Citation: Clin Transl Sci (2020) 13, 985–993; doi:10.1111/cts.12791

ARTICLE

A Single and Multiple Ascending Dose Study of Toll-Like Receptor 7 Agonist (RO7020531) in Chinese Healthy Volunteers

Andrea Luk[1,†], Qiudi Jiang[2,*,†], Katerina Glavini[3], Miriam Triyatni[3], Na Zhao[4], Tomas Racek[3], Yonghong Zhu[2] and Joseph F. Grippo[5]

Toll-like receptor 7 (TLR7) agonists modulate broad spectrum immune activity and are evaluated in the treatment of human diseases, including cancer and chronic viral infection. RO7020531, an oral prodrug of a TLR7 agonist, is in clinical development as part of a curative regimen against chronic hepatitis B. We report the safety, tolerability, pharmacokinetics (PKs), and pharmacodynamics (PDs) of RO7020531 in healthy Chinese volunteers following single and multiple ascending doses (SAD and MAD). PK and PD samples were evaluated from four SAD cohorts and 3 MAD cohorts with 10 subjects each (8 active and 2 placebo). Safety and tolerability were monitored throughout the study. A total of 155 adverse events (AEs) were reported in 49 subjects. Fifty-one AEs in 18 subjects were assessed as treatment-related. Most of the AEs were mild; nine subjects experienced moderate AEs; there were no severe AEs. In two 150 mg MAD cohorts given every other day (q.o.d.), 7 of 20 subjects experienced pyrexia and were discontinued due to transient asymptomatic lymphopenia, which resolved 24–48 hours postdose. The PK of the active metabolite, RO7011785, increased linearly with dose from 40 mg to 170 mg. There was no PK accumulation following q.o.d. dosing. The PK profile is consistent with observations in white subjects in the global first-in-human study. SADs and MADs of RO7020531 resulted in dose-dependent increases in TLR7 response markers at 100 mg or above. Flu-like symptoms were associated with higher interferon-α levels. RO7020531 was safe and acceptably tolerated in healthy Chinese volunteers with a multiple 150 mg q.o.d. dose regimen.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ Due to the therapeutic limitations of the currently available agents for the management of hepatitis B virus (HBV) infection, there is a need for new treatments of chronic hepatitis B (CHB) that can provide clinical cure (surface antigen of the hepatitis B virus loss) and sustained suppression of HBV replication. Toll-like receptor 7 (TLR7) agonists induce broad immuno-enhancing effects and may play a role in overcoming the adaptive and innate immune defects in CHB infection.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ The safety, tolerability, pharmacokinetics, and pharmacodynamics of oral RO7020531 (a double prodrug of specific TLR7 agonist) in healthy Chinese volunteers.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ RO7020531 was safe and acceptably tolerated in healthy Chinese volunteers at the doses tested in this study. The study demonstrated clear evidence of target engagement and manageable safety profile. The unique advantage of every other day dosing regimen is novel in the clinical development of all TLR agonists.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
☑ The study results justify further clinical development of RO7020531 in combination with other antiviral agents.

Chronic hepatitis B (CHB) virus infection is a major global healthcare problem, with ~ 240 million now chronically infected, among whom approximately one-third are in China.[1,2] Nearly 25% of all patients with CHB develop serious liver diseases, such as cirrhosis and primary hepatocellular carcinoma. More than 887,000 people die every year due to the consequences of CHB.[2]

CHB infection is defined by the persistence of hepatitis B surface antigen (HBsAg) in the blood > 6 months. The loss of HBsAg followed by seroconversion to anti-hepatitis

[†]Co-first authors.

[1]Phase 1 Clinical Trial Centre, The Chinese University of Hong Kong, Hong Kong SAR, China; [2]Roche Innovation Center Shanghai, Shanghai, China; [3]Roche Innovation Center Basel, Basel, Switzerland; [4]Roche Pharma Development Shanghai, Shanghai, China; [5]Roche Innovation Center New York, New York, New York, USA. *Correspondence: Qiudi Jiang (qiudi.jiang@roche.com)
Received: January 3, 2020; accepted: March 8, 2020. doi:10.1111/cts.12791

B antibody signaled sustained viral control by the cellular and humoral immune response.[3] Therefore, HBsAg loss is regarded as the optimal treatment end point (functional cure) for the new anti-hepatitis B virus (HBV) therapies. The current standard of care (SOC), such as pegylated interferon (PEG-IFN) and nucleos(t)ide analogues (NUCs), effectively suppress viral replication and reduce the risk of CHB sequelae[4–6]; however, the functional cure rates are low (< 3% after 1 year of therapy).[7]

Furthermore, the existing SOCs have other limitations. For example, NUCs require long-term and possibly lifelong therapy as virologic relapse commonly occurs after NUC discontinuation. IFN therapies have common adverse events (AEs) of flu-like symptoms and can be associated with treatment-limiting adverse effects (e.g., neutropenia and thrombocytopenia). Given these limitations, there is an unmet need for novel treatments with finite duration and higher rates of HBsAg loss.[8–11]

In HBV infection, both liver damage and viral control are immune mediated.[12] During acute infection, robust HBV-specific, hepatic CD8 + T cell responses mediates viral clearance from infected hepatocytes via noncytolytic and cytolytic mechanisms.[13] In contrast, chronic infection is associated with lower frequencies and impaired HBV-specific CD8 + T cells that commonly displayed "exhausted" T cell phenotypes. Persistent exposure to high viral antigen load, such as HBsAg, is considered as one of the main drivers of T-cell exhaustion, providing the rationale to include activation of host immune response as part of therapeutic strategies aiming for functional cure.

Two hypotheses currently exist that explain the potential mechanisms by which HBV establishes and maintains chronic infection.[14] First, HBV is a "stealth" virus, which initially establish infection by avoiding host innate immune response.[15] Second, HBV progresses to persistent infection by actively manipulating the host immune responses. There is mounting evidence that host innate immune responses play key roles in limiting adult-acquired HBV infection and that HBV has evolved numerous strategies to counteract these host defense mechanisms.[14]

One promising strategy to stimulate the immune system in CHB is through toll-like receptors (TLRs). TLRs are a family of pathogen-recognition receptors that activate the innate immune response. Stimulation of TLRs triggers the release of multiple cytokines, including type I and type II IFNs, the induction of pathways and enzymes that destroy intracellular pathogens, and the maturation of professional antigen-presenting cells, resulting in the activation of the adaptive immune response.[16] To date, 11 functional TLRs have been identified in humans. Most TLRs are located in the plasma membrane of immune cells, including antigen-presenting cells, except TLR3, TLR7, TLR8, and TLR9, which are intracellularly expressed, particularly in endosomes. TLR7 receptors are able to recognize viral components and induce IFN production and downstream responses.[17]

A number of small molecule agonists for TLR7 have been identified.[18] TLR7 agonism mediates an endogenous type I IFN response, critical in development of a broad, effective, and protective immunity against hepatitis viruses.[18,19] Compared with PEG-IFN therapy, treatment with a TLR7 agonist induces broader immunomodulatory effects that are likely to lead to more effective control of CHB infection.[20,21] TLR7 agonists induce the production of multiple isotypes of IFN from plasmacytoid dendritic cells, which have been shown *in vitro* to possess additive or synergistic antiviral effects compared with exogenous PEG-IFN.

RO7020531, an oral double prodrug of the TLR7-specific agonist, RO7011785, is being developed as combination therapy for CHB. RO7020531 requires *in vivo* metabolic conversion to the active metabolite RO7011785 via hydrolysis by carboxylesterase (mainly CES2) and oxidation by aldehyde oxidase. A prodrug approach was chosen for oral delivery of the active TLR7 agonist RO7011785 in order to improve bioavailability and potentially limit TLR7 activation in the gastrointestinal tract, which may be associated with gastrointestinal intolerability. In a global first-in-human study, single and multiple doses of oral RO7020531 up to 170 mg were safe and generally well-tolerated in healthy volunteers.[22]

In this paper, we summarize the study results of a phase I study, which evaluated the safety, tolerability, pharmacokinetics (PKs), and pharmacodynamics (PDs) of RO7020531 in healthy Chinese volunteers.

METHODS

Seventy healthy Chinese participants aged 18–60 years (inclusive) and with a body mass index between 19 and less than 28 kg/m^2 were enrolled into this single ascending dose (SAD) and multiple ascending dose (MAD) study. This study was conducted at Phase 1 Clinical Trial Centre, The Chinese University of Hong Kong, Hong Kong SAR, China, and approved by the local ethics committee, in compliance with the clinical study protocol, the International Council for Harmonization—Good Clinical Practice, and additional applicable regulatory approvals, including an umbrella Clinical Trial Application approved by the National Medical Products Administration (NMPA) of China. All the participants provided written informed consent prior to any study-related procedures.

Study design

This study was a randomized, sponsor-open, investigator/subject-blinded, placebo-controlled, SAD and MAD study in healthy Chinese volunteers. Four SAD cohorts of 10 healthy volunteers each had been dosed with 40, 100, 140, and 170 mg RO7020531 placebo (8 active and 2 placebo per cohort) and 3 MAD cohorts of 10 healthy volunteers each had been dosed every other day (q.o.d.) for 2 weeks with 100 mg (1 cohort) and 150 mg (2 cohorts, the results are combined) RO7020531 or placebo (8 active and 2 placebo per cohort). Each dose was given in a fasted state. The details about dietary and special requirements during the study were provided in **Listing S1**. Each cohort included a minimum of two women, with at least one woman receiving active drug. PK, PD, safety, and tolerability data collected in the SAD part of this study were used to determine doses at which to initiate the MAD part of the study with the q.o.d. dosing regimen (14 days). The starting dose of 40 mg was selected for the SAD portion based on the safety and tolerability data in the global

first-in-human study.[22] The maximum dose investigated in this study (170 mg) was considered to be safe and with acceptable tolerability in the global first-in-human (EIH) study. The trigger to start the first cohort in the MAD included documentation of adequate safety and tolerability evaluated in the SAD and evidence that at least two subjects exhibited activity above placebo-defined baseline for select TLR7-responsive PD biomarkers (described in PK/PD assessment section). In the current study, PD effects were observed only with doses ≥ 100 mg and good safety and tolerability were shown with single RO7020531 doses up to 170 mg, so 100 mg was chosen as the starting dose in MAD portion. Mean exposures of the active TLR7 agonist at the 170 mg dose are below the PK exposure associated with adverse effects in the monkey good laboratory practice toxicology studies. Nevertheless, for multiple long-term dosing in further phase II clinical trials, a slightly lower dose of 150 mg q.o.d. is planned to be tested and was evaluated first here in the second MAD dose in this study.

Safety assessment

Vital signs, physical examination, electrocardiograms, safety laboratory variables, and AEs were monitored throughout the study. Laboratory measures included hematology, clinical chemistry, coagulation, and urinalysis.

Pharmacokinetic and pharmacodynamic assessments

In each cohort, blood and urine samples (only in SAD portion) for PK determination of RO7020531 and its metabolites were collected predose and at 0–48 hours postdose on day 1 and day 13 (only in MAD portion). Plasma and urine concentrations of RO7020531 and its metabolites were measured by a specific and validated liquid chromatography-tandem mass spectrometry method. Blood samples were analyzed for PD markers, including immunoassays of cytokines/chemokines (IFN-α, TNF-α, IL-12p40, IL-6, IL-10, and IP-10) and neopterin, a catabolic product of guanosine triphosphate, reflective of pro-inflammatory immune status. Samples were analyzed using the Luminex X-MAP Cytokine/Chemokine Magnetic Beads (Millipore, Burlington, MA, USA) kit, Simoa IFN-α Advantage Kit (Quanterix, Billerica, MA, USA) and Brahms Neopterin ELISA kit (Thermo Fisher Scientific, B·R·A·H·M·S GmbH, Hennigsdorf, Germany), respectively. Additionally, whole blood samples for transcriptional analysis (ISG15, OAS1, MX1, and TLR7 mRNAs) were collected predose and at 0–48 hours postdose. Fluidigm targeted gene expression analysis was performed via TaqMan assay panels: ISG15 (Hs01921425_s1), OAS1 (Hs00973637_m1), MX1 (Hs00895608_m1), and TLR7 (Hs01933259_s1) with PPIB (Hs00168719_m1) and GUSB (Hs00939627_m1) for normalization. The details about sampling collection and processing were provided in **Listing S2**. Total 450 mL of blood from each subject was collected throughout the study.

Statistical analysis

This study was designed to evaluate the safety, tolerability, PKs, and PDs of RO7020531 administered as SADs and MADs in healthy Chinese volunteers with descriptive statistics but no formal statistical hypothesis testing conducted.

Ten subjects were enrolled to each cohort (8 active and 2 placebo). Sample size was primarily determined based on practical consideration. With eight subjects per dose cohort treated with RO7020531, there is a 90% chance to observe at least one AE if the underlying event incidence rate is 25% in the subject population.

Safety and tolerability were analyzed in the safety population that included all subjects who received at least one dose of the study medication, and with at least one postbaseline safety assessment. Subjects were grouped according to the actual treatment they received. All subjects who received placebo were combined into a single placebo control group for safety analysis in SAD and MAD cohorts, respectively. The PK analysis population included all subjects who provided sufficient PK data to obtain at least one of the primary PK variables. The PD analysis population included all subjects receiving at least one dose of the study medication and one postbaseline PD assessment.

Descriptive summary of the incidence of AEs and laboratory abnormalities was provided with SAS 9.4 programming (SAS Institute, Cary, NC,USA).

Plasma PK parameters for RO7020531 and its metabolites RO7011785, RO7018822, and RO7033805 included area under the concentration-time curve from time 0 to infinity (AUC_{inf}), peak plasma concentration (C_{max}), terminal half-life ($t_{1/2}$), and time to maximum concentration (T_{max}). Plasma PK parameters were estimated by a standard noncompartmental method using WinNonlin 6.4 (Pharsight Corporation, Mountain View, CA) and presented with summary statistics. The kinetics of renal elimination of RO7011785 following single oral doses of RO7020531 were provided.

Geometric mean of absolute concentrations for IFN-α and geometric mean of fold-change from baseline for all other PD parameters were summarized. A nonparametric regression method, locally weighted scatterplot smoothing (Loess), was applied to investigate the relationship between selected PD variables and RO7011785 AUC_{inf}. To improve the robustness of the fit in the presence of outliers, iterative reweighting was applied. Ninety-five percent confidence intervals for the estimated geometric mean values were provided.

RESULTS

Demographics and baseline characteristics

The treatment groups were well balanced for all characteristics except for sex (**Table S1**).

Pharmacokinetics

These results focus on the PKs of the active TLR 7 agonist, RO7011785. The inactive prodrug metabolite RO7018822 was found at a lower concentration level (< 10% of the level of the active compound) and the parent compound, RO7020531 was undetectable at all dose levels. Minor metabolite RO7033805 were barely detectable in urine and plasma.

Plasma pharmacokinetics following single dose of RO7020531. Following single oral doses of RO7020531, the active metabolite, RO7011785, appeared rapidly in the blood, and reached plasma C_{max} at ~ 0.5–0.75 hours

(median T_{max}, ranging from 0.50 to 1.50 hours postdose) across all SAD dose cohorts. After C_{max}, RO7011785 concentrations declined with mean $t_{1/2}$ in the range of 2.92–4.24 hours across all SAD cohorts (**Table 1**) and almost cleared within 12 hours.

RO7011785 plasma exposure (AUC_{inf} and C_{max}) increased progressively with higher RO7020531 single doses. Linear regression of the dose-exposure relationship for RO7011785, shown in **Figure S1**, suggests that AUC_{inf} and C_{max} of RO7011785 seemed to increase linearly with doses from 40 mg to 170 mg.

Plasma pharmacokinetics following multiple doses of RO7020531. The mean $AUC_{0\text{-}inf}$ and C_{max} of RO7011785 following multiple doses of 100 mg and 150 mg q.o.d. (day 13) were generally comparable to those following a single dose (day 1). However, for the 150 mg q.o.d. group, this observation was based on the data from 8 subjects and such a conclusion may not be the entirely true reflection of the overall study population as another 8 subjects in this dose group discontinued from the study treatment before day 13. Summary statistics for selected RO7011785 PK parameters on days 1 and 13 following dosing with RO7020531 q.o.d. are provided in **Table 2**.

Renal elimination of RO7020531. RO7011785 was predominantly eliminated by the kidneys following oral administration of single doses of 40–170 mg RO7020531 in healthy Chinese volunteers. Approximately 60–67% of the dose of the double prodrug RO7020531 was found in urine as the active metabolite, RO7011785, in all cohorts (**Figure S2**). Mean renal clearance for RO7011785 appeared to be dose-independent and ranged from 536–659 mL/min. The parent drug RO7020531 was undetectable in urine. No urine sample collection was planned in the MAD cohorts.

Pharmacodynamics

In general, single and multiple doses of RO7020531 at ≥ 100 mg result in dose-dependent increases in TLR7 response marker (IFN-α, neopterin, IP-10 and mRNAs of ISG15, OAS1, MX1, and TLR7) in Chinese subjects. Among the protein biomarkers, little or no dose-dependent PD effect was noted for IL-12p40, IL-10, IL-6, or TNF-α (data not shown). These markers are generally associated with TLR8 agonism.[23]

Pharmacodynamics following single dose of RO7020531. Following single oral doses of RO7020531, the geometric mean of serum IFN-α concentrations increased from 100 mg to 140 mg and tended to plateau at the 170 mg dose (**Table 3**). IFN-α concentrations peaked at ~ 6–12 hours postdose, and the responses to IFN-α were observed up to 24 hours postdose in some subjects at the doses of 100 mg or above. The high geometric mean of IFN-α in the placebo group was due to high IFN-α concentrations in two

Table 2 Selected RO7011785 PK parameters following multiple (q.o.d.) RO7020531 doses

Dose (mg)	PK Parameter arithmetic mean ± SD (range) and CV%	Multiple dose (q.o.d.)	
		Day 1	Day 13
100	C_{max} (ng/mL)	1,290 ± 414 (726–2,180) CV% = 32.2	1,100 ± 480 (566–1,740) CV% = 43.6
	AUC_{inf} (ng*hour/mL)	1,880 ± 333 (1,200–2,250) CV% = 17.8	1,660 ± 311 (1,060–2,150) CV% = 18.7
	$t_{1/2}$ (hour)	3.38 ± 0.976 (2.38–5.33) CV% = 28.9	3.52 ± 0.813 (2.20–4.57) CV% = 23.1
150	C_{max} (ng/mL)	1,900 ± 501 (850–2,710) CV% = 26.4	1,530 ± 382 (860–2,110) CV% = 24.9
	AUC_{inf} (ng*hour/mL)	2,660 ± 389 (1,790–3,440) CV% = 14.5	2,510 ± 523 (1,690–3,170) CV% = 20.8
	$t_{1/2}$ (hour)	4.39 ± 0.918 (2.90–6.37) CV% = 20.9	3.85 ± 0.807 (2.68–5.00) CV% = 20.9

The PK exposure in this study are similar to those in global study.[22]
AUC_{inf}, area under the concentration-time curve from time 0 to infinity; C_{max}, maximum plasma concentration observed; CV%, percent coefficient of variation; PK, pharmacokinetic; $t_{1/2}$, terminal half-life.

Table 1 Selected RO7011785 PK parameters following single RO7020531 doses

Dose (mg)	PK parameter, arithmetic mean ± SD (range), and CV%				
	C_{max} (ng/mL)	AUC_{inf} (ng•hour/mL)	$t_{1/2}$ (hour)	A_e (mg)	CL_r (L/hour)
40	640 ± 227 (376–1,090) CV% = 35.5	714 ± 154 (564–1,000) CV% = 21.6	2.92 ± 0.787 (1.81–4.44) CV% = 27.0	22.2 ± 1.96 (20.0–25.0) CV% = 8.8	32.5 ± 6.2 (24.8–40.3) CV% = 19.1
100	1,380 ± 461 (788–2,140) CV% = 33.3	1,670 ± 229 (1,350–1,990) CV% = 13.7	3.38 ± 0.499 (2.62–4.01) CV% = 14.8	58.3 ± 3.27 (48.9–59.1) CV% = 10.8	35.6 ± 4.8 (28.6–43.6) CV% = 13.4
140	1,940 ± 516 (1,170–2,440) CV% = 26.6	2,510 ± 549 (1,790–3,280) CV% = 21.8	3.96 ± 0.761 (2.90–4.88) CV% = 19.2	87.0 ± 10.1 (69.0–88.7) CV% = 11.6	36.1 ± 8.2 (25.8–48.4) CV% = 22.6
170	2,180 ± 841 (1,130–3,370) CV% = 38.6	2,720 ± 467 (1,940–3,180) CV% = 17.2	4.24 ± 0.667 (3.38–5.16) CV% = 15.7	104 ± 14.9 (73.5–110) CV% = 14.9	39.6 ± 9.5 (31.6–58.1) CV% = 24.0

A_e, cumulative amount recovered in the urine; AUC_{inf}, area under the concentration-time curve from time 0 to infinity; C_{max}, maximum plasma concentration observed; CL_r, renal clearance; CV%, percent coefficient of variation; PK, pharmacokinetic; $t_{1/2}$, terminal half-life

Table 3 Descriptive statistics of PD response following single doses of RO7020531

		Geometric mean (min–max)						
		Concentration (pg/mL)	Fold change from baseline					
Dose (mg)	*n*	IFN-α	IP10	Neopterin	ISG15	OAS1	MX1	TLR7
Placebo	8	0.123 (0.043–37.280)	1.11 (0.59–2.09)	1.08 (0.74– 1.77)	1.05 (0.10–2.47)	1.07 (0.24– 1.95)	1.06 (0.16–2.54)	1.09 (0.26–2.60)
40	8	0.043 (0.043–0.047)	0.95 (0.65–1.55)	1.02 (0.76–1.29)	1.09 (0.38–2.26)	1.13 (0.69–1.72)	1.17 (0.51–1.85)	1.16 (0.59–2.30)
100	8	0.071 (0.043–0.084)	1.00 (0.27–4.64)	1.04 (0.62–1.78)	1.92 (0.40–16.97)	1.61 (0.45–8.12)	2.20 (0.54–12.95)	0.99 (0.47–3.21)
140	8	0.132 (0.043–3.722)	1.53 (0.57–7.89)	1.16 (0.81–3.00)	4.08 (0.27–119.20)	2.93 (0.27–18.98)	3.87 (0.30–46.89)	2.08 (0.60–11.52)
170	8	0.116 (0.043–2.406)	1.62 (0.61–25.96)	1.21 (0.82–3.56)	4.32 (0.52–49.79)	3.07 (0.91–21.05)	4.37 (0.78–46.52)	1.39 (0.30–4.58)

subjects: one had extremely high IFN-α concentration at baseline, whereas no AE was reported, and the other one had increased serum IFN-α postbaseline, possibly due to pyrexia on study days 1–3. The IFN-α concentrations exhibited high intersubject variability by TLR7 agonism.

After single oral doses of RO7020531 at ≥ 100 mg, concentrations of IP-10 demonstrated clear dose-dependent increases, whereas neopterin only showed modest increase compared with the baseline (the maximum fold change from baseline was only twofold vs. placebo). IP-10 exhibited a peak response at ~ 6–12 hours postdose and remained above background for up to 24 hours following the single dose of RO7020531. The neopterin response peak appeared at 24–48 hours postdose and the response was persistent at the last observation time point.

The expression of mRNA PD markers, including ISG15, OAS1, and MX1 exhibited dose-dependent increases at the single dose of 100 mg or above, with high intersubject variability. The expression changes of TLR7 transcript were relatively modest compared with the other genes evaluated. Mean responses for TLR7, ISG15, OAS1, and MX1 mRNA seemed to peak at ~ 12 hours post-RO7020531 dose, and the response remained above background until 24 hours postdose.

Geometric mean of IFN-α concentrations and geometric mean fold change from baseline of IP10, neopterin, and mRNA species, including ISG15, OAS1, MX1, and TLR7 for each dose are shown in **Table 3**.

Pharmacodynamics following multiple doses of RO7020531. Following multiple oral doses of RO7020531, the 150 mg q.o.d. doses induced higher geometric mean concentration of IFN-α and geometric fold changes from baselines of IP-10, neopterin, and mRNA expressions of ISG15, OAS1, MX1, and TLR7 mRNAs than those from 100 mg q.o.d. doses at each time point over 2-week dosing period (**Table 4**). These PD responses seemed to reach their maximum values after the second dose at 100 mg/150 q.o.d. and the response was maintained with subsequent doses (**Figures 1** and **2**, the examples of IFN-α and ISG15). Eight subjects (50%) in the 150 mg q.o.d. cohort discontinued/withdrew from the planned study drug administration, hence, the change of geometric mean of these PD biomarkers over time may not reflect the overall trend in the study population. The intersubject variability of these biomarkers was relatively high over the 2-week dose period compared with that after single dose (**Table 4**).

Safety. A total of 155 AEs were reported in 49 of 70 subjects (70%): 39 AEs in 20 subjects (50%) in SAD cohorts and 116 AEs in 29 subjects (96.7%) in MAD cohorts. Fifty-one AEs were assessed as related to study drug: 7 AEs in 4 subjects in SAD cohorts and 44 AEs in 14 subjects in MAD cohorts. Most of the AEs were of mild intensity; AEs with moderate intensity were reported in SAD (2 subjects) and MAD (7 subjects) cohorts.

An overview of AEs for SAD and MAD cohorts are provided in **Tables S2** and **S3**, respectively.

The most common AEs (reported by ≥ 3 subjects) in SAD cohorts were catheter-site bruise (12.5%), fatigue,

Table 4 Descriptive statistics of PD response following multiple doses of RO7020531

		Geometric mean (min–max)						
		Concentration (pg/mL)	Fold change from baseline					
Dose (mg)	*n*	IFN-α	IP10	Neopterin	ISG15	OAS1	MX1	TLR7
Placebo	6	0.046 (0.043–0.156)	0.809 (0.191–2.08)	0.900 (0.278–1.75)	0.694 (0.043–18.5)	0.632 (0.065–3.92)	0.741 (0.066–6.96)	0.848 (0.275–1.71)
100	8	0.134 (0.043–16.380)	1.40 (0.545–21.2)	1.20 (0.754–3.07)	2.07 (0.284–66.5)	1.94 (0.400–30.9)	1.83 (0.312–30.0)	1.49 (0.442–6.57)
150	16	0.373 (0.043–97.600)	2.16 (0.374–49.9)	1.68 (0.445–7.80)	4.27 (0.108–285)	3.13 (0.242–84.5)	3.02 (0.183–106)	1.54 (0.243–17.4)

and headache (7.5%) (**Table S4**). The most common AEs in MAD cohorts were pyrexia, headache (26.7%), lymphopenia (23.3%), fatigue, aphthous ulcer, oropharyngeal pain (16.7%), catheter-site pain, epistaxis, dizziness (13.3%), diarrhea, dry mouth, somnolence, palpitations, musculoskeletal chest pain, upper respiratory tract infection, and contusion (10.0%; **Table S5**).

A serious adverse event of hand fracture was due to hospitalization for one night in a subject in SAD cohort 170 mg dose group; this serious adverse event was unrelated to study drug.

There were 7 discontinuations due to AEs and a withdrawal from study drug administration by one subject, all of which occurred in the MAD 150 mg dose cohort.

In SAD cohorts with 40, 100, and 140 mg and MAD cohort with 100 mg RO7020531 dose, there were no clusters of AEs related to PD effects expected with TLR7 agonist exposure (i.e., flu-like symptoms or pyrexia). Nine subjects experienced such clusters of AEs (1 subject from the 170 mg dose SAD cohort and 8 subjects from the 150 mg dose MAD cohorts). These 8 subjects from the 150 mg dose MAD cohorts experienced pyrexia between 5.5 and 12 hours after the first, second, or third dose, which resolved within 24–48 hours with paracetamol. Other AEs reported by these subjects included: headache, fatigue (in 3 of 8 subjects), influenza-like illness (in 2of 8 subjects), chills, somnolence, nasal congestion, myalgia, asthenia, peripheral coldness, sinus tachycardia, tachycardia, nausea, aphthous ulcer, dizziness, dry mouth, abdominal pain, abdominal distension, and rectal hemorrhage (each AE occurred in 1 of 8 subjects). Unscheduled laboratory test performed at the time of pyrexia revealed that seven subjects had lymphopenia (grade 1–4; grading according to Division of Acquired Immunodeficiency Syndrome). The investigator decided to stop further administration of RO7020531 to these subjects. The lymphopenia was resolved within 24–48 hours postdose in all subjects.

Other than pyrexia, there were no clinically significant changes in vital signs or patterns in other safety parameters (electrocardiograms, urinalysis, and other safety laboratory tests).

There were no deaths during the study.

Pharmacokinetics/pharmacodynamics/safety relationship. The levels of IFN-α, IP10, neopterin, ISG15, MX1, OAS1, and TLR7 increased with increasing RO7011785 exposure (AUC_{inf}; **Figures S3–S5**). Neopterin response increased more slowly and modestly than IP10 response. For transcriptional markers, TLR7 response increased more slowly than that for the other three genes (ISG15, OAS1, and MX1). No clear difference in these PD biomarkers was found between male and female subjects (**Figures S3–S5**). However, clear interpretation of PK/PD relationships is difficult due to the high variability of these PD biomarker responses.

A PK/PD comparison between subjects experiencing common AEs of flu-like symptoms and those without these AEs was performed. **Table 5** is the summary of the mean RO7011785 exposure, geometric mean of maximum IFN-α concentrations, and geometric mean of maximum fold change from baseline of IP10 following 150 mg q.o.d.

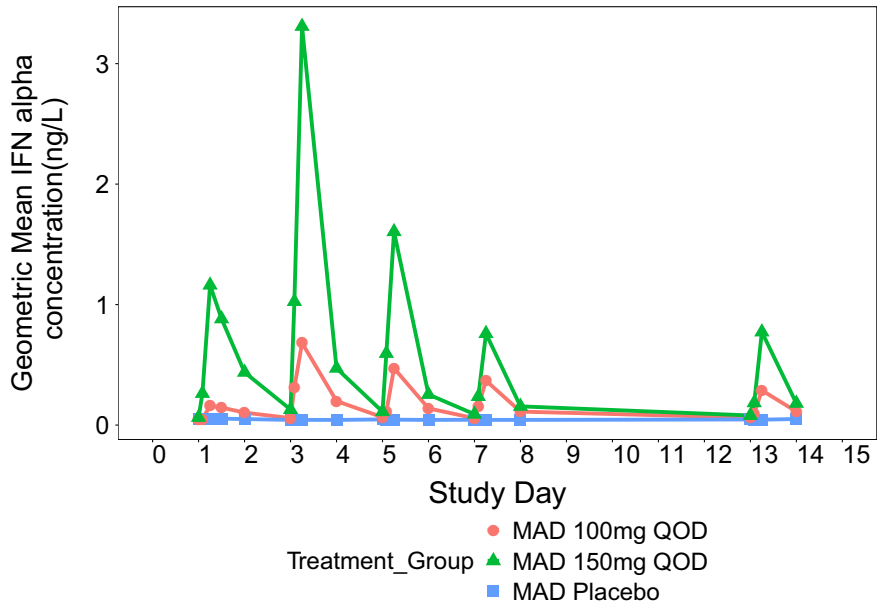

Figure 1 Geometric mean interferon (IFN)α concentrations (ng/L) vs. time following multiple ascending doses (MADs) of RO7020531.

of RO7020531 in the subjects with or without flu-like symptoms.

Although the individual AUC_{inf} values exhibited high overlap between subjects with flu-like symptoms and those without flu-like symptoms, the mean RO7018785 AUC_{inf} was ~ 15% higher in the subjects with flu-like symptoms. In addition, the geometric mean of maximum IFN-α concentration and geometric mean of the maximum fold change from baseline of IP10 in these subjects are higher than those without flu-like symptoms. These results suggest that the higher PD response is associated with increased probability of flu-like symptoms occurrence.

DISCUSSION

This phase I study aimed to characterize the safety, tolerability PKs, and PDs of the TLR7 agonist, RO7020531, in healthy Chinese volunteers, and to bridge the PK/PD results in global EIH study. Oral RO7020531 was safe and acceptably tolerated after single and multiple q.o.d. doses triggering TLR7 agonism. Prior to the start of this study, a EIH global study has been initiated that evaluated single and multiple q.o.d. doses of RO7020531 covering a wider range of doses up to 170 mg, primarily in white healthy volunteers.[22] Dose escalation was not designed in this study to examine the maximum tolerated

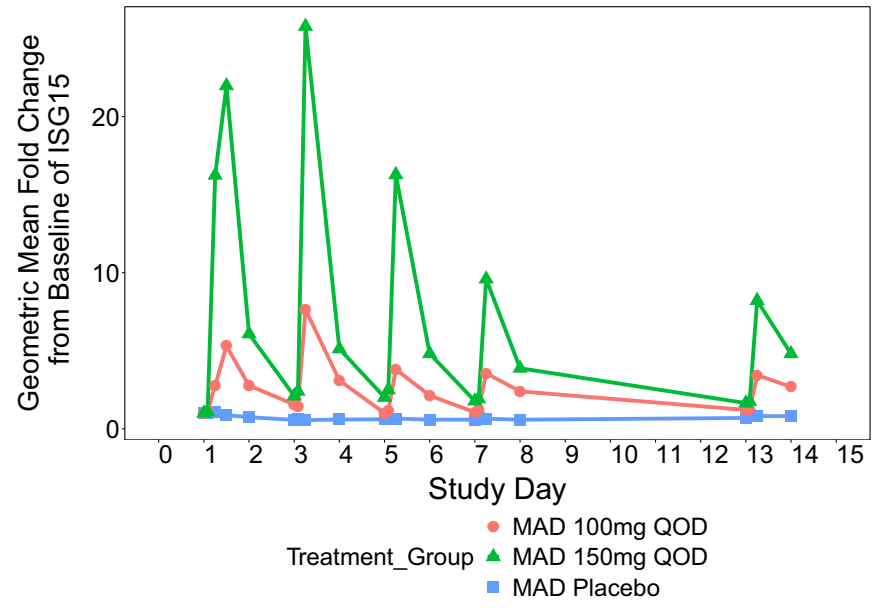

Figure 2 Geometric mean fold change from baseline of ISG15 vs. time following multiple ascending doses (MADs) of RO7020531.

Table 5 Summary of mean RO7011785 exposure, geometric mean of maximum IFN-α concentration and geometric mean fold change from baseline of IP10 following 150 mg q.o.d. of RO7020531

Subjects experiencing flu-like symptoms	*N*	AUC_{inf} on day 1 (ng*hour/mL, arithmetic mean ± SD)	Geometric mean of maximum IFN-α[a] (pg/mL, min–max)	Geometric mean of maximum fold change from baseline of IP10[a] (min–max)
Yes	8	2,840 ± 312	26.7 (1.98–97.6)	23.6 (5.63–49.9)
No	8	2,478 ± 388	2.34 (0.57–14.5)	4.10 (1.49–12.5)
Placebo	6	n/a	0.06 (0.043–0.156)	1.40 (1.07–2.09)

[a]Maximum IFN-α concentration and IP-10 fold change from baseline measured over 2-week interval for the individual subject.

dose in humans. Rather, doses were escalated in the SAD and MAD portions of the study to generate mean exposures (AUC) of the active TLR7 agonist, RO7011785, that did not exceed the PK exposure associated with adverse effects in the monkey good laboratory practice toxicology studies. To this end, the maximum single dose evaluated was 170 mg. As mentioned earlier, a slightly lower dose of 150 mg q.o.d. is planned for subsequent clinical trial investigation to maintain the best relationship between safety and efficacy. The individual and mean RO7011785 exposure (C_{max} and AUC_{inf}) in healthy Chinese volunteers in the current study are consistent with exposure found in healthy volunteers from the global EIH study across all SAD cohorts covering a similar RO7020531 dose range, in which the majority of the healthy volunteers were white[22] This EIH global study also has evaluated multiple q.o.d. doses in CHB patients for safety, tolerability, and PDs (study is ongoing and select data from the patient has been reported).[24]

Current concept of combination treatment focuses on that direct-acting antiviral agents and immune modulators may be the best approach to achieve functional cure in patients with CHB.[25] Such combination treatments will likely be evaluated in patients with CHB who are currently undergoing treatment with SOC (nucleoside/nucleotide analogs; e.g., tenofovir and entecavir). These antiviral agents are typically eliminated by active renal transport utilizing the OAT system[26] and it is important to understand how the TLR7 agonist may be eliminated. In the current study, RO7011785 was eliminated by the kidneys with urinary recovery of unchanged drug of ~ 60–67% of the administered dose across all single dose levels. *In vitro* studies with transporters indicated renal transporter inhibition (mainly for OAT1 and OAT3 inhibition) by RO7011785 was weak,[27] suggesting that the potential interaction with drugs eliminated by renal transport, such as entecavir and tenofovir, is low.

Evaluation of TLR7 agonists in monotherapy or in combination with nucleos(t)ides has not resulted in significant HBsAg decline in patients with CHB (Roche study with previous TLR7 molecule RO6864018[28] and Gilead GS-9620[29,30]). However, preclinical work in mouse models have suggested that combination of TLR7 agonists with other direct acting antivirals may promote significant HBsAg decline.[31] Although the translatability of this mouse data to humans has to be demonstrated, combinations of direct-acting antivirals with immune modulators like TLR7 agonists represent a plausible approach for HBV cure. The key issue for phase I is how to define the best dose and regimen of TLR7 agonist. One underlying hypothesis is to choose the safe dose that demonstrates PD activity in the majority of subjects in a cohort. This approach was evaluated in the current study. In general, single and multiple doses of RO7020531 result in dose-dependent increases in TLR7 response markers, including IFN-α, IP10, neopterin, and mRNAs of ISG15, OAS1, MX1, and TLR7, consistent with activation of the TLR7 pathway. PD data from this study and from the global study,[22,32] suggest that no TLR7 activation is found with doses lower than 100 mg and at that dose only modest activity is found. Although no direct comparison of PD profile has been performed between healthy Chinese volunteers in the current study and white healthy volunteers in the global EIH study, an exploratory PD and PK/PD analyses in a previous clinical trial with another structurally related TLR7 agonist RO6864018 did not show significant differences in the overall PD profile between Asian and white healthy volunteers with some biomarkers (IFN-α, neopterin, MX1, and OAS1) exhibiting higher responses in Asians.[33]

Based on the presented data, a dose of 150 mg in combination treatment would be a reasonable choice for further development in patients with CHB.

During the 2-week dosing period of RO7020531, there was an increase in PD response with the second dose and the response was maintained with subsequent doses. This may be explained by TLR7-response priming (i.e., increase in PD effects with the second dose and tachyphylaxis; reduced response with chronic and frequent doses) with the q.o.d. schedule. A similar phenomenon was also observed in the previous clinical trial in another TLR7 agonist RO6864018 (manuscript in preparation). The q.o.d. dose schedule provides additional priming of the TLR7 response and is considered a dose regimen that may optimize the benefit-risk ratio for HBV treatment.[24]

In the current study, greater PD responses (IFN-α and IP10) were observed in subjects with flu-like symptoms compared with those without flu-like symptoms, although no significant differences in PK exposures were observed between subjects with flu-like symptoms vs. those without flu-like symptoms. These results suggest that the sensitivity of PD activation to TLR7 agonist may be individually different.

In regard to clinical safety, the majority of the moderate AEs, 13 of 15, occurred in the 150 mg dose cohorts. At this dose, seven subjects reported pyrexia and other clusters of AEs collectively considered flu-like symptoms. Unscheduled laboratory tests performed at the time of pyrexia revealed asymptomatic lymphopenia and all seven subjects were discontinued from further dosing by the investigator, not due to mild-to-moderate pyrexia/flu-like symptoms reported by the subjects. Lymphocyte counts returned to normal ranges within 24–48 hours in all subjects. The transient nature of lymphopenia suggest that this effect is unlikely due to myelosuppression. In mice, TLR agonism and administration of IFN-α have been shown to trigger transient lymphopenia due to redistribution of lymphocytes to secondary lymphoid

organs, and this effect is mediated via IFN-α signaling.[34] Of note, in the global study, several subjects who received multiple 150 mg q.o.d. doses of RO7020531 experienced flu-like symptoms and no lymphopenia was observed during weekly laboratory tests (no unscheduled laboratory test at the time of pyrexia was conducted).[24] In both global and Chinese studies, all subjects with flu-like symptoms experienced AEs after the first, second, or the third dose. In most cases, flu-like symptoms are attenuated (milder) or disappear with the subsequent doses, consistent with the IFN-α profiles (tachyphylaxis effect). Therefore, as a mitigating strategy, an enhanced safety monitoring plans after each dose during the first week of dosing are currently implemented for RO7020531 related trials.

Flu-like symptoms are part of the expected and known safety profile of therapeutic interferons and are consistent with their mechanism of action. Therefore, these AEs are also expected for TLR7 agonists at the doses inducing serum interferon. In the case of RO7020531, this dose was ≥ 100 mg, as confirmed by this study and the global EIH study, NP39305. Similar types of AEs have been reported with other TLR7 agonists.

CONCLUSION

Oral RO7020531 was safe and acceptably tolerated in healthy Chinese volunteers at single doses of up to 170 mg and multiple doses of up to 150 mg q.o.d. RO7011785 exposure correlated with an increase in biomarkers of TLR-7 pathway activation at doses of 100 mg and the higher. PD response is associated with increased probability of flu-like symptoms occurrence.

Supporting Information. Supplementary information accompanies this paper on the *Clinical and Translational Science* website (www.cts-journal.com).

Acknowledgments. The authors thank all healthy volunteers and their families for participation in this trial; and the Roche and Covance employees who contributed to the subject enrolment, study conduct, and analysis.

Funding. The study was funded by F. Hoffmann-La Roche Ltd.

Conflict of Interest. A.L. has no conflicts of interest regarding the contents of this article to disclose; Q.J., K.G., M.T., N.Z., T.R., Y.Z., and J.F.G. are employees of F. Hoffmann-La Roche and have no additional competing interests for this work.

Author Contributions. Q.J., J.G., M.T., K.G., and Y.Z. wrote the manuscript. Q.J. and J.G. designed the research. A.L. performed the research. N.Z., T.R., and Q.J. analyzed the data.

1. World Health Organization. Hepatitis B. Global Alert and Response (GAR) (2002) <https://www.who.int/csr/resources/publications/hepatitis/WHO_CDS_CSR_LYO_2002_2/en/>.
2. World Health Organization. Hepatitis B fact sheet. Updated July 2018 <https://www.who.int/en/news-room/fact-sheets/detail/hepatitis-b>.
3. Heim, K., Neumann-Haefelin, C., Thimme, R. & Hofmann, M. Heterogeneity of HBV-specific CD8+ T-cell failure: implications for immunotherapy. *Front. Immunol.* **10**, 2240 (2019).
4. Papatheodoridis, G. *et al.* EASL clinical practice guidelines: management of chronic hepatitis B virus infection. *J. Hepatol.* **57**, 167–185 (2012).
5. Sarin, S.K., *et al.* Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. *Hepatol. Int.* **10**, 1–98 (2016).
6. Terrault, N.A. *et al.* AASLD guidelines for treatment of chronic hepatitis B. *Hepatol.* **63**, 261–283 (2016).
7. European Association for the Study of the Liver. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. *J. Hepatol.* **67**, 370–398 (2017).
8. Liu, J. *et al.* Advancing the regulatory path on hepatitis B virus treatment and curative research: stakeholders consultation. *J. Virus Erad.* **3**, 1–6 (2017).
9. Durantel, D. & Zoulim, F. New antiviral targets for innovative treatment concepts for hepatitis B virus and hepatitis delta virus. *J. Hepatol.* **64**, S117–S131 (2016).
10. Lok, A.S. *et al.* Hepatitis B cure: from discovery to regulatory approval. *J. Hepatol.* **67**, 847–861 (2017).
11. Wang, X.Y. & Chen, H.S. Emerging antivirals for the treatment of hepatitis B. *World J. Gastroenterol.* **20**, 7707–7717 (2014).
12. Trepo, C., Chan, H.L. & Lok, A. Hepatitis B virus infection. *Lancet* **384**, 2053–2063 (2014).
13. Thimme, R. *et al.* CD8_T Cells mediate viral clearance and disease pathogenesis during acute hepatitis B virus infection. *J. Virol.* **77**, 68–76 (2003).
14. Revill, P. & Yuan, Z. New insights into how HBV manipulates the innate immune response to establish acute and persistent infection. *Antiviral Ther.* **18**, 1–15 (2013).
15. Wieland, S.F. & Chisari, F.V. Stealth and cunning: hepatitis B and hepatitis C viruses. *J. Virol.* **79**, 9369–9380 (2005).
16. Iwasaki, A. & Medzhitov, R. Toll-like receptor control of the adaptive immune responses. *Nat. Immunol.* **5**, 987–995 (2004).
17. Lester, S.N. & Li, K. Toll-like receptors in antiviral innate immunity. *J. Mol. Biol.* **426**, 1246–1264 (2014).
18. Horscroft, N.J., Pryde, D.C. & Bright, H. Antiviral applications of toll-like receptor agonists. *J. Antimicrob. Chemother.* **67**, 789–801 (2012).
19. Funk, E., Kottilil, S. & Gilliam, B. Tickling the TLR7 to cure viral hepatitis. *J. Transl. Med.* **12**, 129 (2014).
20. Strader, D.B. *et al.* Diagnosis, management and treatment of hepatitis C. *Hepatology* **39**, 1147–1171 (2004).
21. Isogawa, M. *et al.* Toll-like receptor signaling inhibits hepatitis B virus replication in vivo. *J. Virol.* **79**, 7269–7272 (2005).
22. Gane, E. *et al.* RO7020531, a novel prodrug of a toll-like receptor 7 agonist, is safe, well tolerated and activates TLR signalling in healthy volunteers. EASL 2018; poster FRI-337.
23. Gorden, K.B. *et al.* Synthetic TLR agonists reveal functional differences between human TLR7 and TLR8. *J. Immunol.* **174**, 1259–1268 (2005).
24. Yuen, M.F.*et al.* Safety, pharmacokinetic, pharmacodynamic and viral data after 6-weeks of dosing with TLR7 agonist RO7020531 in chronic hepatitis B patients. AASLD 2019; abstract 0692 (2018).
25. Lok, A. *et al.* Hepatitis B cure: from discovery to regulatory approval. *J. Hepatol.* **67**, 847–861 (2017).
26. Bifano, M. *et al.* Absence of a pharmacokinetic interaction between entecavir and adefovir. *J. Clin. Pharmacol.* **47**, 1327–1334 (2007).
27. Investigator's Brochure, RO7020531, fourth version, June 2019.
28. Investigator's Brochure, RO6864018, eighth version, June 2016.
29. Agarwal, K. *et al.* Safety and efficacy of vesatolimod (GS-9620) in patients with chronic hepatitis B who are not currently on antiviral treatment. *J. Viral. Hepat.* **25**, 1331–1340 (2018).
30. Janssen, H.L.A. Safety, efficacy and pharmacodynamics of vesatolimod (GS-9620) in virally suppressed patients with chronic hepatitis B. *J. Hepatol.* **68**, 431–440 (2018).
31. Dai, L.*et al.*Combination treatment of a TLR7 agonist RO7020531 and a capsid assembly modulator RO7049389 achieved sustainable viral load suppression and HBsAg loss in an AAV-HBV mouse model. EASL 2018; abstract PS-028.
32. Luk, A. *et al.* A single and multiple ascending dose study of toll-like receptor 7 (TLR 7) agonist (RO7020531) in Chinese healthy volunteers. APASL 2019; abstract 188.
33. Kamphuis, E., Junt, T., Waibler, Z., Forster, R. & Kalinke, U. Type I interferons directly regulate lymphocyte recirculation and cause transient blood lymphopenia. *Blood* **108**, 3253–3261 (2006).
34. Jiang, Q., *et al.* Ascending dose pharmacokinetic (PK)/pharmacodynamic (PD) bridging study of toll-like receptor 7 (TLR 7) agonist (RO6864018) in Asian and Caucasian healthy subjects. APASL 2017; abstract OP-117.