be phoned the day after Visit 6; Visits 7 and 8 should be done 7 (± 1 day) and 28 days (±1 day) respectively after Visit 6
- ***follow-up:*** Visit 9 must be done 24 (± 4 weeks) weeks after the last dose (Visit 6)

The scheduled assessments may shift in instances where a visit window is used; subsequent visit dates should be recalculated from the date of the new visit. If a second dose visit is missed, the dose can be given 6 weeks after the first dose and noted as out of window. If the dose is later than 6 weeks, the second dose visit can be skipped and given at Visit 3 at 8 weeks as per schedule to avoid a delay in the schedule.

7.10 Withdrawal criteria

Participation in the study is strictly voluntary. A subject has the right to withdraw from the study at any time and for any reason. If he/she chooses to withdraw, the Investigator must be informed immediately. The Investigator has the right to terminate participation of any subject at any time if the Investigator deems it in the subject's best interest. The reason and circumstances for premature discontinuation will be documented in the subject's eCRF.

In general, all subjects should be encouraged to continue follow up until the end of the study even if they are withdrawn from continued study treatment, to allow collection of late safety data as well as immunogenicity and virology data.

7.10.1 Possible reasons for discontinuing subjects from continued study treatment

Examples of possible reasons for premature withdrawal of subjects:

- Major protocol violations;
- Pregnancy;
- Serious intercurrent illness;
- AEs which, in the judgement of the PI or Co-Investigator and subject to discussion with the Sponsor's medical advisor, justifies discontinuation;
- Non-compliance with study requirements;
- Withdrawal of consent by the subject;
- Subjects experiencing clinically significant injection site reactions or clinically significant changes in haematology and serum biochemistry results, considered possibly related to administration of the IMP, after a dose may not receive the subsequent dose of medication, at the discretion of the PI, in discussion with the Chief Investigator (CI) or the Sponsor's medical advisor. However, safety, virology and immunogenicity data will continue to be collected until the end of the study unless the subject withdraws his/her consent.

If a subject is withdrawn from study treatment, relevant study procedures e.g. physical examination, clinical laboratory analyses for safety and immunogenicity will be performed wherever possible. The reason(s) for discontinuation will be recorded in the appropriate section of the eCRF. Any subject who prematurely terminates participation and who has received at least one injection of the study treatment will undergo relevant post-study procedures for reasons of medical prudence, including a final examination and safety laboratory studies, unless the subject has withdrawn his/her consent and refuses to be examined and/or have blood drawn.

If a subject terminates participation due to an AE possibly related to the study medication or study procedures, the subject should be followed up by additional examinations according to the medical judgment of the Investigator until the abnormal condition is resolved or the Investigator deems further observations or examinations to be no longer medically indicated. If the subject refuses to be followed up the Investigator should explain the importance of the follow-up and encourage the subject to allow follow up. Subjects who elect to discontinue dosing should be encouraged to continue with other study procedures until the end of study if they are willing.

Study stopping rules

Individual subjects

An individual subject will have treatment discontinued if he/she experiences any one or more of the following events:

- ALT ≥ 10 times the ULN
- INR > 1.3
- Total bilirubin >3 times the ULN

Study as a whole

Dosing will be stopped for all subjects and enrolment discontinued if 20% or more of subjects experience one or more of the following events

- ALT ≥ 10 times the ULN
- INR > 1.3
- Total bilirubin >3 times the ULN
- Serious adverse events considered related to investigational products

- Severe systemic reactions.

7.10.2 Site discontinuation

A site may be closed for the following reasons:

- The site is unlikely to be able to recruit sufficient patients within the agreed time frame
- The site does not respond to study management requests
- Repeated protocol violations

7.10.3 Study termination

The Sponsor reserves the right to modify or terminate the study at any time. All subjects recruited to the study prior to termination, including those who have been withdrawn, will be followed until 225 days after their last injection.

Possible reasons for termination are:

- Safety reasons –the incidence of AEs in this or any other study using the same IMP indicates a potential health risk for the subjects.
- New scientific knowledge becomes known that makes the objectives of the study no longer feasible/valid
- Unsatisfactory enrolment of patients

7.10.4 Replacement of subjects

Eligible subjects who are randomised and withdrawn before their second dose of IMP will be replaced. Subjects who have received two doses of IMP who are subsequently withdrawn from the study will not be replaced.

7.11 Storage and analysis of samples

It is the responsibility of the trial site to ensure that samples are appropriately labelled in accordance with the trial procedures to comply with local legislation. Biological samples collected from participants as part of this trial will be transported, stored, accessed and processed in accordance with local legislation relating to the use and storage of human tissue for research purposes. Information for specific handling and storage conditions can be found in the laboratory manual.

7.11.1 Safety laboratory

Blood and urine samples will be collected for safety laboratory tests and screening for alcohol, drugs of abuse and HIV, HDV and HCV infection at the times indicated in the flow chart (see Table 1 Flow chart). The analytes listed in Table 5 will be determined.

Clinical laboratory tests (see Table 5) will be performed at local site hospital laboratories. Procedures for sample collection, processing, and storage will be described in a separate laboratory manual. Additional and repeat testing may be performed at the discretion of the Investigator.

Table 5 Clinical laboratory safety test analytes

Panel	**Parameters**	
Haematology	White blood cells (leukocytes) Red blood cells (erythrocytes) Haemoglobin Haematocrit Mean corpuscular volume Mean corpuscular haemoglobin Mean corpuscular haemoglobin concentration	Neutrophils [a] Lymphocytes [a] Monocytes [a] Eosinophils [a] Basophils [a] Platelets
Serum biochemistry	Sodium Potassium Urea Creatinine Albumin Calcium Phosphate Glucose Total protein C-reactive protein	Aspartate transaminase (AST) Alkaline phosphatase (ALP) [b] Alanine aminotransferase (ALT) Creatine kinase [c] Gamma-glutamyl transferase (gamma glutamyl transpeptidase) (GGT) Lactate dehydrogenase Total and direct bilirubin
Coagulation	International normalized ratio of prothrombin time (INR)	
Urinalysis	Protein Bilirubin Urobilinogen Ketones Glucose	Leukocyte esterase Red blood cells pH Nitrites Specific gravity
Urine microscopy [d]	Microscopy to be performed if clinically indicated (as per local procedures)	
Viral serology [d]	Human immunodeficiency virus (HIV I and II)	Hepatitis C virus (HCV) antibodies Hepatitis D virus (HDV) antibodies
Drugs of abuse and alcohol screen [d]	Amphetamine/ecstasy Ethanol Cannabinoids Cocaine Urine creatinine	Opiates Benzodiazepines Methadone metabolites Barbiturates
Serum/urine pregnancy test [e]	Beta human chorionic gonadotropin	

[a] Given as both absolute count and as a percentage of total leukocyte count.

[b] Alkaline phosphatase iso-enzymes (liver-specific and bile duct specific) will be measured if original alkaline phosphatase test result is above the upper limit of normal.

[c] Creatine kinase MB or Troponin T will be measured where clinically indicated.

[d] At Screening Visit only.

[e] A serum pregnancy test will be performed in all women of childbearing potential at the Screening Visit. Serum βHCG concentrations above 5 mIU/mL. Urine pregnancy tests will be performed during the remainder of the study at the times indicated in the flow chart.

7.11.2 Immunology

Blood samples will be processed to isolate, cryopreserve and store PBMC. This may be conducted at a central laboratory at Altimmune or at Investigator sites. Further details can be found in the laboratory manual.

Subsequent primary immunological analysis (IFNγ ELISpot) will be conducted at Altimmune. Any cryopreserved PBMC prepared and stored at Investigator sites will be transferred to Altimmune prior to analysis.

Other specialist laboratories may be used for exploratory immunological analyses and for HLA genotyping. A PBMC sample from either the screening visit or Visit 1 will used for HLA genotyping in consenting subjects. PBMC samples isolated at other time points may be used for other genotyping as required.

7.11.3 Virology

Blood samples will be collected for the assessment of virological parameters (HBsAg, HBsAb, HBV DNA) at the times indicated in the flow chart (see Table 1 Flow chart). Isolation of serum or plasma will be performed at the clinical site following methods detailed in the laboratory manual. Serum and plasma will be stored at the clinical site under appropriate conditions (further details are provided in the laboratory manual) until shipment to Altimmune laboratories. Batched samples will be sent from Altimmune laboratories to King's Liver Labs for virological analysis as detailed in the laboratory manual.

7.11.4 Sampling handling and storage

The handling of the samples will be described in a separate laboratory manual.

Samples will be retained for a period up to 10 years and will only be used for purposes described in this protocol.

7.11.5 Blood volume

The estimated blood volumes collected from each subject during the study for safety, immunology and virology tests as described in Sections 7.11.1, 7.11.2, and 7.11.3, respectively, are presented in Table 6 Approximate Blood volumes [a].

Table 6 Approximate Blood volumes [a].

	Sample Volume	Number of Samples	Total Volume (mL)
Safety			
Serum biochemistry [b]	5.0	8	40.0
Haematology [b]	2.0	8	16.0
Coagulation [b]	2.0	8	16.0
Serum pregnancy test [b,c]	3.5	1	3.5
Serology (HCV/HIV I and II, HDV) [b]	3.5	1	3.5
Immunoanalysis			
Cellular immunoanalysis (PBMC) [d]	40	9	360.0
Virology			
HBsAg, HBsAb, HBV DNA quantitative [e]	4.0/8.0 [f]	6	32.0
Total [a]			
Male subjects			467.5
Female subjects			471.0

HBsAg = hepatitis B surface antigen; HBsAb = antibody to hepatitis B surface antigen; HCV = hepatitis C virus; HDV = hepatitis D virus; HIV = human immunodeficiency virus; PBMC = peripheral blood mononuclear cells.
[a] Sample volumes are based on direct venipuncture; where a cannula is used an extra 1mL will be drawn and discarded. Specific volumes for safety analysis may vary from site to site.
[b] Analysis to be performed by site-specific Hospital Laboratories.
[c] All women of childbearing potential; serum pregnancy test will be conducted at the Screening Visit.
[d] Isolation of PBMC may be performed at a central laboratory, at Altimmune, or at the Investigator site.
[e] Analysis to be performed by King's Liver Labs which is a Clinical Pathology Accreditation UK Ltd. (CPA UK Ltd.) enrolled Laboratory.
[f] 4.0 mL of blood will be collected for virology except at screening and visit 9 when 8.0mL of blood will be collected.

The planned blood volume is not greater than 150mL each month. However, the number of samples collected and the volume required for each analysis may change during the study (i.e. if additional samples are collected for repeated assessments). Extra blood samples for safety assessments will be taken only as and when needed. No more than an extra 50mL blood will be taken each month for immunology and virology assessments.

7.12 End of trial

The end of the active study period is defined as the last Day 85 visit of the last subject participating in the trial. The end of the trial includes the late safety follow up period and is defined as the last Day 225 visit for the last subject participating in the trial. If the trial is terminated early, the trial ends when the Sponsor notifies the Investigator in writing that the trial has finished, or when the last subject attends the Day 255 visit, whichever is later. The Sponsor will notify the relevant competent authorities (CAs) and main Ethics Committees (ECs) of the end of a clinical trial within 90 days of its completion or 15 days if the trial is terminated early. A supplementary follow-up notification will be submitted to the relevant CAs and ECs after the last Day 225 visit.

8 TRIAL MEDICATION

8.1 Name and description of investigational medicinal product(s)

Vaccine FP-02.2

Vaccine FP-02.2 is a lyophilised product containing 0.6mg of nine different synthetic peptides (designated P877, P151, P113, P856(K), P753(K), P376, P797(K), P277(K) and P1266(K)), each covalently linked to a single fluorocarbon moiety on the N-terminus under a controlled process and 30mg Mannitol (EP grade). The nine peptides are 32 to 40 amino acids in length and correspond to conserved regions of Hepatitis B viral proteins. The fluorocarbon conjugated peptides, called fluoropeptides (the active Drug Substances), are designed to enhance the delivery of the antigens to the human immune system and induce therapeutic T cell responses against infection.

Vaccine FP-02.2 is a white to off-white powder presented in a 2mL clear glass vial sealed with a metal crimp. The vaccine is intended to be reconstituted with 600µl of 28mM L-histidine (EP grade) and 600µl sterile NaCl 0.9% in a total 1200µl to achieve a sterile, isotonic, neutral pH, clear to slightly translucent solution with no visible particles which will be injected intramuscularly. 1mL of the reconstituted Vaccine FP-02.2 will administer a 500µg/peptide dose. The reconstituted Vaccine FP-02.2 can be diluted with placebo component (see below) in order to administer 150µg/peptide dose in 1mL. Where applicable, Vaccine FP-02.2 can be prepared with IC31® to produce either a 500µg/peptide or 150µg/peptide dose both containing IC31® at a strength of 500nmol/mL KLK and 20nmol/mL ODN1a. Full preparation instructions and details of shelf life will be described in a separate pharmacy manual.

Placebo

The Placebo is comprised of placebo component (50mg/mL mannitol (EP grade) in 28mM L-histidine (EP grade)) diluted 1:1 in sterile NaCl 0.9%.

Placebo component is stored in 2mL glass vials containing 1.225mL of placebo component which, after thawing, is diluted 1:1 with sterile NaCl 0.9% to achieve an isotonic, neutral pH, clear and colourless solution, free from particulate matter which will be injected intramuscularly. Full preparation instructions and details of shelf life will be detailed in a separate pharmacy manual.

IC31®

IC31® is a two-component adjuvant composed of the synthetic peptide KLK and the synthetic oligodeoxynucleotide ODN1a. IC31® is packaged in 2mL clear glass vials and contains 1mL of a sterile turbid suspension, neutral pH at a strength of 1000nmol/mL KLK and 40nmol/mL ODN1a in 5mM phosphate and sterile NaCl 0.9%. IC31® is prepared by diluting 1:1 with placebo component. Full preparation instructions and details of shelf life will be detailed in a separate pharmacy manual.

8.2 Legal status of the drug

The trial is being carried out under a Clinical Trial Authorisation (CTA). Vaccine FP-02.2 and IC31® are therefore only to be used by the named Investigators, for the patients specified in this protocol, and within the trial.

8.3 Summary of Product Characteristics (SmPC)

Vaccine FP-02.2 and IC31® are not approved and there is no SmPC for the IMPs. Details of Vaccine FP-02.2 and IC31® can be found in the latest version of the IB.

8.4 Drug storage and supply

The Sponsor will supply Vaccine FP-02.2, placebo component, 28mM L-histidine and IC31® to the central Pharmacy site or the local hospital pharmacy with the relevant documentation including certificate of analysis. Further details will be provided in the pharmacy manual. Transport of lyophilised Vaccine FP-02.2, 28mM L-histidine, placebo component and IC-31® from the central pharmacy to sites will be via an approved courier and at monitored temperatures appropriate for each substance.

When the Pharmacist receives the materials for use in the study, he/she will check the conditions and contents of the package. The pharmacy staff will acknowledge receipt of the trial materials and report any anomalies in the inventory to both the Sponsor and GMP storage facility. The supplies will then be logged in to the pharmacy and the records will be updated in accordance with the pharmacy SOPs.

Lyophilised Vaccine FP-02.2 and frozen placebo component will be stored at minus 20°C (-20°C) (±5°C). Liquid IC31® adjuvant and 28mM L-histidine will be stored refrigerated, at 2-8°C. Liquid sterile NaCl 0.9% must be stored at room temperature. It is anticipated that temporary changes in temperature will occur when the refrigerator or freezer door is open for the removal of IMP as detailed in the pharmacy manual.

Records of the temperatures that Vaccine FP-02.2, IC31®, 28mM L-histidine or placebo component are stored will be maintained throughout the study. If deviations occur from the recommended storage conditions, these will be notified to the Sponsor's contact. The clinical material affected will not be used until the Sponsor has assessed the impact of the deviation and has determined whether the material can be used.

The central or hospital Pharmacy will prepare injections for all clinical sites upon receipt of a suitably signed trial-specific prescription or similar. All study drugs will be handled according to Pharmacy standard operating procedures (SOPs) and pharmacy manual. An unblinded pharmacy monitor will be permitted to check the supplies, storage and dispensing procedures. During the preparation, the Pharmacist will ensure that records are kept in sufficient detail for the sequence of operations to be determined. Sufficient reconciliation will take place in order to ensure that the correct quantity of treatment has been accounted for at each stage of the process.

Once reconstituted the IMP will be stored in the refrigerator at 2-8°C until transport to clinical sites as detailed in the pharmacy manual.

The receipt, dispensing and return of any trial material to and from pharmacy will be logged on the appropriate forms in the pharmacy.

At a time convenient to both the Sponsor and the Pharmacy all unused drugs will be returned to the Sponsor together with an account of the quantities supplied and used during the study. If required by the Sponsor, the Pharmacist will arrange for the recorded destruction of unused study drugs.

8.5 Dosage schedules

Each dose of Vaccine FP-02.2, Vaccine FP-02.2/IC31®, Placebo or IC31® will be administered as an IM injection (1.0mL) in the deltoid muscle, where possible, of the non-dominant arm by appropriately trained clinical staff members. The first dose will be administered on Day 1, the second dose approximately 4 weeks later on Day 29 and the third dose approximately 4 weeks after the second dose on Day 57. Subjects will stay in the unit for at least 4 hours following administration of the first dose and at least 2 hours following administration of the second and third doses.

8.6 Preparation and labelling of Investigational Medicinal Product

Detailed reconstitution instructions will be provided to the Pharmacies by the Sponsor. In brief:

The bulk drug product will be reconstituted at the Pharmacy as described in separate pharmacy handling instructions provided by the Sponsor. Individual subject treatments will be dispensed by the pharmacist and labelled in accordance with local Good Manufacturing Practice legislation.

Reconstitution instructions will be detailed in the Pharmacy manual.

8.7 Dosage modifications

No dosage modifications for individual subjects are allowed. The SRC may recommend reducing the dose in any treatment arm after the first dose in sentinel subjects; or the dose(s) in the next treatment arm after review of safety data at Day 36 from the previous treatment arm, as described in Section 4. In either case, the Sponsor will decide whether the study is stopped or whether to continue with a lower dose. If the dose(s) is reduced after the first dose in the sentinel subjects, those subjects will be withdrawn; if the Sponsor decides to continue, the remaining subjects in the treatment arm will be given the reduced dose(s).

8.8 Known drug reactions and interaction with other therapies

Please refer to Section 2.1.2 and the IB for an overview of the risks associated with the IMP including information about potential drug reactions. No interactions with other therapies are known so far.

8.9 Concomitant medication

In the interests of subject safety and acceptable standards of medical care the Investigator will be permitted to prescribe additional treatment(s) at his/her discretion in addition to treatment with entecavir or tenofovir.

All treatments, including vaccinations, must be recorded in the subjects' eCRFs (medication, dose, treatment duration and indication).

8.10 Trial restrictions

Women of childbearing potential must use a highly effective contraceptive method within the time frames mentioned below.

Women of childbearing potential are defined as:

- Not surgically sterile
- Not post-menopausal (post-menopausal is defined as at least one year amenorrhea in woman over 50 years. Women younger than 50 years should have laboratory confirmation of menopausal status)

Women of childbearing potential not surgically sterilised or with laboratory confirmed menopausal status are required to use a highly effective contraceptive measure with low used dependency such as:

- Combined (oestrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation
- Progestogen-only hormonal contraception implants associated with inhibition of ovulation
- Intrauterine device (IUD)
- Intrauterine hormone-releasing system (IUS)
- Bilateral tubal occlusion
- Vasectomised partner – must have had medical assessment of successful surgery.

From screening until one menstrual cycle after the last dose of IMP (Day 57).

Males should fulfil one of the following criteria:

- Surgically sterile
- Willing to abstain from sexual intercourse or use a reliable form of contraception (e.g. condom), if having sex with a pregnant or non-pregnant woman of childbearing potential, from screening until 3 months after the final injection of IMP/Placebo.
- Surgically sterile female partner

8.11 Assessment of compliance

The injections will be administered by study personnel and therefore IMP compliance is not a concern. The study personnel will be appropriately trained on IMP administration procedures and documentation requirements prior to study start.

Non-compliance with study procedures will be reported to the Sponsor who will decide if persistent non-compliant subjects should be withdrawn from continued study treatment.

8.12 Name and description of each Non-Investigational Medicinal Product (NIMP)

As part of the study inclusion criteria, trial participants will currently receive either entecavir (brand name Baraclude®, Bristol-Myers Squibb) or tenofovir disoproxil furamate (brand name Viread®, Gilead) as standard of care medicine.

Additional information on these products can be found in the respective, regulated local specific documents.

Tenofovir

Tenofovir is recommended as an option for the treatment of adults with chronic HBeAg-positive or HBeAg-negative hepatitis B with compensated liver disease, with evidence of active viral replication, persistently elevated serum ALT levels and histological evidence of active inflammation and/or fibrosis. Tenofovir is a NA which works by blocking the enzyme reverse transcriptase, which is

responsible for hepatitis B virus (HBV) replication. Full details of uses, side effects and contraindications are presented in regulated local specific documents.

Entecavir

Entecavir is recommended as an option for the treatment of adults with chronic HBeAg-positive or HBeAg-negative hepatitis B with compensated liver disease, with evidence of active viral replication, persistently elevated ALT levels and histological evidence of active inflammation and/or fibrosis. Entecavir is a NA which works by inhibiting the viral DNA polymerase responsible for HBV replication, which is responsible for hepatitis B virus (HBV) replication. Full details of uses, side effects and contraindications are presented in regulated local specific documents.

9 PHARMACOVIGILANCE

9.1 Definitions

The definitions of an adverse event (AE), adverse reaction (AR), serious adverse event (SAE), serious adverse reaction (SAR), and suspected unexpected serious adverse reaction (SUSAR) are provided in Table 7 Pharmacovigilance definitions.

Table 7 Pharmacovigilance definitions

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.
Adverse Reaction (AR)	An untoward and unintended response in a participant to an investigational medicinal product which is related to any dose administered to that participant. The phrase "response to an investigational medicinal product" means that a causal relationship between a trial medication and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out. All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the trial medication qualify as adverse reactions.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • results in death • is life-threatening • requires inpatient hospitalisation or prolongation of existing hospitalisation • results in persistent or significant disability/incapacity • consists of a congenital anomaly or birth defect Other ‘important medical events’ may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences. NOTE: The term "life-threatening" in the definition of "serious" refers to an

	event in which the participant 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.
Serious Adverse Reaction (SAR)	An adverse event that is both serious and, in the opinion of the reporting Investigator, believed with reasonable probability to be due to one of the trial treatments, based on the information provided.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	A serious adverse reaction, the nature and severity of which is not consistent with the information about the IMP set out in the Investigator's Brochure (IB) relating to this trial.
Adverse Event of Special Interest (AESI)	AESI refers to adverse events of significant scientific, medical, and public interest among pandemic vaccines. For this study AESI are hepatic flares and autoimmune conditions

9.2 Operational definitions for (S)AEs

The occurrence of AEs will be assessed by non-directive questioning of the subject at each visit. Additionally, AEs volunteered by the subject during or between visits or detected through observation, physical examination, laboratory test or other assessments will be documented. Subjects will be instructed that they must immediately report any AEs, subjective complaints or objective changes in their well-being to the Investigator or the clinic personnel, regardless of the perceived relationship between event and test product.

All AEs must be fully recorded throughout the entire study period (from Screening Visit to Day 85) and will be entered into the subjects' eCRF, whether or not they are considered to be drug-related. Each AE should be described in detail: onset time and date, offset time and date, description of event, severity, relationship, action taken and outcome. From Day 85 and Day 225 only SAEs and AESIs will be collected.

Adverse events should be followed until recovery to the normal state has been achieved. In the event of a subject not returning to the clinical unit, every attempt will be made to contact the subject up to Day 85 after which the outcome of this event will be recorded as lost at follow-up.

9.2.1 Assessment of seriousness

The criteria for seriousness are provided in the definition of an SAE given in Table 7.

9.2.2 Categorisation of adverse events

The intensity of an AE will be categorised as follows:

- Mild: the event causes a minor discomfort, does not interfere with daily activity of the subject or does not lead to establishment of a correcting treatment;
- Moderate: the event perturbs the usual activity of the subject and is of a sufficient severity to make the subject uncomfortable;

- Severe: the event prevents any usual routine activity of the subject and causes severe discomfort. The term "severe" is used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious," which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning.

9.2.3 Assessment of causality

Causal relationship assessment to drug treatments is required for purposes of reporting AEs. To promote consistency, the following guidelines should be taken into consideration along with good clinical and scientific judgment when determining the relationship of Vaccine FP-02.2 to an AE:

- Reasonable Possibility: a causal relationship with the IMP cannot be excluded
- No Reasonable Possibility: there is no reasonable evidence to suggest a causal relationship between Vaccine FP-02.2 and the AE.
- No Possibility: there is no possibility for a causal relationship. This assessment will likely only be applied to AEs recorded prior to administration of IMP.

9.2.3.1 Action Taken

Action taken will be defined as:

- None;
- Clinical management of the AE;
- Dosing stopped.

9.2.3.2 Outcome

Outcome will be defined as:

- Resolved;
- Ongoing;
- Lost to follow up.

9.3 Recording and reporting of SAEs and SUSARs

The expectedness of the SAEs in relation to the IMP will be determined using the IB.

SAEs occurring on this study will be reported to the Sponsor Pharmacovigilance provider (PRA Health Sciences) and the Sponsor's medical representative within 24 hours of discovery or notification of the event. For contact details refer to the Key trial contacts section in this protocol. The Investigator will be requested to complete a separate SAE reporting form in addition to the information on the eCRF.

A SUSAR that is fatal or life-threatening must be reported to the CA and EC immediately (within 7 days) after the Sponsor becomes aware of the event. Any additional information must be reported within 8 days of sending the first report.

A SUSAR which is not fatal or life-threatening must be reported to the CA and EC according to country specific requirements after the Sponsor becomes aware of the event.

9.4 Responsibilities

Principal Investigator (PI):

- Checking for AEs when participants attend for treatment / follow-up and ensuring that all AEs are recorded.
- Using medical judgement in assigning seriousness and causality and providing an opinion on expectedness using the IB approved for the trial.
- Ensuring that all SAEs are recorded and reported to the Sponsor within 24 hours of becoming aware of the event and provide further follow-up information as soon as available. Ensuring that SAEs are followed up with Sponsor if a record of receipt is not received within 2 working days of initial reporting.

Chief Investigator (CI) or delegate:

- Clinical oversight of the safety of patients participating in the trial, including an ongoing review of the risk / benefit.
- Using medical judgement in assigning seriousness and causality where it has not been possible to obtain local medical assessment.
- Immediate review of all SUSARs, if the study is unblinded.
- Review of specific SAEs in accordance with the trial risk assessment and protocol as detailed in the Trial Monitoring Plan.
- Assisting in assigning Medical Dictionary for Regulatory Activities (MedDRA) or Body System coding to all SAEs and SARs.

Sponsor or delegate:

- Central data collection and verification of AEs, ARs, SAEs, SARs and SUSARs according to the trial protocol.
- Reporting safety information to the CI, delegate or independent clinical reviewer for the ongoing assessment of the risk / benefit according to the Pharmacovigilance Plan.
- Reporting safety information to the SRC according to the Trial Monitoring Plan.
- Expedited reporting of SUSARs to the CAs and ECs within required timelines.
- Assigning expectedness.
- Notifying Investigators of SUSARs that occur within the trial, if the study is unblinded.
- The unblinding of a participant for the purpose of expedited SUSAR reporting.
- Checking for (annually) and notifying PIs of updates to the IB for the trial.
- Preparing standard tables and other relevant information for the development safety update report (DSUR) in collaboration with the CI
- Assigning MedDRA or Body System coding to all SAEs in consultation with the CI or delegate.

Safety review committee (SRC)

- The SRC will review safety data from a sentinel group of each cohort before commencement to the remaining cohort. In addition, the SRC will review safety data after two injections in each cohort, prior to progression from Cohort 1 to Cohort 2, and prior to progression from Cohort 2 to 3.
- For review of data from the sentinel group, the SRC will consist of the PI(s) from the site(s) involved with sentinel dosing, the CI and the Sponsor's medical advisor.
- For review of data to allow progression between cohorts the SRC will consist of the CI, all PIs and the Sponsor's medical advisor. In addition, representative(s) from the Sponsor, or their delegate, may attend but without voting rights.

9.5 Notification of deaths

Any untoward medical occurrence resulting in death fulfils the definition of an SAE and is subject to expedited reporting as described in Section9.3.

9.6 Pregnancy reporting

All pregnancies within the trial (either the trial participant or the participant's partner) should be reported to the Sponsor's Pharmacovigilance provider (PRA Health Sciences) in the first instance using the relevant Pregnancy Reporting Form for follow up and the Clinical Lead and the Medical Monitor should also be notified.

Pregnancy is not considered an AE unless a negative or consequential outcome is recorded for the mother or child/foetus. If the outcome meets the serious criteria, this would be considered an SAE.

If a subject is confirmed to be pregnant, use of the IMP will be discontinued immediately, however, the subject may continue with study follow up at the discretion of the investigator and obstetrician. The Investigator must provide follow-up information about the course of the pregnancy and outcome.

9.7 Overdose

An overdose is not expected, as the IMP is administered via IM injection. Should subjects allocated to the low dose of the IMP receive a higher dose than the allocated dose this should be reported in the eCRF and the Sponsor should be informed. Any deviations from the assigned dose will be handled as a protocol deviation.

9.8 Reporting urgent safety measures

If any urgent safety measures are taken the CI/Sponsor shall immediately and in any event no later than 3 days from the date the measures are taken, give written notice to the CAs and the relevant ECs of the measures taken and the circumstances giving rise to those measures.

9.9 The type and duration of the follow-up of subjects after adverse events

The Investigator will initiate appropriate treatment according to his/her medical judgment and will decide whether to discontinue dosing if an AE or a concurrent health condition occurs.

The subject may be followed up by additional examinations according to the medical judgment of the Investigator and the nature of the AE, until the abnormal condition is resolved or the Investigator deems further observations or examinations to be no longer medically indicated.

Any SUSAR will need to be reported to the Sponsor irrespective of how long after IMP administration the reaction has occurred.

9.10 Development safety update reports

The Sponsor will submit DSURs once a year throughout the clinical trial, or on request to the CAs, ECs and Investigative sites.

The report will be submitted within 60 days of the Developmental International Birth Date (DIBD; 8 June 2015) of the trial each year until the trial is declared ended.

10 STATISTICS AND DATA ANALYSIS

10.1 Treatment Arms

There are six treatment arms:

1. Low-dose FP-02.2 (Cohort 1)
2. High-dose FP-02.2 (Cohort 2)
3. Low-dose FP-02.2 + adjuvant (IC31®) (Cohort 2)
4. High-dose FP-02.2 + adjuvant (IC31®) (Cohort 3)
5. Placebo (pooled Placebo subjects)
6. IC31® only (pooled IC31® subjects)

10.2 Sample size calculation

No formal sample size calculation has been performed. It is planned to randomise approximately 10 subjects into each of the six treatment arms, which is considered an appropriate study size at this phase of development of Vaccine FP-02.2.

10.3 Statistical analysis plan

The proposed statistical analyses for this study will be detailed in a separate statistical analysis plan (SAP), which will be finalised prior to database lock. Any changes to the statistical analysis described in this protocol do not require a protocol amendment but must be described as a ‘change to the protocol planned statistical analysis’ in the SAP and the clinical study report.

The statistical analysis and report will conform to the relevant ICH requirements.

Data listings and summary tables will be generated using the software SAS© Version 9.3 or later. All data recorded in the eCRFs will be included in data listings. The SAS datasets and programs will be made available to the Sponsor at study closure.

Summary tables and data listings will be presented by treatment arm and time point.

The standard summary statistics for continuous variables will comprise number of valid observations, arithmetic mean, standard deviation, median, quartiles, minimum, and maximum. The geometric mean and coefficient of variation will also be presented for continuous variables which are positive and substantively positively skewed (such as liver enzyme levels, ELISpot counts and measures of viral load). The standard summary statistics for categorical data will comprise absolute and relative frequencies (proportions).

If continuous variables are assessed both at and after baseline, change from baseline will be calculated and summarised. If categorical variables are assessed both at and after baseline, then cross-tabulations will be presented by category at baseline.

10.4 Analysis Sets

Membership of analysis sets will be determined at a blinded data review meeting convened by the Sponsor.

10.4.1 Safety Analysis Set

The safety analysis set will contain all subjects who were randomised and received at least 1 injection of Vaccine FP-02.2, Vaccine FP-02.2/IC31®, placebo or IC31®.

10.4.2 Full Analysis Set (FAS)

The FAS comprises of all members of the safety analysis set with at least one complete post-vaccination immunogenicity result.

10.4.3 Immunogenicity Analysis Set

The immunogenicity analysis set comprises of all members of the FAS with immunogenicity assessments at screening followed by days 1, 8, 29, 36, 57, 64 and 85 and with no major protocol violations. Subjects with missing day 1 PBMC may be included if screening PBMC data are available to calculate a baseline.

10.5 Baseline data

Baseline data will be tabulated using the standard summary statistics over all randomised subjects.

10.6 Procedure(s) to account for missing or spurious data

There will be no imputation for missing data. Summaries will be over all valid cases at that time point in the applicable analysis set.

The nature and extent of missing data or spurious data will be reviewed at a blinded data review meeting.

10.7 Subject Disposition and flow of patients

Subject disposition tabulation will display number of patients screened and randomised, number of subjects in each analysis population, and having completed or withdrawn, as well as reasons for withdrawal/drop-out.

A subject will be considered enrolled at the time of randomisation. Subjects will be considered to have completed the study if they have taken all of the scheduled doses of IMP and have completed all applicable safety assessments and other study-related procedures.

The disposition table will be detailed enough to enable construction of a CONSORT-style flow diagram.

10.8 Study Conduct

10.8.1 IMP and NUC exposure

IMP and NUC exposure will be tabulated using the standard summary statistics.

10.8.2 Protocol Violations

Protocol violations will be listed and tabulated using the standard summary statistics. Violations with the potential for compromising the integrity or interpretation of the study will be referred to as 'major', other violations will be referred to as 'minor'. Whether or not each protocol deviation is categorised as 'major' will be determined at a blinded data review meeting convened by the Sponsor.

10.9 Safety analysis

Safety data in general will be listed and summarised by treatment arm and time point using the standard summary statistics over the safety analysis set.

10.9.1 Liver function

Liver function test results will be presented using the standard summary statistics and shift tables.

10.9.2 Adverse Events

Adverse events shall be coded according to the current version of MedDRA.

The subject incidence, severity, duration, relatedness and outcome of all treatment emergent AEs will be tabulated by treatment arm, system organ class and preferred term. Separate tables will be provided for fatal events, events leading to withdrawal, serious events and SUSARs.

Treatment emergent AEs will be flagged in the subject data listings.

10.9.3 Local tolerability

Injection site tolerability will be tabulated by treatment group, time point, reaction, grade (Section 7.6.1.7).

10.9.4 Vital signs

Vital signs test results will be presented using the standard summary statistics and shift tables.

Listings of safety laboratory results and vital signs will have assessments below and above the reference range highlighted, together with an indicator for clinical significance.

10.9.5 ECG

ECG assessments will be tabulated by treatment arm, time point and whether the ECG trace was normal, abnormal (not clinically significant) and abnormal (clinically significant). 12-lead ECG parameters will be tabulated using the standard summary statistics.

10.9.6 Safety laboratory assessments

Safety laboratory assessments will be presented using the standard summary statistics and shift tables.

Listings of safety laboratory results from local laboratories will have assessments below and above the reference range highlighted, together with an indicator for clinical significance.

10.9.7 Other safety assessments

The Physical Examination will be summarised by treatment arm and time point.

Concomitant medications will be summarised by treatment arm, WHO drug class, generic name and indication.

10.10 Immunogenicity analysis

Immunological outcome variables will be summarised by treatment arm and by time point tabulated using the standard summary statistics over the immunogenicity analysis set.

A detailed description of the planned analysis of each immunogenicity and virological endpoint will be presented in a separate SAP.

10.11 Timing of analysis

After all participants have completed Day 85, the primary analysis of the safety data collected up to Day 85, immunogenicity (except HLA genotyping) and virology data will be performed. A secondary follow-up analysis of the immunology and safety data collected between Day 86 and Day 225 will be conducted. The Investigators, all site staff, the subjects and trial monitors will remain blinded between

the two analyses. Details of the analyses will be documented in the SAP. The information from the primary analysis is required to inform the design of the next clinical study and confirm the formulation and dose to use.

11 DATA HANDLING

11.1 Data collection tools and source document identification

All the information collected during the study will be recorded in the eCRFs, patient diaries or other source documents, if applicable, which are identified by subject number. The Investigator will ensure that the eCRFs are correctly completed.

11.2 Data handling and record keeping

Clinical data will be entered into DataLabs. PRA processes allow adherence to 21 CFR Part 11. DataLabs is part of that process, and is therefore compliant with Part 11. DataLabs includes password protection and internal quality checks, such as range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Data reported in the eCRF, derived from source documents, should be consistent with the source documents or the discrepancies should be explained. Where the data violate validation checks, queries will be generated for resolution by clinical staff. All edits made to the database upon resolution of queries will be recorded in an electronic audit trail.

eCRFs will be used for all patients. The Investigator's data will be accessible from the Investigator's site throughout the trial. eCRFs must be kept current to reflect patient status at each phase during the course of the trial. The eCRF will not capture personalised data. The Investigator must make a separate confidential record of personalised details (name and initials) on the subject identification and screening enrolment log.

It is the responsibility of the PI of the respective site to ensure that all subject discontinuations or changes in study or other medications entered on the subject's eCRF are also made on the subject's medical records.

The eCRFs for any subject leaving the study should be completed at the time of the final visit or shortly thereafter.

11.3 Access to Data

Direct access will be granted to authorised representatives from the Sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

11.4 Archiving

Records and documents pertaining to the conduct of the study and the distribution of the investigational product (e.g. ICFs, eCRFs (if used), laboratory slips, medication inventory records, and other pertinent information) must be retained by the Investigator according to local requirements.

11.5 Clinical study report

After completion of the study, the results will be tabulated, evaluated and issued as a complete final clinical study report according to the ICH-E3 Note for guidance on structure and content of clinical study reports.

The Sponsor will send a summary of this clinical study report to the ECs and CAs within one year after the end of the trial.

12 MONITORING, AUDIT & INSPECTION

The Investigator will permit trial-related monitoring, IEC review, and regulatory inspections, providing direct access to source data/documents. Sponsor-authorised quality assurance personnel may carry out audits for which the Investigator must provide support.

The study will be supervised by a monitor. The study monitor will contact the Investigator regularly to discuss the progress of the study and to check the study documents including the ICFs for completeness and consistency. In regards to an unblinded monitor, the monitor will review the drug delivery information at the pharmacy.

The Sponsor, or delegate, will conduct pre-study and start-up meeting site visits. The study monitor, or a representative of the Sponsor, will cross-check the data entered in the eCRFs with the source data at the study site and observe the study procedures in order to verify adherence to the study protocol. The eCRFs will be checked for completeness and correctness by the monitor and data management department of the CRO and any queries will be resolved by the Investigator. The Sponsor, or delegate, will conduct post study site visits.

13 ETHICAL AND REGULATORY CONSIDERATIONS

13.1 Ethics Committee (EC) review & reports

Favourable opinions from the relevant ECs will be obtained for the trial protocol, ICFs and other relevant documents prior to the start of the study.

The ECs will be notified about the end of the trial by the CI within 90 days of its completion and a report summarising the study results will be sent to the ECs within one year after the end of the trial. If the trial is terminated early, the ECs will be notified within 15 days. Periodic Reports to CAs and ECs will be submitted as required by local regulations.

13.2 Peer review

The protocol has been reviewed by representatives of the Sponsor, the Sponsor's Medical advisor, the Sponsor's Statistician, the CI, and a CRO assigned by the Sponsor.

13.3 Public and Patient Involvement

The public and study participants will not be involved in the research.

13.4 Regulatory Compliance

Prior to the start of the study, appropriate regulatory authority approvals will be obtained, according to local country requirements. This study will be conducted in accordance with the protocol and ethical principles stated in the applicable guidelines on Good Clinical Practice (GCP), and all applicable local and/or regional laws, rules, and regulations.

The Investigator will provide the Sponsor or its designee with documentation of the Institutional Review Board/Independent Ethics Committee approval of the protocol and the informed consent document before the study may begin at the investigative site(s).

13.5 Protocol compliance

The Investigator agrees to conduct the trial in compliance with the protocol. Any amendments should be agreed by both the Sponsor and the CI, with the appropriate written and approved protocol amendments made to reflect the changes agreed upon.

Any deviation from the clinical study protocol in the conduct of the clinical trial will be notified to the monitor on an ongoing basis.

13.6 Notification of Serious Breaches to GCP and/or the protocol

A “serious breach” is a breach which is likely to affect to a significant degree

- the safety or physical or mental integrity of the subjects of the trial; or
- the scientific value of the trial

The Sponsor will be notified immediately of any case where the above definition applies during the trial conduct phase. The Sponsor will notify the licensing authority in writing of any serious breach of the conditions and principles of GCP in connection with that trial; or the protocol relating to that trial, as amended from time to time, within 7 days of becoming aware of that breach.

13.7 Data protection and patient confidentiality

The ICF will incorporate wording that complies with relevant local data protection and privacy legislation. Records containing patient medical information will be handled in accordance with local and national laws, rules, and regulations and consistent with the terms of the patient authorisation contained in the informed consent document for the study.

The Investigators and trial site staff will comply with these requirements with regard to the collection, storage, processing and disclosure of personal information.

For the purposes of the study (recording of data in the eCRF or other documents), personal subject data such as name, birth date or address will be replaced by an identification number (pseudonymous data recording). The Investigator makes and archives a decode list, by means of which the pseudonymous data can be allocated to the subject if necessary in accordance with legal requirements. Only pseudonymous study data may be transferred to the Sponsor or a CRO for the purpose of analysis and to the relevant authorities according to legal requirements.

Authorised representatives from the Sponsor or an institution assigned by the Sponsor and the responsible authorities may review the subjects' personal data stored at the study site as far as this is necessary for the inspection of the proper conduct of the study. All persons involved in the review of personal data will guarantee complete confidentiality.

The study results will be analysed by the Sponsor and may be published in a professional scientific journal. However, in no case can the subject be identified from this data.

13.8 Financial and other competing interests for the CI, PIs at each site and committee members for the overall trial management

The disclosed financial interest of the Investigator must be collected before screening of the first patient, following study completion at the Investigator site and 1 year following overall study completion. The Investigator should promptly update this information if any relevant changes occur during this period.

13.9 Indemnity

In accordance with Statutory Instrument 1031 and amendments section 15 (5i, j) and the EU Clinical Trials Directive 2000/20/EC Article 3 (2f), provision is to be made for:

- The indemnity or compensation in the event of injury or death attributable to the clinical trial; and
- Insurance or indemnity to cover the liability of the Investigator or Sponsor.

Therefore the Sponsor, Altimmune, will indemnify the CI/PI from all and any claims arising out of this study except for those that arise from their negligence or malpractice and providing that the study is conducted according to the standards established by the protocol.

In the event that it can be demonstrated that a subject suffers any significant deterioration in health or well-being or any harmful susceptibility or toxicity as a direct result of their participation in this study then Altimmune will agree to abide by the current Association of the British Pharmaceutical Industry Guidelines with regard to compensation payable to the subject. The amount of compensation will be calculated by reference to the level of damages commonly awarded in local law for similar injuries at the time when such injury occurred.

The Investigator declares to having insurance cover for the malpractice and/or negligence of their employees and agents.

13.10 Amendments

Amendments to this study protocol may be made following the procedures specified by local laws and regulations. Substantial amendments to this study protocol may be implemented only if the approval of the CA and a favourable opinion of the EC(s) have been obtained.

Substantial amendments to the conduct of the clinical trial may arise from changes to the protocol or from new information relating to the scientific documents in support of the trial. Amendments to the trial are regarded as "substantial" where they are likely to have a significant impact on:

- the safety, physical health and mental integrity of the subjects;

- the scientific value of the trial;
- or be otherwise significant.

If a new event occurs related to the conduct of the trial or the development of the investigational product, which may affect the safety of the subjects, the Sponsor and the Investigator will take appropriate safety measures to protect the subjects against any immediate hazard. The Sponsor will immediately inform the CAs and EC(s) of the new events and the measures taken.

13.11 Post-trial care

After the end of the trial, the study participants will return to the care of their treating physician(s).

13.12 Access to the final trial dataset

The original eCRFs and the data generated from the eCRFs or otherwise obtained during the study under this study protocol will become the property of the Sponsor. Access to the final trial dataset will be restricted to the Sponsor or authorised representatives of the Sponsor.

13.13 Authorship eligibility guidelines and any intended use of professional writers

Authorship of scientific publications will be in accordance with the guidelines of the International Committee of Medical Journal Editors. Publication of the results of this study by the Investigator is possible only after written consent has been obtained from the Sponsor. Any material intended for publication will be given to the Sponsor at least 4 weeks before submission for publication. The Sponsor will have the right to comment on the intended publication and to take any reasonable measures for patent protection.

13.14 Ethical conduct of the study

The study will be conducted in accordance with the ethical principles set forth in the Declaration of Helsinki (including amendments). The Sponsor will ensure that the study complies with all local, federal, or country regulatory requirements as applicable.

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