CLINICAL STUDY OF SINGLE STRANDED OLIGONUCLEOTIDE RO7062931 IN HEALTHY VOLUNTEERS AND PATIENTS WITH CHRONIC HEPATITIS B

Supporting Information

Title: Clinical Study of Single Stranded Oligonucleotide RO7062931 in Healthy Volunteers and Patients with Chronic Hepatitis B

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Supplementary Methods

Participants

In Part 1, exclusion criteria included a history of alcohol or other substance abuse in the past 6 months, any major illness within the month before the screening visit, or any febrile illness within the 2 weeks before the screening visit. Volunteers were required to have no evidence of active or chronic disease on the basis of a detailed medical and surgical history, physical examination, vital signs, electrocardiogram, blood chemistry, serology, and urinalysis. Other than hormone replacement therapy, concomitant drug use was not permitted (including herbal remedies, vitamins, fish oils, and protein powders) within 2 weeks before administration of study drug.

In Part 2, a liver biopsy, Fibroscan® or equivalent test obtained within the past 6 months demonstrating liver disease consistent with chronic hepatitis B infection (CHB) without evidence of bridging fibrosis or cirrhosis was required. Any patients with hepatitis A, hepatitis C, HIV, or documented history of hepatitis D infection, active or suspected cancer or a history of malignancy other than adequately treated basal cell carcinoma, or a history or evidence of a medical condition associated with chronic liver disease other than hepatitis B infection were excluded. Patients with abnormal renal function (serum creatinine >upper limit of normal [ULN] or calculate creatinine clearance <70 mL/min) were also excluded, as were those with a history or evidence of alcohol abuse and/or drug abuse within 1 year of randomization. Patients must not have had any significant acute infection or other clinically significant illness within 2 weeks

of randomization.

Safety Assessments

For Part 1 in healthy volunteers, treatment-emergent elevated alanine transaminase (ALT) or aspartate transaminase (AST) >3 × ULN in combination with either total bilirubin >2 × ULN or clinical jaundice was classified as an adverse event (AE). For Part 2 in CHB patients, treatment-emergent ALT >3 × baseline and >3 × ULN in combination with total bilirubin >2 × ULN or clinical jaundice was classified as an AE.

Pharmacokinetic Assessments

For healthy volunteers in Part 1 of the study, plasma samples for analysis of RO7062931 levels and determination of pharmacokinetic (PK) parameters were collected pre-dose, and post-dose at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 18, 24, 30, 36, 48, 72, 96, 120, and 168 hours. Urine collection periods were at 0–4 hours, 4–8 hours, 8–12 hours, and 12–24 hours post-dose.

For CHB patients in Part 2 of the study, plasma samples for analysis of RO7062931 levels and determination of PK parameters were collected pre-dose, and post-dose at 0.5, 1, 2, 4, 6, 8, 24, 168, 336, and 504 hours. Urine collection periods were at 0–4 hours and 4–8 hours after the first dose. To directly compare PK data in healthy volunteers and CHB patients, patient plasma PK assessment time points were used.

Plasma and urine PK parameters included maximum plasma concentration (C_{max}), time to reach maximum plasma concentration (t_{max}), area under the plasma concentration-time curve from time zero to infinity ($AUC_{0\text{-}inf}$), AUC up to 24 hours post-dose ($AUC_{0\text{-}}$

$_{24}$), terminal half-life ($t_{1/2}$), cumulative amount of drug excreted in urine (Ae).

Pharmacodynamic Assessments

Plasma HBV DNA was quantitatively assessed at Cenetron Diagnostics (Austin, USA) using the COBAS® AmpliPrep/COBAS® TaqMan® HBV Test, v2.0 (Roche Diagnostics) with a linear range from 20 IU/mL to 1.7 x 10^8 IU/mL. Circulating HBV RNA was quantified by real-time PCR using the Roche HBV RNA investigational assay (IA) for use on the cobas® 6800/8800 Systems (Roche Diagnostics International Ltd, Rotkreuz, Switzerland). The HBV RNA assay is a quantitative nucleic acid test (LLOQ 10 cp/ml; linearity range 10 to 10^9cp/ml on armored RNA template) to enable the detection and quantification of HBV RNA in EDTA plasma or serum of HBV-infected patients. Serum HBcrAg was quantitatively assessed at DDL Diagnostic Laboratory (Rijswijk, The Netherlands) using the Lumipulse® G HBcrAg assay (Fujirebio) with an LOD of 2.6 $\log_{10}$ U/mL, an LLOQ of 3.0 $\log_{10}$ U/mL and a ULOQ of 7.0 $\log_{10}$ U/mL. Serum levels of HBsAg and HBeAg were quantitatively or qualitatively assessed at Q2 Solutions (Singapore) using their respective Elecsys® assays as described by the manufacturer (Roche Diagnostics): HBsAg II, HBsAg II quant II, HBeAg.

Modelling and Simulation

A mechanistic modelling approach has been developed and implemented to better understand the PK/PD relationship (Supplementary Figure 1A) as well as project longer term effect. With regards to the PK component, the model describes the key processes

that determine plasma, liver, and kidney exposure. It was found that a combination of an empirical 2-compartment model with first-order absorption from the subcutaneous compartment plus the liver and kidney compartment performs best. The liver and kidney are modeled as compartments by fixing their volume to the physiological values for the different species. This makes the model a mix of a physiologically based and empirical model.

The uptake into the kidney is modeled as a first-order process while the uptake in the liver is described by a Vmax, Km model to represent the saturable uptake via the ASGPR. The clearance of the molecule is described by a first-order rate from the central compartment as well as first-order processes from the liver and kidney.

The PK component has then been plugged into the model as driving the effect of the drug in a PD viral kinetic model. Disease parameters such as clearance of HBsAg, production rate, and clearance of hepatocytes have been estimated, showing good agreement with literature values. The drug effect has been added as inhibiting the production rate of HBsAg in an expected liver concentration-dependent manner, this concentration being derived from the PK model.

Simulations could show that the model was able to describe the observed individual time course of HBsAg, the mean time course, the variability in the data (baseline and effect size) with a very good overlap with the observed data as illustrated for 3.0 mg/kg weekly cohort in Supplementary Figure 1B. And, because of the mechanistic component integrating the disease underlying processes, long-term effect could be projected for this similar dosing regimen as illustrated in Supplementary Figure 1C.

Statistical Analysis

In the Power model, a regression model was fitted, in which the log-transformed PK parameter (AUC) was fitted as the response variable and the log-transformed dose, treated as continuous data, was fitted as a fixed effect. Dose-proportional increases in exposure to RO7062931 could be concluded if 90% CIs of the estimated slope of the linear regression of the log-transformed PK variable versus the log-transformed dose were within acceptance ranges.

Supplementary Tables

Supplementary Table 1. Demographics of healthy volunteers in Part 1 of the study.

Characteristics	RO7062931 0.1 mg/kg (n = 8)	RO7062931 0.3 mg/kg (n = 8)	RO7062931 1.0 mg/kg (n = 8)	RO7062931 2.0 mg/kg (n = 8)	RO7062931 3.0 mg/kg (n = 8)	RO7062931 4.0 mg/kg (n = 8)	Placebo (n = 12)
Age, mean (SD) years	30 (12)	29 (11)	34 (11)	30 (14)	25 (18)	23 (4)	32 (9)
Weight, mean (SD) kg	73 (9)	78 (11)	81 (15)	75 (12)	69 (4)	70 (11)	75 (12)
BMI, mean (SD) kg/m^2	24 (3)	23 (3)	26 (3)	23 (3)	23 (2)	23 (2)	24 (3)
Male, n (%)	8 (100)	8 (100)	7 (88)	8 (100)	8 (100)	8 (100)	12 (100)
Ethnicity, n (%)							
Asian	1 (13)	1 (13)	1 (13)	2 (25)	3 (38)	4 (50)	3 (25)
White	4 (50)	5 (63)	6 (75)	5 (63)	4 (50)	4 (50)	9 (75)
Other[a]	3 (37)	2 (25)	1 (13)	1 (13)	1 (12)	0	0

[a]Black or African American, Native Hawaiian or other Pacific Islander, multiple or unknown.
BMI, body mass index.

Supplementary Table 2. Adverse events occurring in ≥5% of healthy volunteers overall in Part 1 of the study.

Patients with ≥ 1 adverse event, n (%)	RO7062931						Placebo (n = 12)
	0.1 mg/kg (n = 8)	0.3 mg/kg (n = 8)	1.0 mg/kg (n = 8)	2.0 mg/kg (n = 8)	3.0 mg/kg (n = 8)	4.0 mg/kg (n = 8)	
Total	6 (75.0)	7 (87.5)	3 (37.5)	6 (75.0)	7 (87.5)	4 (50.0)	7 (58.3)
Mild	6 (75.0)	7 (87.5)	3 (37.5)	6 (75.0)	7 (87.5)	4 (50.0)	7 (58.3)
Moderate	1 (12.5)	0	1 (12.5)	0	0	0	1 (8.3)
Treatment-related	1 (12.5)	0	1 (12.5)	1 (12.5)	4 (50.0)	4 (50.0)	1 (8.3)
Mild	0	0	1 (12.5)	1 (12.5)	4 (50.0)	4 (50.0)	1 (8.3)
Moderate	1 (12.5)	0	0	0	0	0	0
Injection site reaction	0	0	1[a] (12.5)	1[a] (12.5)	4[a] (50.0)	4[a] (50.0)	1[a] (8.3)
URTI	2 (25.0)	1 (12.5)	0	1 (12.5)	0	1 (12.5)	1 (8.3)
Headache	1 (12.5)	2 (25.0)	0	1 (12.5)	0	0	1 (8.3)
Vessel puncture site bruise	2 (25.0)	0	0	0	0	1 (12.5)	0
Vessel puncture site hematoma	0	1 (12.5)	1 (12.5)	0	0	1 (12.5)	0
Back pain	1 (12.5)	0	2 (25.0)	0	0	0	0

[a]Mild treatment-related.
URTI, upper respiratory tract infection.

Supplementary Table 3. RO7062931 plasma and urine pharmacokinetic parameters following single doses in healthy volunteers in Part 1 of the study.

	RO7062931					
	0.1 mg/kg (n = 8)	**0.3 mg/kg (n = 8)**	**1.0 mg/kg (n = 8)**	**2.0 mg/kg (n = 8)**	**3.0 mg/kg (n = 8)**	**4.0 mg/kg (n = 8)**
Plasma PK parameters[a]						
C_{max} (nmol/L)	7.78 (40.2%)	19.53 (28.1%)	41.69 (30.8%)	103.68 (34.0%)	153.53 (25.3%)	312.53 (22.2%)
T_{max} (h)[b]	1.75 (1.00–3.00)	2.00 (1.50–2.00)	3.00 (1.50–6.00)	2.00 (2.00–6.00)	2.00 (2.00–6.00)	3.00 (2.00–6.00)
$AUC_{0–24}$ (h·nmol/L)	38.95 (13.5%)	92.34 (14.0%)	307.81 (13.3%)	778.99 (19.6%)	1240.08 (19.3%)	2417.98 (7.9%)
AUC_{inf} (h·nmol/L)	39.21 (14.0%)	92.72 (14.1%)	308.48 (13.2%)	792.36 (19.1%)	1257.23 (19.4%)	2478.56 (8.7%)
$t_{1/2}$ (h)	3.77 (65.4%)[d]	3.99 (38.3%)[d]	44.32 (170.4%)[d]	97.06 (35.9%)	85.70 (29.7%)	115.24 (60.4%)
Urine PK parameters[c]						
$Ae_{0–8h}$ (nmol)	9.97 (4.88)	26.26 (6.01)	131.02 (64.89)	234.47 (97.16)	467.54 (100.75)	998.87 (399.56)
$Ae_{0–12h}$ (nmol)	11.14 (4.58)	29.20 (7.39)	154.25 (64.12)	282.70 (105.70)	597.62 (82.25)	1217.34 (443.41)
$Ae_{0–24h}$ (nmol)	11.76 (4.59)	30.18 (8.02)	165.25 (62.92)	309.12 (110.48)	651.54 (79.58)	1294.43 (420.83)
$Fe_{0–8h}$ (%)	0.98 (0.52)	0.77 (0.18)	1.17 (0.61)	1.10 (0.50)	1.59 (0.39)	2.49 (0.89)
$Fe_{0–12h}$ (%)	1.08 (0.48)	0.86 (0.26)	1.37 (0.61)	1.32 (0.51)	2.03 (0.34)	3.03 (0.95)
$Fe_{0–24h}$ (%)	1.14 (0.48)	0.89 (0.29)	1.47 (0.60)	1.44 (0.52)	2.22 (0.33)	3.21 (0.87)

[a]Presented as geometric mean (coefficient of variation).
[b]Presented as median (range).

[c]Presented as mean (SD).
[d]Value reported is mixed half-life of distribution and terminal phase.

Ae, cumulative amount excreted unchanged in urine; AUC_{0-24}, area under concentration time curve up to 24 hours post-dose; AUC_{inf}, area under concentration time curve extrapolated to infinity; Fe, fraction of cumulative amount excreted unchanged in urine over total dose; PK, pharmacokinetic; $t_{1/2}$, terminal half-life.

Supplementary Table 4. Pharmacokinetic parameters for RO7062931 in patients with CHB (Part 2 of the study).

	RO7062931 QM dosing (Part 2a)			RO7062931 Q2W or QW dosing (Part 2b)		
Parameter	**0.5 mg/kg QM (n = 6)**	**1.5 mg/kg QM (n = 7)**	**3.0 mg/kg QM (n = 6)**	**3.0 mg/kg QW (n = 14)**	**3.0 mg/kg Q2W (n = 7)**	**4.0 mg/kg QW (n = 4)**
C_{max}[a] (nmol/L)	21.2 (46.4)	72.4 (57.8)	169.6 (43.9)	218.0 (64.4)	158.6 (37.9)	232.5 (10.6)
T_{max}[b] (h)	3.0 (0.5-7.9)	2.0 (2.0-4.0)	2.0 (1.9-8.0)	4.0 (1.9-5.9)	4.0 (1.9-6.0)	2.0 (1.9-2.0)
$AUC_{0–24}$[a] (h·umol/L)	145.9 (28.4)	512.6 (37.3)	1510.8 (30.2)	1858.0 (41.2)	1434.4 (13.6)	2371.7 (15.2)
$R_{acc}AUC_{0–24}$[c]	1.0 (6.4)	1.1 (26.7)	1.0 (38.2)	0.8 (61.9)	1.0 (31.8)	1.0 (23.4)
Ae 0–8h[d] (nmol)	48.4 (22.5)	183.4 (67.5)	587.0 (294.6)	790.5 (402.1)	615.3 (432.8)	1146.0 (177.6)
Fe 0–8h[d] (%)	0.9 (0.5)	1.3 (0.6)	1.9 (0.8)	2.7 (1.7)	2.1 (1.6)	2.9 (0.6)

[a]Geometric mean (coefficient of variation) first dose.
[b]Median (range) first dose.
[c]Geometric mean (coefficient of variation) last dose/first dose.
[d]Arithmetic mean (standard deviation) first dose.

Ae, cumulative amount of drug excreted in urine; $AUC_{0–24}$, area under the plasma concentration-time curve up to 24 hours post-dose; CHB, chronic hepatitis B; Fe, fraction of unchanged drug excreted in urine; Q2W, once every 2 weeks; QM, once monthly; QW, once weekly; $R_{acc}AUC_{0–24}$, accumulation ratio of AUC_{0-24} equals AUC of last dose/AUC of first dose.

Supplementary Table 5. Summary of MMRM Analysis: Change from baseline in HBsAg with RO7062931 versus placebo in patients with CHB (Part 2 of the study).

Change in HbsAg versus placebo, difference in adjusted[a] mean (90% CI) log_{10} IU/mL	**RO7062931 QM dosing (Part 2a)**			**RO7062931 Q2W or QW dosing (Part 2b)**		
	0.5 mg/kg QM (n = 6)	**1.5 mg/kg QM (n = 6)**	**3.0 mg/kg QM (n = 6)**	**3.0 mg/kg QW (n = 14)**	**3.0 mg/kg Q2W (n = 6)**	**4.0 mg/kg QW (n = 4)**
Day 8	-0.01 (-0.13, 0.1)	-0.14 (-0.25, -0.02)	-0.14 (-0.25, -0.02)	-0.07 (-0.16, 0.02)	-0.11 (-0.23, 0)	-0.03 (-0.16, 0.11)
Day 15	-0.03 (-0.14, 0.09)	-0.20 (-0.31, -0.08)	-0.20 (-0.32, -0.09)	-0.22 (-0.31, -0.13)	-0.14 (-0.26, -0.03)	-0.10 (-0.23, 0.04)
Day 22	-0.02 (-0.13, 0.1)	-0.17 (-0.29, -0.06)	-0.18 (-0.3, -0.07)	-0.28 (-0.37, -0.19)	-0.21 (-0.33, -0.09)	-0.09 (-0.22, 0.05)
Day 29	-0.01 (-0.12, 0.11)	-0.13 (-0.25, -0.01)	-0.15 (-0.27, -0.04)	-0.34 (-0.43, -0.25)	-0.19 (-0.31, -0.08)	-0.10 (-0.23, 0.03)
Day 36[b]	-0.05 (-0.17, 0.06)	-0.31 (-0.42, -0.19)	-0.24 (-0.35, -0.12)	-0.40 (-0.49, -0.31)	-0.34 (-0.45, -0.22)	-0.11 (-0.25, 0.02)
Day 43[b]	-0.04 (-0.15, 0.08)	-0.31 (-0.42, -0.19)	-0.25 (-0.36, -0.13)	-0.39 (-0.48, -0.3)	-0.25 (-0.36, -0.13)	-0.13 (-0.26, 0.01)
Day 50[b]	-0.05 (-0.16, 0.07)	-0.22 (-0.34, -0.11)	-0.22 (-0.33, -0.1)	-0.33 (-0.42, -0.24)	-0.21 (-0.33, -0.1)	-0.15 (-0.28, -0.02)
Day 57[b]	-0.02 (-0.14, 0.1)	-0.19 (-0.31, -0.08)	-0.21 (-0.33, -0.09)	-0.28 (-0.37, -0.19)	-0.13 (-0.24, -0.01)	NE
Day 78/85[b]	0.00 (-0.12, 0.11)	-0.06 (-0.18, 0.06)	-0.08 (-0.20, 0.04)	-0.12 (-0.22, -0.03)	-0.06 (-0.17, 0.06)	-0.12 (-0.38, 0.14)

Day 106/113[b]	-0.03 (-0.14, 0.09)	-0.05 (-0.17, 0.07)	-0.02 (-0.14, 0.09)	-0.08 (-0.17, 0.02)	0.00 (-0.11, 0.12)	-0.15 (-0.41, 0.14)

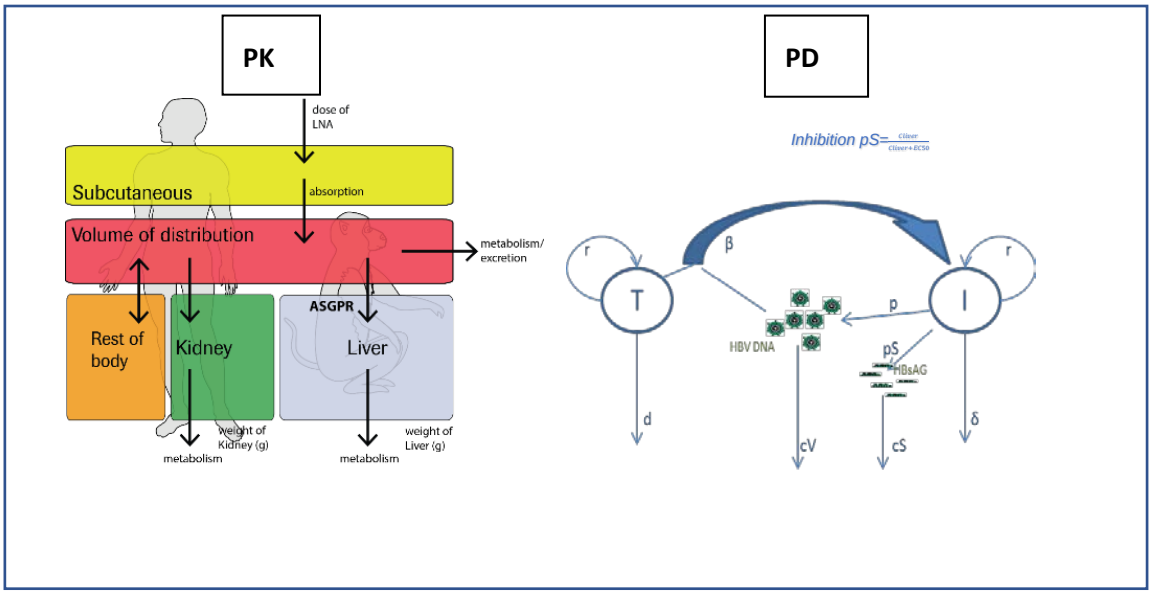
[a]MMRM with treatment, visit, and treatment by visit interaction fitted as fixed effects (between subject effects), baseline HBsAg and baseline body weight as covariates and visit within subject fitted as a random effect (within subject effect).
[b]Post-treatment follow-up.
MMRM, mixed model for repeated measures; NE, not evaluable; Q2W, once every 2 weeks; QM, once monthly; QW, once weekly

Supplementary Figures

Supplementary Fig 1. Mechanistic PK/PD model for simulating HBsAg decline. A) One model for monkey and humans developed based on preclinical and clinical data, including interindividual variability and describing the PK of LNAs. B) Simulation of HBsAg decline for RO7062931 3.0 mg/kg QW, compared to observed data C) Extrapolation of long term effect for RO7062931 3.0 mg/kg QW

LNA, locked nucleic acid; PD, pharmacodynamics; PK, pharmacokinetics; QW, once weekly.

A



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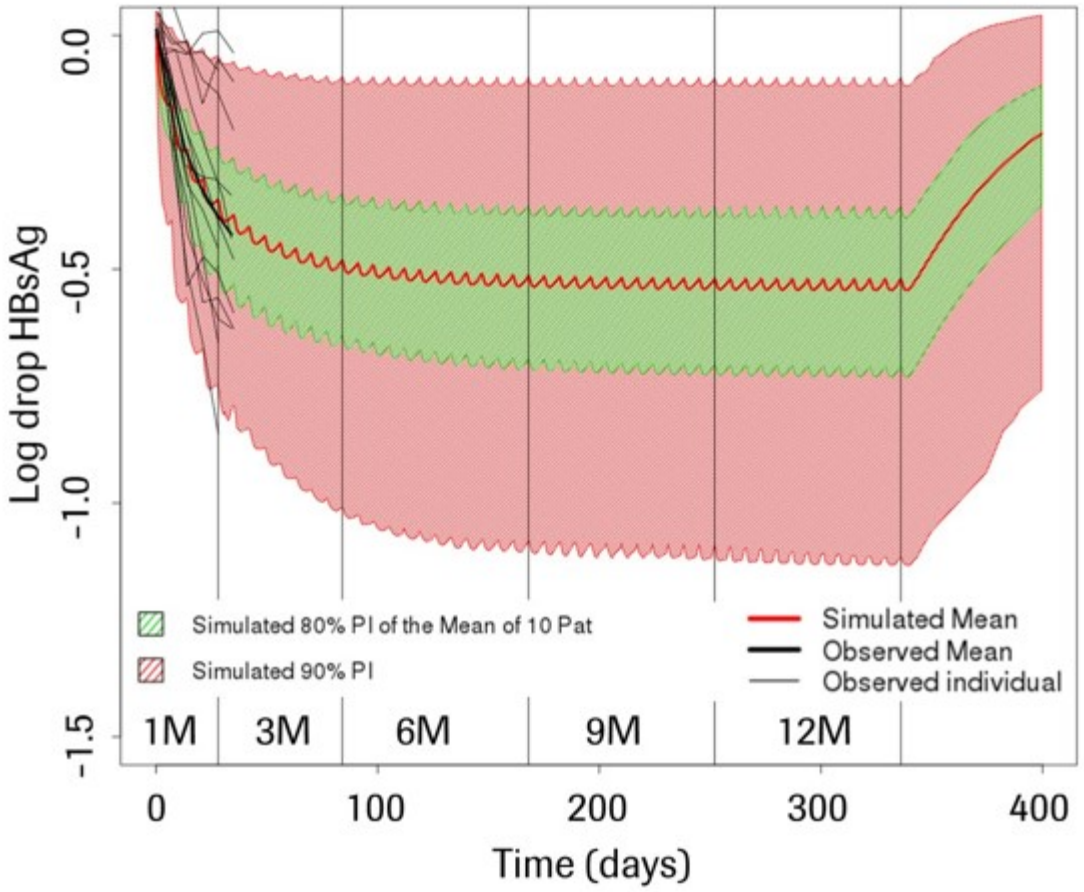
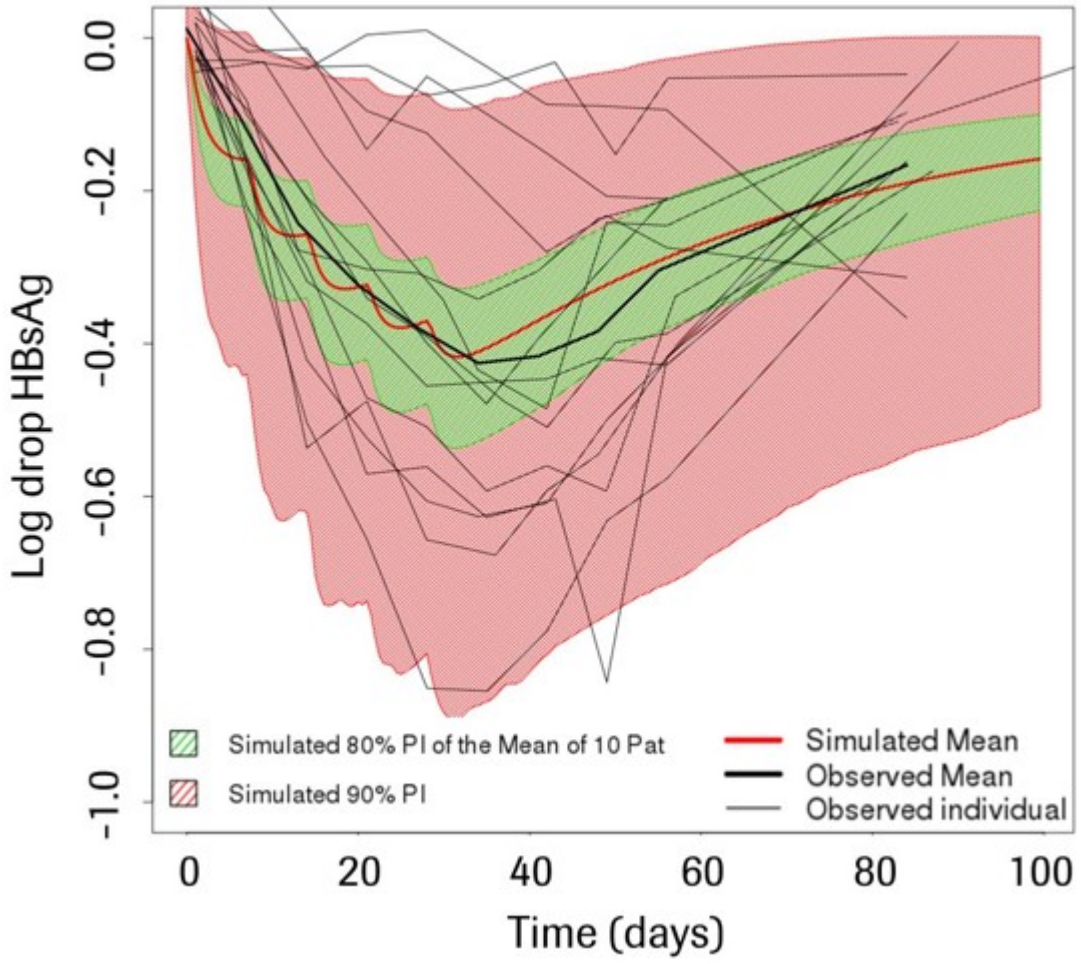

Log drop HBsAg
0.0
-0.5
-1.0
-1.5
Simulated 80% PI of the Mean of 10 Pat
Simulated 90% PI
Simulated Mean
Observed Mean
Observed individual
1M
3M
6M
9M
12M
0
100
200
300
400
Time (days)

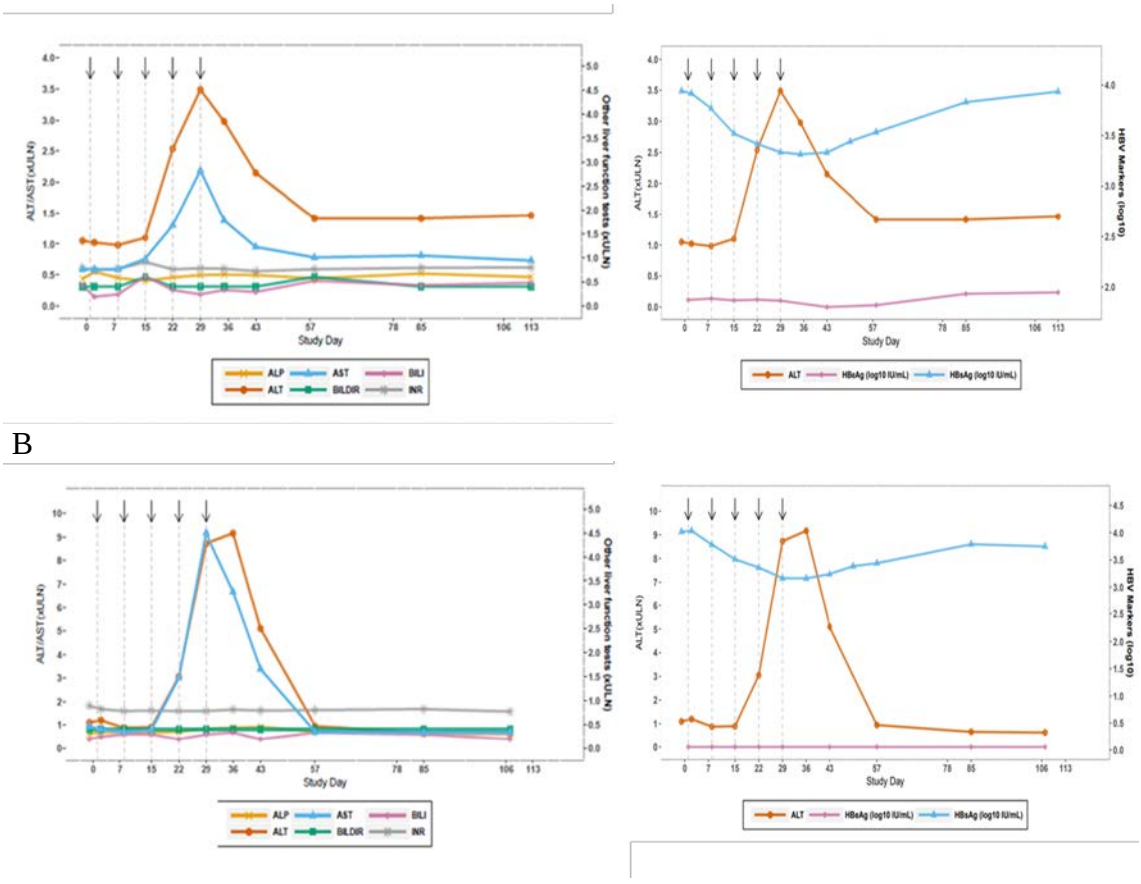
C


Log drop HBsAg
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
0
20
40
60
80
100
Time (days)
Simulated 80% PI of the Mean of 10 Pat
Simulated 90% PI
Simulated Mean
Observed Mean
Observed individual

Supplementary Fig 2. Laboratory profiles in two patients (A and B) with ALT flares (>3 × ULN) who received RO7062931 3.0 mg/kg QW in Part 2b of the study. Left panels illustrate the liver function tests and right panels compare ALT with HBsAg and HBeAg levels.

ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase; BILI, bilirubin; BILDIR, direct bilirubin; INR, international normalized ratio; ULN, upper limit of normal.

A



B

Supplementary Fig 3. Mean change from baseline in viral PD markers: HBV RNA in $\log_{10}$ copies/mL (Panel A) and HBcrAg in $\log_{10}$ U/mL (Panel B) and HBsAg in $\log_{10}$ IU/mL (Panel C)