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Research Article

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Mechanistic PK/PD Modeling of In Vivo Dose–Exposure–Response and Antiviral Persistence in HBV siRNA Therapy

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Abstract

Small-interfering RNA (siRNA) therapies can produce antiviral effects that persist beyond the dosing interval, but the quantitative relationship among administered dose, tissue exposure, endpoint-specific potency, and response duration is difficult to determine directly in vivo. Here, we developed a mechanistic pharmacokinetic/pharmacodynamic (PK/PD) model for the HBV-targeting siRNA KC13-M2G2 using published longitudinal mouse data obtained after three weekly subcutaneous doses of 0.3, 1, and 3 mg/kg. The model links subcutaneous absorption and systemic distribution to hepatic uptake, liver exposure, and three endpoint-specific inhibitory functions describing suppression of HBsAg, HBeAg, and HBV DNA production. By combining longitudinal in vivo biomarker trajectories with mechanistic PK/PD modeling, we reconstructed quantitative dose–exposure–effect relationships within the experimentally studied dose range, thereby extending the dose-response concept beyond concentration-controlled in vitro assays. The fitted model reproduced the principal dose-dependent patterns of all three virological markers. HBsAg and HBV DNA inhibition were predicted to reach high effect levels at substantially lower hepatic exposure than HBeAg inhibition, consistent with marked endpoint-specific differences in the estimated EC_{50} values. After the final dose, liver exposure declined gradually, whereas nonlinear pharmacodynamics maintained prolonged HBsAg and HBV DNA inhibition. Local sensitivity analysis identified hepatic clearance and liver distribution volume as the major determinants of the time to 50% antiviral effect, while endpoint-specific potency became increasingly influential near the pharmacodynamic threshold. These results provide a mathematical framework for extracting an in vivo dose-response relationship from longitudinal antiviral data and for separating dose effects, exposure–response relationships, and post-dose persistence after finite siRNA treatment.

Keywords: hepatitis B virus; siRNA; pharmacokinetics/pharmacodynamics; dose–exposure–response; response persistence; quantitative analysis

1 Introduction

Chronic hepatitis B virus (HBV) infection remains a major global health problem and a major cause of cirrhosis and hepatocellular carcinoma [1]. Although nucleos(t)ide analogues effectively suppress viral replication, sustained loss of hepatitis B surface antigen (HBsAg), a central component of functional cure, remains uncommon [2, 3]. This limitation has motivated finite therapies that directly reduce viral transcripts and antigens.

Small-interfering RNAs (siRNAs) are attractive for this purpose because they selectively degrade HBV transcripts. Hepatocyte-targeted N-acetylgalactosamine (GalNAc)-siRNA conjugates undergo efficient hepatic uptake after subcutaneous administration and can produce pharmacodynamic effects that persist substantially longer than plasma exposure [4, 5]. This

separation between systemic exposure and response duration motivates mechanistic pharmacokinetic/pharmacodynamic (PK/PD) models that distinguish administered dose, hepatic exposure, and pharmacodynamic effect [6].

KC13-M2G2 is a recently reported GalNAc-conjugated siRNA targeting the HBV S region. In HBV-transgenic mice, three weekly subcutaneous doses of 0.3, 1, or 3 mg/kg produced dose-dependent changes in HBsAg, HBV DNA, and HBeAg over longitudinal follow-up [7]. These data provide a useful setting for mathematical analysis because the administered dose is observed, whereas the effective hepatic exposure is latent and must be inferred through a mechanistic model. In vivo dose–response relationships therefore cannot be interpreted directly from the nominal dose; they arise from the combination of absorption, distribution, hepatic uptake, persistence, and endpoint-specific pharmacodynamic sensitivity [8, 9]. Related kinetic-pharmacodynamic approaches have been used to infer latent biophase behavior from longitudinal response data when direct target-organ concentrations are unavailable [10, 11].

Mathematical models have long been used to quantify HBV within-host dynamics, beginning with coupled hepatocyte–virus systems and later extensions incorporating refractory states, intracellular or production delays, hepatocyte proliferation, cell-to-cell transmission, and HBeAg dynamics [12, 13, 14, 15, 16, 17]. Of particular relevance, Kadelka et al. developed a multi-compartment PK/PD model for ARC-520 and jointly described HBV DNA, HBsAg, and HBeAg under RNAi therapy [18]. Mechanistic GalNAc-siRNA models have also quantified subcutaneous absorption, systemic distribution, receptor-mediated hepatic uptake, and prolonged liver disposition [19, 6]. However, a compact framework that reconstructs the KC13-M2G2 dose–exposure–effect relationship and analytically connects hepatic disposition with endpoint-specific response persistence has not yet been developed.

In the present study, we combine a mechanistic subcutaneous PK model with a reduced HBV within-host model and three endpoint-specific $E_{\max}$ functions, and fit the resulting system jointly to HBV DNA, HBsAg, and HBeAg using nonlinear mixed-effects modeling. Our aims are to reproduce the observed dose-dependent trajectories, reconstruct the model-based in vivo dose–exposure–effect relationship within the studied dose range, compare endpoint-specific pharmacodynamic potency, derive an explicit measure of post-dose persistence based on a 50% inhibition threshold, and identify the parameters that most strongly control persistence.

2 Mathematical model

We developed a mechanistic pharmacokinetic/pharmacodynamic (PK/PD) model to describe the relationship between repeated subcutaneous administration of KC13-M2G2, systemic drug distribution, hepatic exposure, and inhibition of HBV-related virological markers. The viral-dynamic component follows the general lineage of reduced within-host HBV models in which infected hepatocytes generate intracellular HBV-associated DNA, circulating HBV DNA, and viral antigens [12, 13, 15]. The explicit representation of HBsAg and HBeAg as separate treatment-responsive outputs is motivated in particular by prior RNAi PK/PD modeling of ARC-520 [18], while the hepatic disposition structure is informed by mechanistic GalNAc-siRNA PK models [19, 6]. The model consists of a subcutaneous absorption depot, central and peripheral systemic compartments, a liver compartment, and three endpoint-specific pharmacodynamic effects corresponding to HBsAg, HBeAg, and HBV DNA (Fig. 1).

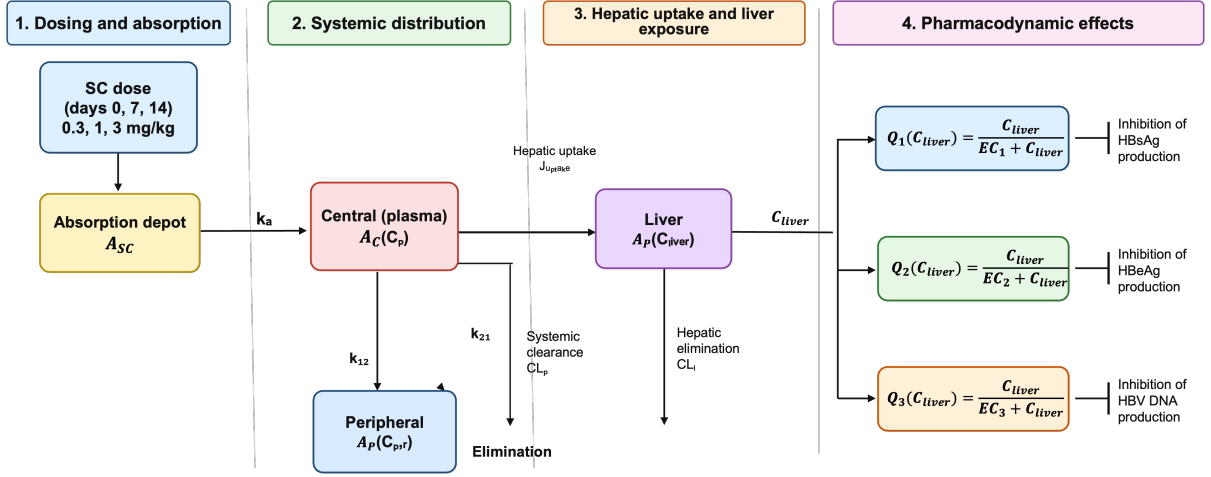


Figure 1: Schematic of the mechanistic PK/PD model. Repeated subcutaneous dosing is followed by systemic distribution and hepatic uptake. The resulting liver concentration C_{liver} drives three endpoint-specific effects: Q_1 for HBsAg, Q_2 for HBeAg, and Q_3 for HBV DNA.

Let A_{sc} denote the amount of drug in the subcutaneous absorption depot, A_c the amount in the central compartment, A_p the amount in the peripheral compartment, and A_l the internalized amount in the liver. Drug amounts are expressed in nmol, whereas compartment volumes are expressed in mL.

Subcutaneous absorption is assumed to follow first-order kinetics:

$$\frac{dA_{sc}}{dt} = -k_a A_{sc}, \quad (1)$$

where k_a is the first-order absorption rate constant. Repeated subcutaneous administrations are represented as instantaneous additions to the depot. If t_j denotes a dosing time, then

$$A_{sc}(t_j^+) = A_{sc}(t_j^-) + \text{Dose}_j D_{conv}, \quad t_j \in \{0, 7, 14\} \text{ days}, \quad (2)$$

where Dose_j is the administered dose in mg/kg. Between dosing events, A_{sc} evolves according to Eq. (1).

To express drug concentrations in nM, we introduce the unit-conversion factor

$$\kappa = 10^3, \quad (3)$$

because

$$1 \text{ nmol/mL} = 10^3 \text{ nM}.$$

For an administered dose expressed in mg/kg, the conversion from dose to the amount entering the subcutaneous depot is

$$D_{conv} = F_{sc} \left(\frac{BW}{1000} \right) \frac{10^6}{MW}, \quad (4)$$

where F_{sc} is the subcutaneous bioavailability, BW is body weight in grams, and MW is the molecular weight in g/mol. With dose in mg/kg, Eq. (4) converts the administered dose to nmol. In the present implementation, $F_{sc} = 1$, $BW = 26.7 \text{ g}$, and $MW = 16,000 \text{ g/mol}$.

Accordingly, the total central and peripheral concentrations are

$$C_{tot} = \kappa \frac{A_c}{V_C}, \quad C_{per} = \kappa \frac{A_p}{V_P}, \quad (5)$$

where V_C and V_P denote the central and peripheral distribution volumes, respectively.

A quasi-steady-state binding relationship is used to describe reversible binding between free plasma drug and the hepatic uptake receptor. The receptor-bound concentration is

$$RC = \frac{R_{\text{tot}}C_p}{K_{\text{SS}} + C_p}, \quad (6)$$

where R_{tot} is the total receptor concentration and K_{SS} is the quasi-steady-state binding constant. The total central concentration is assumed to be the sum of free and receptor-bound drug,

$$C_{\text{tot}} = C_p + RC. \quad (7)$$

Substituting Eq. (6) into Eq. (7) gives

$$C_{\text{tot}} = C_p + \frac{R_{\text{tot}}C_p}{K_{\text{SS}} + C_p}.$$

Multiplying by $K_{\text{SS}} + C_p$ and rearranging yields the quadratic equation

$$C_p^2 + (R_{\text{tot}} + K_{\text{SS}} - C_{\text{tot}})C_p - K_{\text{SS}}C_{\text{tot}} = 0. \quad (8)$$

The physically relevant nonnegative solution of Eq. (8) is used to determine C_p in the simulations.

Drug transport between the systemic and hepatic compartments is written directly in the compartmental balance equations below. Here, D_{conv} converts the administered dose to drug amount, CL_p denotes systemic clearance, Q_{pk} denotes intercompartmental distribution clearance, k_{int} is the hepatic internalization rate constant, CL_l is hepatic elimination clearance, and V_L is the effective liver distribution volume.

Because KC13-M2G2 plasma and liver concentration data were not available, the systemic and hepatic PK parameters were not re-estimated from the efficacy data. Instead, the PK component was fixed to the mouse GalNAc-siRNA framework reported for JNJ-73763989 by Sandra et al. [19]. The parameter values used in the present simulations are summarized in Table 1. For the reference mouse body weight $BW = BW_{\text{ref}} = 26.7$ g, the body-weight scaling factor is unity; hence the values shown in the table are the numerical values used directly in the model. These borrowed PK quantities should therefore be interpreted as reference exposure parameters rather than KC13-M2G2-specific estimates.

Table 1: Fixed PK parameters used in the mechanistic exposure model. The GalNAc-siRNA PK quantities were taken from the mouse JNJ-73763989 framework of Sandra et al. [19] and were not re-estimated from the KC13-M2G2 efficacy data.

Parameter	Description	Value used	Source/role
k_a	First-order SC absorption rate	5.6184 day^{-1}	Sandra et al. [19]
V_C	Central distribution volume	1.992 mL	Sandra et al. [19]
V_P	Peripheral distribution volume	2.252 mL	Sandra et al. [19]
V_L	Effective liver distribution volume	0.8711 mL	Sandra et al. [19]
Q_{pk}	Intercompartmental clearance	41.424 mL/day	Sandra et al. [19]
CL_p	Systemic clearance	264 mL/day	Sandra et al. [19]
CL_l	Hepatic elimination clearance	0.04704 mL/day	Sandra et al. [19]
K_{SS}	Quasi-steady-state binding constant	143.2 nM	Sandra et al. [19]
k_{int}	Hepatic internalization rate	46.2 day^{-1}	Sandra et al. [19]
R_{tot}	Total receptor concentration	647 nM	Sandra et al. [19]
BW_{ref}	Reference mouse body weight	26.7 g	Model implementation
F_{sc}	SC bioavailability	1	Model assumption
MW	Molecular weight for dose conversion	16,000 g/mol	Illustrative assumption

117 The central, peripheral, and hepatic compartments are then described directly by

$$\frac{dA_c}{dt} = k_a A_{sc} D_{conv} - \frac{CL_p C_p}{\kappa} - \frac{Q_{pk}(C_p - C_{per})}{\kappa} - \frac{k_{int} V_C RC}{\kappa}, \quad (9)$$

$$\frac{dA_p}{dt} = \frac{Q_{pk}(C_p - C_{per})}{\kappa}, \quad (10)$$

$$\frac{dA_l}{dt} = \frac{k_{int} V_C RC}{\kappa} - \frac{CL_l}{V_L} A_l. \quad (11)$$

118 The liver concentration used as the pharmacodynamic driver is

$$C_{liver} = \kappa \frac{A_l}{V_L}. \quad (12)$$

119 The antiviral activity of KC13-M2G2 is represented using three endpoint-specific Emax-type
120 functions:

$$Q_1(C_{liver}) = \frac{C_{liver}}{EC_1 + C_{liver}}, \quad (13)$$

$$Q_2(C_{liver}) = \frac{C_{liver}}{EC_2 + C_{liver}}, \quad (14)$$

$$Q_3(C_{liver}) = \frac{C_{liver}}{EC_3 + C_{liver}}. \quad (15)$$

121 The three pharmacodynamic functions represent endpoint-specific inhibition by the model-
122 defined hepatic exposure. Specifically, Q_1 suppresses HBsAg production, Q_2 suppresses HBeAg
123 production, and Q_3 suppresses intracellular HBV-associated DNA production and thereby
124 reduces downstream HBV DNA production. Each function ranges from 0 to 1, with $Q_k = 0.5$
125 when $C_{liver} = EC_k$. Thus, EC_1 , EC_2 , and EC_3 are model-defined hepatic exposure levels
126 corresponding to 50% inhibition of the respective processes.

127 To describe the longitudinal HBV dynamics, we followed the reduced within-host HBV
128 formulation previously used for RNAi therapy by Kadelka et al. [18]. Let I denote the infected-
129 cell population, D an intracellular HBV-associated DNA state, V plasma HBV DNA, S HBsAg,
130 and E HBeAg. Because the analysis begins at the initiation of KC13-M2G2 treatment in
131 HBV-transgenic mice, we consider an already established HBV-producing cell population and
132 do not explicitly model de novo infection of susceptible hepatocytes during the treatment period.
133 Accordingly, no new-infection term is included in the infected-cell equation, and the infected-cell
134 population is assumed to decline according to $dI/dt = -\delta I$. The treatment-period model is

$$\frac{dI}{dt} = -\delta I, \quad (16)$$

$$\frac{dD}{dt} = (1 - Q_3)e^{-gt}\alpha - (p + c_D)D, \quad (17)$$

$$\frac{dV}{dt} = \xi p D I - cV, \quad (18)$$

$$\frac{dS}{dt} = (1 - Q_1)p_s I - d_s S, \quad (19)$$

$$\frac{dE}{dt} = (1 - Q_2)p_e I - d_e E. \quad (20)$$

135 The biological interpretation of the viral-dynamic terms is as follows. The parameter δ is
136 the loss rate of the pre-existing HBV-producing cell population. The intracellular state D is
137 generated at rate α , modulated by the empirical factor e^{-gt} and by the siRNA effect $(1 - Q_3)$,
138 and is removed through virion-associated utilization at rate pD and intracellular loss at rate
139 $c_D D$. The factor e^{-gt} represents a gradual, empirical decline in intracellular HBV-associated

DNA production over time; g is therefore an empirical production-decline rate rather than a direct measurement of a specific molecular process. Serum HBV DNA is produced from the intracellular state through the term ξpDI and cleared at rate cV , where ξ converts intracellular DNA-associated virion production to the serum HBV DNA scale used in the model. HBsAg and HBeAg are produced by the pre-existing HBV-producing cell population at rates $p_s I$ and $p_e I$, respectively, with the corresponding production processes reduced by $(1 - Q_1)$ and $(1 - Q_2)$, and are cleared at rates $d_s S$ and $d_e E$.

Because the analysis starts at treatment initiation in HBV-transgenic mice, the model focuses on the decay and treatment response of an already established HBV-producing cell population. De novo infection of susceptible hepatocytes is not explicitly represented during the treatment period; accordingly, the infected-cell equation contains no new-infection term and reduces to $dI/dt = -\delta I$. The model also does not explicitly resolve target-cell dynamics, cccDNA, integrated HBV DNA, or immune-cell populations. These processes are absorbed into the reduced-order representation used here.

The observation functions are

$$Y_V = \log_{10} V, \quad Y_S = \log_{10} S, \quad Y_E = \log_{10} E. \quad (21)$$

The systemic and hepatic PK parameters were adopted from a published GalNAc-siRNA PK framework for JNJ-73763989 rather than directly estimated from KC13-M2G2 concentration measurements [19]. The resulting compartmental structure is consistent with the broader mechanistic understanding of rapid hepatic uptake and prolonged intracellular disposition of GalNAc-siRNAs [4, 6]. Therefore, C_{liver} should be interpreted as a model-defined surrogate of hepatic exposure rather than as a directly measured KC13-M2G2 liver concentration or a direct measurement of RISC-loaded active siRNA.

3 Data and parameter estimation

3.1 Experimental data

The model was fitted to published longitudinal data from the HBV-transgenic mouse experiment reported by Huang et al. [7]. The HBV-transgenic model carries a 1.28-mer linearized HBV genotype A construct and constitutively expresses the three virological markers considered in the present analysis—HBsAg, HBeAg, and HBV DNA—in serum. For the KC13-M2G2 dose-ranging experiment, mice were randomly allocated to treatment groups with comparable baseline levels of HBsAg, HBeAg, and HBV DNA. Each KC13-M2G2 dose group contained **six individual mice** ($n = 6$ per dose group), giving a total of 18 KC13-M2G2-treated animals included in the present modeling analysis.

KC13-M2G2 was administered by subcutaneous injection once weekly for three consecutive doses at 0.3, 1, or 3 mg/kg. The first administration was defined as day 0, and the second and third administrations were given on days 7 and 14, respectively. Thus, the dataset contains three experimentally observed dose levels and repeated measurements from six animals at each dose, which enabled the model to characterize both dose dependence and between-animal variability rather than relying only on group-mean responses. Although the original experiment also included elebsiran, bepirovirsen, entecavir, and PBS control groups, the present analysis was restricted to the three KC13-M2G2 dose groups because the objective was to reconstruct the dose-exposure-effect relationship of KC13-M2G2 itself.

Blood was sampled repeatedly during follow-up, with the published experiment extending through day 70 after the first dose. In the source study, plasma HBsAg, HBV DNA, HBeAg, and ALT were monitored longitudinally. The present PK/PD analysis used the three virological outcomes HBsAg, HBV DNA, and HBeAg. HBsAg and HBeAg were quantified by chemiluminescence immunoassays on commercial analytical systems, whereas HBV DNA was quantified

using a PCR-fluorescence probing assay [7]. The published data were reported on a logarithmic scale and included lower limits of quantification, which is important when interpreting strongly suppressed measurements during treatment.

The source experiment showed clear dose-dependent antiviral activity. At 3 mg/kg, nearly all treated mice (5–6 of 6) achieved HBsAg loss between days 14 and 42, and HBsAg remained more than 2 log₁₀ IU/mL below baseline by day 70 [7]. Rapid reductions in HBV DNA were also observed after treatment initiation, whereas HBeAg displayed a more modest response profile. These distinct longitudinal patterns motivated the use of separate endpoint-specific pharmacodynamic functions rather than a single common antiviral-effect term for all three biomarkers. Importantly, our analysis used the individual longitudinal trajectories of the six mice in each dose group, not only the published group mean and SEM, so that inter-individual differences in the inferred pharmacodynamic parameters could be represented explicitly within the nonlinear mixed-effects framework.

For model fitting, the three longitudinal outcomes were mapped in the Monolix project as HBV DNA (y_1), HBsAg (y_2), and HBeAg (y_3), and all observations were analyzed on the log₁₀ scale. The Monolix data file contained the mouse identifier (`id`), observation time (`time`), log₁₀-transformed observation (`log10observation`), censoring indicator (`censoring`), observation type (`observationID`), administered amount (`amt`), and administration identifier (`adm`). This structure allowed the three endpoints to be fitted jointly while preserving repeated measurements and individual animal identity across the 70-day observation period.

3.2 Nonlinear mixed-effects parameter estimation

Model fitting was performed using a nonlinear mixed-effects (NLME) framework implemented in Monolix. The population approach was used to estimate the typical response pattern while simultaneously accounting for between-animal variability across the 18 KC13-M2G2-treated mice. Population parameters were estimated by maximum likelihood using the Stochastic Approximation Expectation Maximization (SAEM) algorithm, which is well suited to nonlinear mixed-effects models defined by systems of ordinary differential equations [20, 21]. Individual-level parameter estimates were then obtained from the fitted population model and were used in the subsequent endpoint-specific exposure–response and persistence analyses.

Parameters that were allowed to vary among animals were represented by log-normal random effects. For a generic positive parameter θ_i in animal i ,

$$\theta_i = \theta_{\text{pop}} \exp(\eta_i), \quad \eta_i \sim \mathcal{N}(0, \omega_\theta^2), \quad (22)$$

where θ_{pop} denotes the population typical value and ω_θ quantifies between-animal variability on the log scale. This formulation preserves parameter positivity and allows individual mice to deviate multiplicatively from the population trajectory.

Several quantities were fixed or initialized before estimation in order to reduce structural non-identifiability. The intracellular HBV DNA loss rate was fixed at 0.69 day^{−1}, the conversion factor between intracellular HBV DNA copies and serum IU was fixed at 1/5.3, and the infected-hepatocyte loss rate was fixed at 0.004 day^{−1}. The reference infected-hepatocyte density was set to one half of 1.4 × 10⁷ cells/mL, and the baseline intracellular HBV DNA content was set to 450 copies per infected cell, consistent with the assumptions used in the preceding HBV modeling framework on which the viral-dynamic component was based [18]. The systemic and hepatic siRNA PK constants were not re-estimated from the KC13-M2G2 efficacy data because drug concentration measurements were unavailable. They were fixed to the mouse JNJ-73763989 GalNAc-siRNA PK framework of Sandra et al. [19], as detailed in Table 1. Consequently, the inferred liver concentration should be interpreted as a model-based exposure surrogate rather than a directly measured KC13-M2G2 concentration. A reference body weight of 26.7 g, complete subcutaneous bioavailability, and an illustrative molecular-weight assumption of 16,000 g/mol were used for dose-to-molar conversion.

234 The analysis was initiated at day 0, which corresponds to the day of the first KC13-M2G2
 235 administration, and the day-0 measurements were treated as observed data together with the
 236 subsequent longitudinal measurements. Baseline HBV DNA, HBsAg, and HBeAg levels were
 237 therefore not fixed directly to the observed day-0 values. Instead, the corresponding baseline
 238 parameters l_{V0} , l_{S0} , and l_{E0} were estimated within the nonlinear mixed-effects framework, with
 239 model initial conditions defined as

$$V_0 = 10^{l_{V0}}, \quad S_0 = 10^{l_{S0}}, \quad E_0 = 10^{l_{E0}}.$$

240 The empirical intracellular-production decline rate was parameterized as $g = 10^{-l_g}$, with l_g
 241 estimated within the same nonlinear mixed-effects framework. All drug-related PK compartments
 242 were initialized at zero immediately before the first subcutaneous administration at day 0.

243 Pretreatment viral and antigen dynamics were calibrated under a quasi-steady-state assump-
 244 tion. Using the estimated baseline states, intracellular HBV DNA production was balanced
 245 by intracellular loss and virion production, serum HBV DNA production was balanced by
 246 viral clearance, and HBsAg and HBeAg production were balanced by their respective clearance
 247 processes. Consequently, the production-related parameters were not estimated independently
 248 but were determined algebraically as

$$p = \frac{cV_0}{\xi D_0 I_{\text{ref}}}, \quad \alpha = (p + c_D)D_0, \quad p_s = \frac{d_s S_0}{I_{\text{ref}}}, \quad p_e = \frac{d_e E_0}{I_{\text{ref}}},$$

249 where $D_0 = 450$ and $I_{\text{ref}} = 0.5 \times 1.4 \times 10^7$. This steady-state calibration reduces the number
 250 of independently estimated production parameters while maintaining consistency between the
 251 fitted baseline states and the viral-dynamic equations.

252 The three observation models were specified as Gaussian models with constant additive
 253 residual errors on the $\log_{10}$ scale:

$$y_{1,ij} = V_{\text{out},ij} + \varepsilon_{1,ij}, \quad (23)$$

$$y_{2,ij} = S_{a,ij} + \varepsilon_{2,ij}, \quad (24)$$

$$y_{3,ij} = E_{a,ij} + \varepsilon_{3,ij}, \quad (25)$$

254 with

$$\varepsilon_{k,ij} \sim \mathcal{N}(0, a_k^2), \quad k = 1, 2, 3. \quad (26)$$

255 For transparency, the individual parameter estimates used in the post-fitting analyses are
 256 reported in Table 2. These values correspond to the conditional-mode estimates obtained from
 257 the fitted nonlinear mixed-effects model and are referred to here simply as individual estimated
 258 parameters.

Table 2: Individual estimated parameters used in the post-fitting analyses. Values are conditional-mode estimates from the nonlinear mixed-effects model.

Individual	l_g	c	d_s	d_e	EC_1	EC_2	l_{V0}	l_{S0}	l_{E0}	EC_3
3 mg/kg-1	301.98	0.83808	0.89391	0.06756	2.3061	2779.5	7.9239	3.5136	2.2176	5.7550
3 mg/kg-2	301.98	0.61491	0.83954	0.16941	2.4905	3022.9	7.9252	3.5906	2.2400	6.9314
3 mg/kg-3	301.98	0.60881	0.89228	0.06742	3.1010	2770.6	7.9269	3.6016	2.2151	12.017
3 mg/kg-4	302.12	0.87620	0.92029	0.10871	2.7687	2995.7	7.9222	3.5332	2.2285	6.6719
3 mg/kg-5	301.98	0.66867	0.85676	0.08315	4.4421	2645.8	7.9247	3.5889	2.1449	15.784
3 mg/kg-6	301.99	0.78399	0.93876	0.21896	4.6821	3067.7	7.9256	3.6690	2.2677	11.115
1 mg/kg-1	301.97	0.20842	0.64205	0.16860	6.5639	2913.0	7.9218	3.7160	2.2325	13.691
1 mg/kg-2	301.98	0.28483	0.67803	0.12022	6.0863	2813.9	7.9245	3.7300	2.2468	39.258
1 mg/kg-3	301.98	0.19395	0.31840	0.12825	3.1465	2670.5	7.9240	3.4418	2.1996	1.4213
1 mg/kg-4	302.01	0.24332	0.40514	0.18027	1.8390	2863.5	7.9204	3.4279	2.2552	0.59558
1 mg/kg-5	301.99	0.28571	0.74182	0.20441	8.1305	2564.0	7.9326	3.7654	2.2642	63.195
1 mg/kg-6	301.94	0.31659	0.62664	0.15715	9.6241	2694.4	7.9293	3.7316	2.2638	71.933
0.3 mg/kg-1	301.99	0.03875	0.07786	0.11553	8.1201	2876.0	7.9317	3.5426	2.2769	4.9418
0.3 mg/kg-2	301.98	0.03487	0.13358	0.13290	11.178	2870.3	7.9189	3.5914	2.2891	3.8727
0.3 mg/kg-3	301.97	0.05848	0.07050	0.17790	2.2564	2748.2	7.9268	3.3083	2.1694	1.4080
0.3 mg/kg-4	301.98	0.34388	0.54213	0.19296	17.286	2791.1	7.9266	3.7625	2.2602	261.57
0.3 mg/kg-5	301.98	0.12618	0.16453	0.15514	2.7106	2700.0	7.9281	3.3165	2.1314	0.43054
0.3 mg/kg-6	301.99	0.08660	0.45557	0.14094	14.360	2796.4	7.9288	3.8244	2.2849	109.64

4 Mathematical analysis

4.1 Terminal liver-exposure dynamics

After the final dose, when continued hepatic uptake becomes small relative to hepatic elimination,

$$\frac{dA_l}{dt} \simeq -\frac{CL_l}{V_L} A_l. \quad (27)$$

Defining

$$k_l = \frac{CL_l}{V_L}, \quad (28)$$

the terminal liver concentration is approximated by

$$C_{\text{liver}}(t) = C_0 e^{-k_l(t-t_0)}, \quad (29)$$

where $C_0 = C_{\text{liver}}(t_0)$.

The corresponding terminal liver-exposure half-life is

$$t_{1/2, \text{liver}} = \frac{\ln 2}{k_l} = \frac{V_L \ln 2}{CL_l}. \quad (30)$$

4.2 Nonlinear decay of antiviral activity

For a generic endpoint with potency parameter EC ,

$$Q(t) = \frac{C_0 e^{-k_l(t-t_0)}}{EC + C_0 e^{-k_l(t-t_0)}}. \quad (31)$$

Differentiating Eq. (31) gives

$$\frac{dQ}{dt} = -k_l Q(1 - Q). \quad (32)$$

Equation (32) shows that antiviral activity does not decay exponentially even when liver exposure does. When Q is close to one, the factor $(1 - Q)$ is small and the decline in antiviral activity is strongly buffered. As Q decreases, the loss of pharmacodynamic activity accelerates relative to the saturated regime.

4.3 Effect-persistence threshold

For a threshold $q \in (0, 1)$, solving $Q(t_q) = q$ gives

$$t_q - t_0 = \frac{1}{k_l} \ln \left[\frac{C_0(1 - q)}{ECq} \right]. \quad (33)$$

For $q = 0.5$,

$$t_{50} - t_0 = \frac{1}{k_l} \ln \left(\frac{C_0}{EC} \right). \quad (34)$$

Thus, the duration of antiviral activity is determined jointly by (i) the PK timescale $1/k_l$ and (ii) the exposure-to-potency ratio C_0/EC . Equation (34) also predicts a logarithmic increase in persistence with increasing post-dose exposure.

4.4 Dose-dependent antiviral-effect distribution

To characterize antiviral activity within the experimentally studied dose range without extrapolating to unobserved dose levels, model-predicted Q_1 , Q_2 , and Q_3 values were calculated for each fitted individual at all simulated time points from day 0 to day 70. The resulting distributions were summarized separately for 0.3, 1, and 3 mg/kg. This analysis incorporates both between-individual variation in the estimated pharmacodynamic parameters and temporal variation in model-predicted liver exposure.

4.5 Local sensitivity of response persistence

A one-at-a-time local sensitivity analysis was performed for t_{50} . Each model parameter p was perturbed by $\pm 20\%$ while all remaining parameters were held fixed. The normalized sensitivity index was defined as

$$\mathcal{S}_p = \frac{t_{50}(1.2p) - t_{50}(0.8p)}{0.4 t_{50}(p)}. \quad (35)$$

Positive values indicate that increasing the parameter prolongs the predicted effect, whereas negative values indicate that increasing the parameter shortens it. For the dose-comparison sensitivity analysis, common endpoint-specific EC values were used to isolate the model-implied effect of dose from between-individual variation in potency. When the simulated antiviral effect did not reach 50%, normalized t_{50} sensitivity was not interpreted.

5 Numerical results

5.1 Model fitting

Figure 2 shows the fitted individual trajectories and observations for HBV DNA, HBsAg, and HBeAg at 3, 1, and 0.3 mg/kg. The model reproduces the principal dose-dependent features of all three endpoints. At 3 mg/kg, HBV DNA and HBsAg exhibit pronounced declines followed by gradual recovery, whereas suppression is weaker at lower doses. HBeAg changes are comparatively modest, particularly in the 1 and 0.3 mg/kg groups.

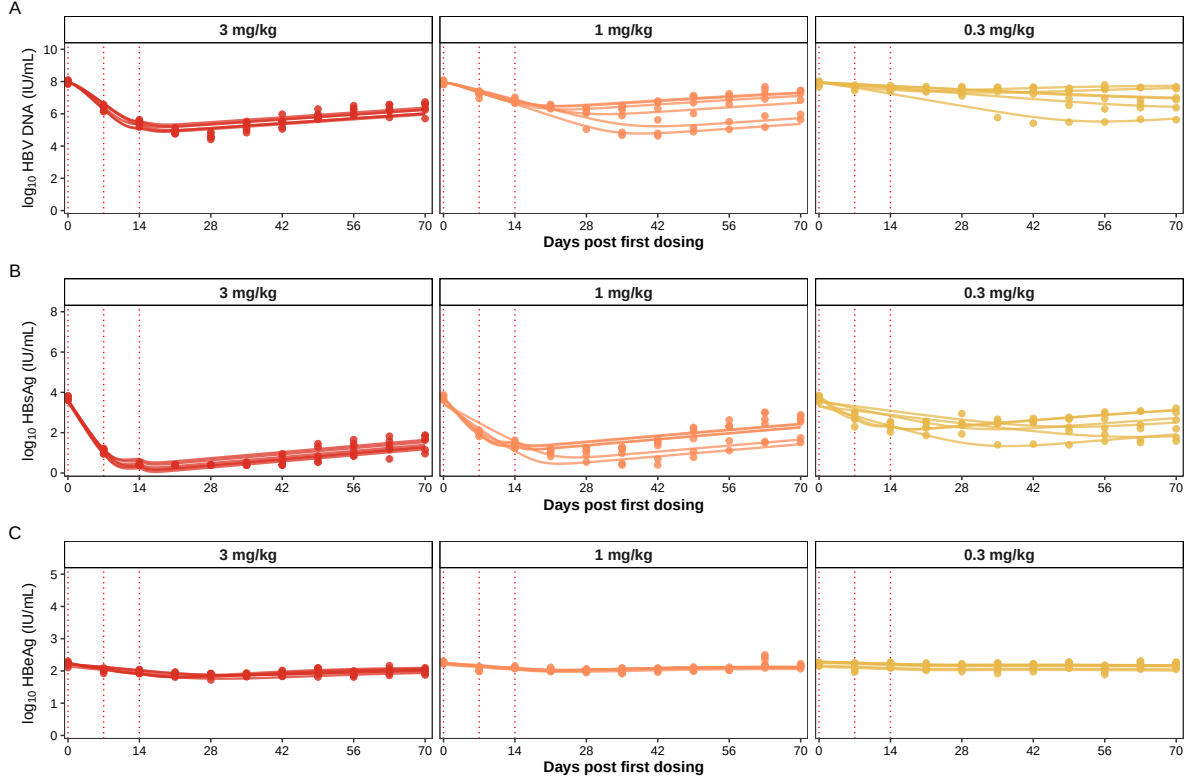


Figure 2: Joint model fitting to longitudinal HBV markers after KC13-M2G2 treatment. (A) HBV DNA, (B) HBsAg, and (C) HBeAg. Columns correspond to 3, 1, and 0.3 mg/kg. Points denote observations and solid curves denote individual model predictions. Vertical dotted lines indicate weekly dosing.

5.2 In vivo dose-response and endpoint-specific antiviral effects

The longitudinal in vivo data were used to reconstruct the dose-dependent antiviral effect through the mechanistic PK/PD mapping

$$\mathcal{D} \xrightarrow{\text{PK}} C_{\text{liver}}(t; \mathcal{D}) \xrightarrow{\text{PD}} Q_j(t; \mathcal{D}), \quad j \in \{1, 2, 3\},$$

where $\mathcal{D}$ denotes the administered dose and Q_j denotes the inhibitory effect for HBsAg, HBeAg, or HBV DNA. This construction differs from a conventional in vitro concentration–response experiment: the effective concentration is not externally fixed but emerges from the estimated in vivo PK process. Consequently, the dose-response relationship incorporates absorption, distribution, hepatic uptake, persistence, and between-individual pharmacodynamic variability.

Figure 3 summarizes the distributions of the model-predicted time-dependent antiviral effects, $Q_1(t)$, $Q_2(t)$, and $Q_3(t)$, over the entire 70-day observation period for the experimentally studied doses of 0.3, 1, and 3 mg/kg. Each distribution pools predictions across the six fitted animals within a dose group and across all simulated time points from day 0 to day 70. HBsAg inhibition (Q_1) remained high over a substantial portion of the observation period even at the lower doses and shifted further toward saturation as dose increased. HBV DNA inhibition (Q_3) showed a broadly similar dose-dependent pattern but a broader distribution, reflecting both temporal variation in hepatic exposure and between-individual variability in EC_3 . In contrast, HBeAg inhibition (Q_2) remained substantially lower at 0.3 and 1 mg/kg and increased most markedly at 3 mg/kg.

The population typical potency estimates quantified this separation: $EC_1 = 5.33$ nM for HBsAg, $EC_3 = 8.43$ nM for HBV DNA, and $EC_2 = 2875.67$ nM for HBeAg. Thus, within

the model-defined liver-exposure scale, the estimated HBeAg EC_{50} was approximately 539-fold higher than that for HBsAg and 341-fold higher than that for HBV DNA. These values should not be interpreted as direct experimental liver concentrations, but they quantitatively explain why the same in vivo dosing regimen can produce strong HBsAg and HBV DNA suppression while HBeAg inhibition remains incomplete.

Importantly, the source study also reported classical in vitro concentration–response curves for KC13-M2G2, with nanomolar or sub-nanomolar IC_{50} values measured under controlled cell-culture exposure conditions [7]. The present analysis addresses a different question: it derives an in vivo dose–exposure–effect relationship from longitudinal whole-animal data. Direct numerical equivalence between the in vitro IC_{50} values and the present model-derived EC_j values is not expected because the experimental systems and exposure definitions differ.

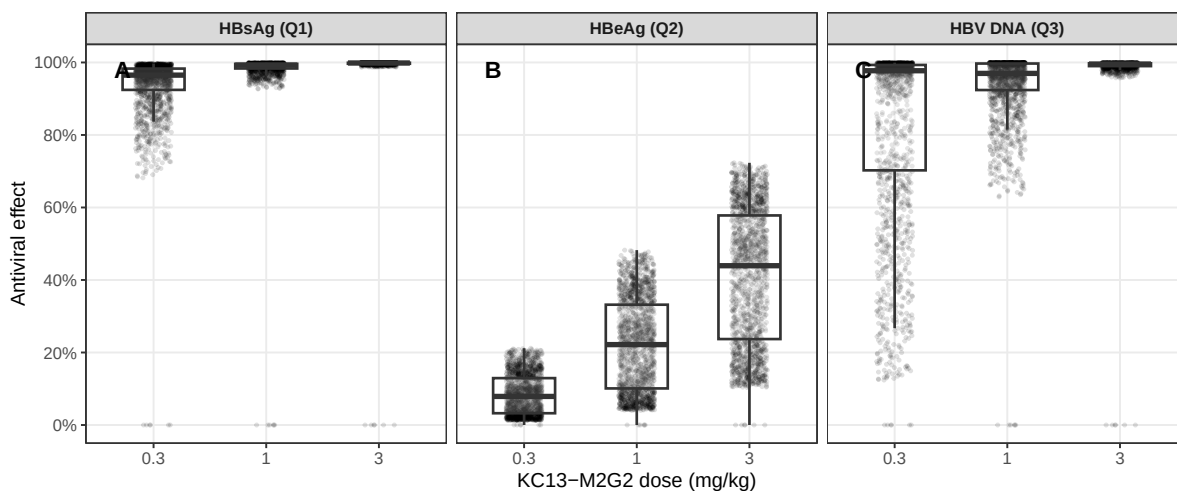


Figure 3: Dose-dependent distributions of model-predicted antiviral effects. Boxplots and scatter points summarize $Q_1(t)$, $Q_2(t)$, and $Q_3(t)$ over days 0–70 for the six fitted animals in each KC13-M2G2 dose group. Thus, each distribution reflects both temporal changes in predicted liver exposure and between-individual variability in endpoint-specific EC parameters. Only the experimentally studied dose groups (0.3, 1, and 3 mg/kg) are shown.

5.3 Endpoint-specific concentration–effect relationships

The dose-dependent distributions can be interpreted through the distinct concentration–effect relationships shown in Fig. 4. HBsAg inhibition (Q_1) reaches high effect levels at relatively low liver concentrations. HBV DNA inhibition (Q_3) behaves similarly overall but exhibits broader between-individual variation. By contrast, the HBeAg relationship (Q_2) is markedly shifted toward higher liver concentrations.

This separation implies that a common liver-exposure trajectory can simultaneously produce near-saturated HBsAg and HBV DNA inhibition while HBeAg remains only partially inhibited.

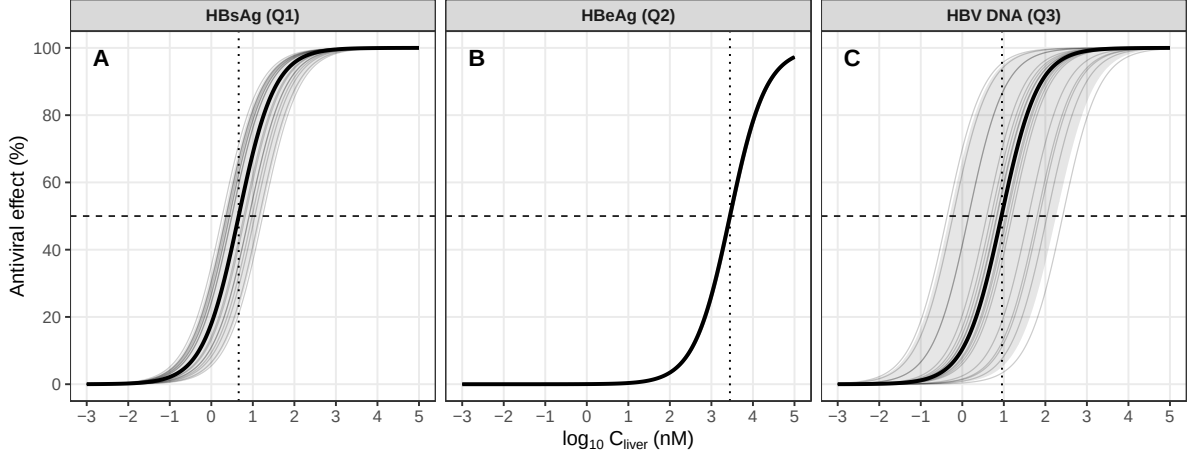


Figure 4: Concentration-dependent antiviral effects. (A) HBsAg inhibition Q_1 , (B) HBeAg inhibition Q_2 , and (C) HBV DNA inhibition Q_3 as functions of $\log_{10} C_{\text{liver}}$. Thin curves represent individual concentration–effect relationships, thick curves denote the median relationship, horizontal dashed lines indicate 50% inhibition, and vertical dotted lines indicate the median EC_{50} .

5.4 Post-dose persistence

Figure 5A shows the simulated hepatic exposure following three weekly doses. Liver exposure accumulates after repeated dosing and then declines gradually after the final injection.

Despite declining exposure, HBsAg inhibition remains high for an extended period, particularly at the higher doses (Fig. 5B). HBeAg inhibition is substantially shorter-lived and remains below 50% at the lower doses (Fig. 5C). HBV DNA inhibition also remains prolonged, although dispersion is larger because of between-individual variability in EC_3 (Fig. 5D).

Using common endpoint-specific potency values to isolate the pure dose effect, predicted t_{50} increased monotonically with dose for both HBsAg and HBV DNA. For HBeAg, only the 3 mg/kg group exceeded the 50% inhibition threshold.

Table 3: Model-predicted time from the final dose to the first decline below 50% inhibition (t_{50}) using common endpoint-specific potency values. NR indicates that the post-dose inhibitory effect did not reach the 50% threshold and t_{50} was therefore not defined.

Endpoint	0.3 mg/kg	1 mg/kg	3 mg/kg
HBsAg (Q_1)	94.8	116.8	136.5
HBeAg (Q_2)	NR	NR	17.7
HBV DNA (Q_3)	82.1	104.2	123.9

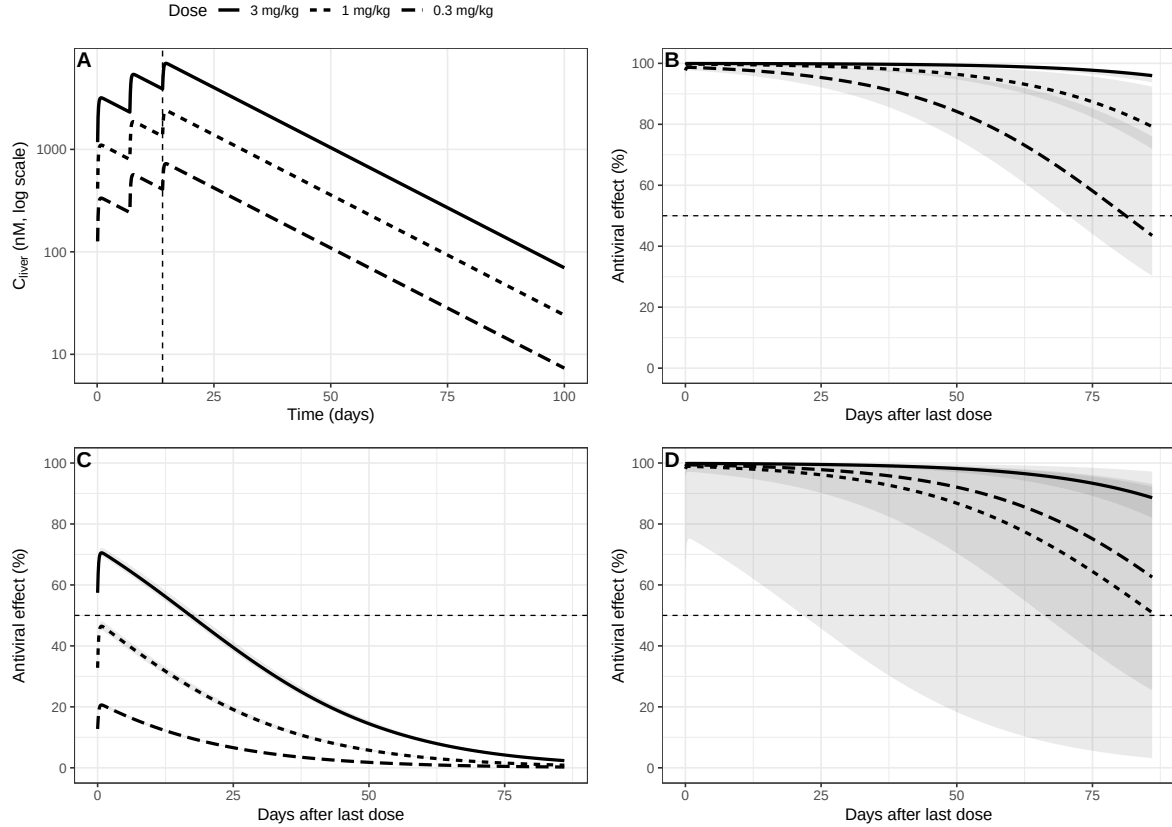


Figure 5: Post-dose persistence of liver exposure and antiviral activity. (A) Simulated liver concentration after three weekly doses. (B) HBsAg inhibition, (C) HBeAg inhibition, and (D) HBV DNA inhibition after the final dose. The horizontal dashed line in panels B–D marks 50% inhibition. Shaded regions represent variability induced by individual endpoint-specific EC estimates.

5.5 Local sensitivity analysis

The local sensitivity analysis identified terminal hepatic disposition as the primary determinant of post-dose response persistence. For both HBsAg and HBV DNA, hepatic clearance CL_l showed the largest negative sensitivity, whereas liver volume V_L showed the largest positive sensitivity, consistent with the terminal hepatic elimination rate $k_l = CL_l/V_L$. The corresponding baseline model-predicted t_{50} values decreased with dose, from 136.5 to 94.8 days for HBsAg and from 123.9 to 82.1 days for HBV DNA across 3 to 0.3 mg/kg. Endpoint-specific potency became progressively more influential at lower doses, with the absolute sensitivities of EC_1 and EC_3 increasing as exposure approached the corresponding EC_{50} thresholds.

HBeAg displayed a distinct threshold-limited pattern. Only the 3 mg/kg regimen produced a post-dose inhibitory effect above 50%, yielding a finite t_{50} of 17.7 days. At this dose, CL_l remained the strongest determinant, but EC_2 and molecular-weight-dependent exposure scaling were also highly influential, reflecting the much larger HBeAg EC_{50} . Hepatic uptake and systemic disposition parameters had only secondary effects for HBsAg and HBV DNA but were more influential for HBeAg near threshold. In contrast, the intercompartmental distribution term Q_{pk} and the absorption-rate constant k_a had little influence on long-term persistence.

Overall, the sensitivity results indicate that response duration is governed mainly by hepatic retention, whereas pharmacodynamic potency becomes increasingly important when exposure is close to the endpoint-specific response threshold. These findings explain why the same dosing

regimen can produce substantially different persistence across HBsAg, HBV DNA, and HBeAg.

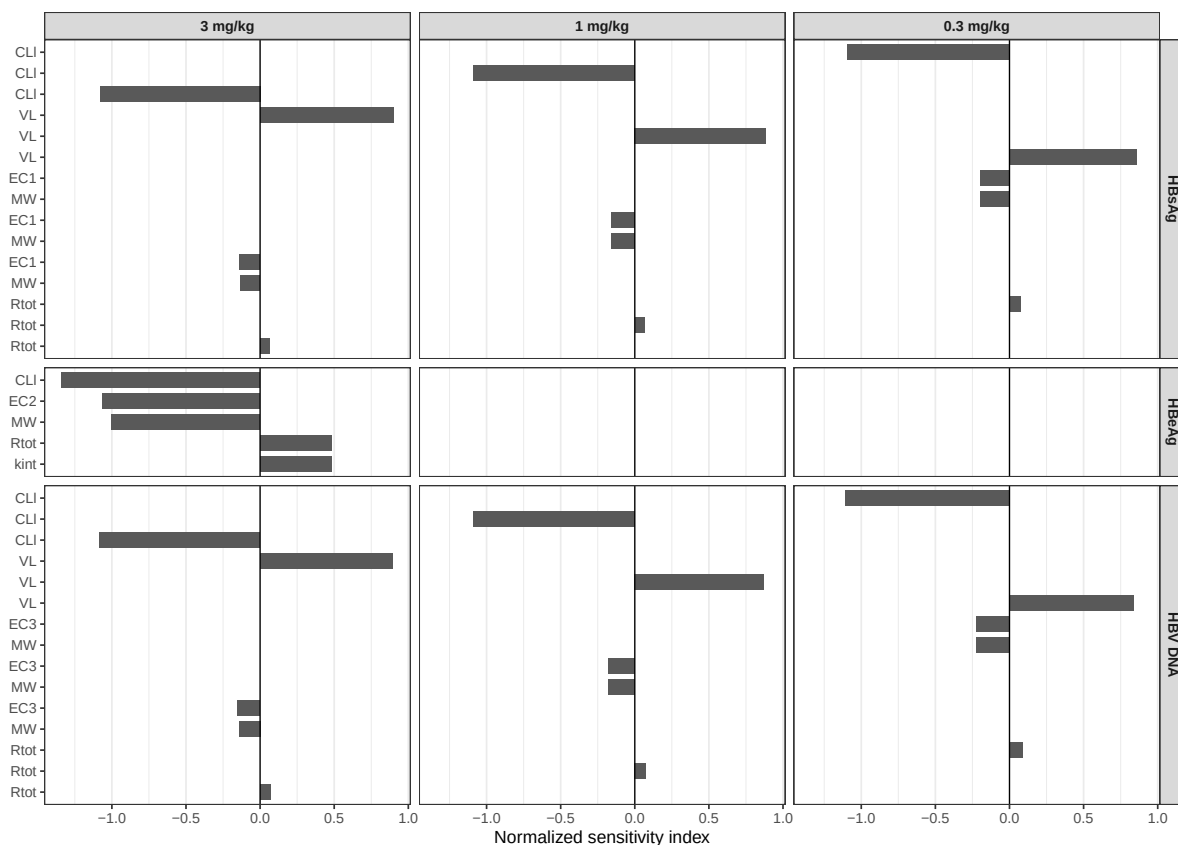


Figure 6: Local sensitivity of t_{50} to model parameters. Bars show normalized sensitivity indices after $\pm 20\%$ one-at-a-time perturbations. Negative values indicate that increasing the parameter decreases t_{50} , whereas positive values indicate prolongation. The five largest absolute sensitivities are displayed for each dose–endpoint combination. For HBeAg at 1 and 0.3 mg/kg, normalized sensitivities are not reported because the post-dose inhibitory effect did not reach the 50% threshold and the baseline t_{50} was therefore undefined.

6 Discussion

This study develops a mathematical framework that separates three components of the KC13-M2G2 response: dose-dependent exposure, endpoint-specific pharmacodynamic potency, and post-dose persistence. This separation is important for GalNAc-siRNAs because pharmacodynamic activity can persist well beyond systemic exposure [4, 5]. The mechanistic hepatic PK model therefore acts as the bridge between administered dose and the three Emax-type response functions.

The reconstructed dose–exposure–effect relationship was restricted to the experimentally studied doses of 0.3, 1, and 3 mg/kg. Increasing dose shifted the predicted antiviral-effect distributions upward, but the magnitude of this shift differed among HBsAg, HBeAg, and HBV DNA. The fitted EC_1 and EC_3 values were of similar order, whereas EC_2 was substantially larger, indicating that HBsAg and HBV DNA were more sensitive to the model-defined hepatic exposure than HBeAg. This endpoint ordering is qualitatively consistent with the source experiment, although the present model-derived EC values should not be interpreted as direct equivalents of in-vitro IC_{50} values because the exposure definitions and experimental systems differ [7].

A central mathematical result concerns the decay of pharmacodynamic effect after dosing

387 stops. For

$$Q = \frac{C}{EC + C},$$

388 and exponential terminal hepatic elimination, the inhibitory effect satisfies

$$\frac{dQ}{dt} = -k_l Q(1 - Q).$$

389 Consequently, the time required for the effect to cross a threshold can be written explicitly. For
390 the 50% threshold,

$$t_{50} - t_0 = \frac{1}{k_l} \ln \left(\frac{C_0}{EC} \right).$$

391 This relation shows that persistence is determined jointly by the hepatic elimination timescale
392 $1/k_l$ and the exposure-to-potency ratio C_0/EC . It also explains why strongly saturated endpoints
393 can remain suppressed even as liver exposure declines and why further dose increases produce
394 only logarithmic gains in persistence once exposure substantially exceeds the relevant EC_{50} .

395 The local sensitivity analysis was consistent with this analytical result. Hepatic clearance
396 CL_l had the strongest negative influence on t_{50} , whereas liver distribution volume V_L had a
397 strong positive influence through the terminal relation $k_l = CL_l/V_L$. Endpoint-specific EC
398 parameters became increasingly influential as exposure approached the corresponding response
399 threshold. Thus, hepatic retention dominates response duration when the system is well above
400 threshold, whereas pharmacodynamic potency becomes increasingly important near threshold.

401 The model-derived t_{50} is intended as a mathematical persistence measure rather than a clinical
402 cure endpoint. It summarizes how long the modeled inhibitory function remains above 50% after
403 the final dose and allows comparisons across doses, endpoints, and parameter perturbations.
404 Although sustained HBsAg loss is clinically important in HBV therapy [2, 3], the present
405 mouse-scale t_{50} values should not be interpreted as predictions of human functional cure.

406 Several limitations qualify the quantitative interpretation. First, the analysis is based on
407 only three dose levels and six animals per dose, with repeated measurements of three virological
408 outputs [7]. The nonlinear mixed-effects framework uses all longitudinal observations jointly,
409 but the limited sample size restricts precise characterization of between-animal variability and
410 makes some individual EC estimates uncertain. Second, KC13-M2G2-specific plasma and liver
411 concentration data were unavailable; therefore, the systemic and hepatic PK parameters could not
412 be re-estimated from the present dataset and were instead fixed to the published JNJ-73763989
413 mouse GalNAc-siRNA PK framework [19]. Consequently, C_{liver} , hepatic exposure half-life, and
414 t_{50} should be interpreted as model-derived quantities conditional on the assumed reference PK
415 structure and parameter set rather than as KC13-M2G2-specific pharmacokinetic estimates.
416 The molecular-weight assumption used for dose-to-molar conversion introduces additional scale
417 uncertainty.

418 Third, C_{liver} is a lumped surrogate for hepatic exposure rather than a measured intracellular
419 pharmacodynamic driver. The model does not explicitly describe endosomal trafficking, cytosolic
420 escape, RISC loading and turnover, target-mRNA degradation, or active intracellular metabolites,
421 even though such processes may contribute to GalNAc-siRNA persistence [4, 5, 6]. Introducing
422 these mechanisms would require additional measurements to avoid unsupported parameter
423 expansion [22, 23, 24]. Similarly, the HBV component is intentionally reduced: infected cells
424 decay exponentially, and the intracellular HBV-associated DNA production term contains the
425 empirical factor e^{-gt} . The model therefore does not explicitly resolve cccDNA, integrated HBV
426 DNA, hepatocyte proliferation, distinct genomic sources of HBsAg, or adaptive immunity.

427 Fourth, formal structural and practical identifiability analyses were not performed. The local
428 one-at-a-time sensitivity analysis measures output changes around the fitted parameter set and
429 should not be interpreted as an identifiability test. Because KC13-M2G2 concentrations were not
430 measured together with the virological markers, assumed PK exposure and endpoint-specific EC

parameters may compensate for one another. The fitted EC_1 , EC_2 , and EC_3 should therefore be interpreted as effective parameters conditional on the assumed PK structure. Future work should include profile-likelihood, bootstrap, posterior, or Fisher-information-based identifiability analyses [23, 24].

Finally, some predicted t_{50} values extend beyond the 70-day observation window, and the sensitivity analysis is local, using $\pm 20\%$ one-at-a-time perturbations. These results should therefore be interpreted as mechanistic extrapolations around the fitted parameter set rather than as globally robust uncertainty bounds. The present model is also preclinical and mouse-specific; quantitative translation to humans will require KC13-M2G2-specific PK measurements and additional intracellular or virological data. Such data would allow the current exposure surrogate to be replaced by experimentally anchored states and would provide a stronger basis for translational prediction.

7 Conclusion

A mechanistic PK/PD model was developed to connect repeated KC13-M2G2 dosing with systemic distribution, hepatic exposure, and three HBV-related virological endpoints. The fitted model reproduced the principal dose-dependent dynamics of HBV DNA, HBsAg, and HBeAg. Analysis of antiviral-effect distributions showed that the experimentally studied doses generate distinct inhibition profiles across endpoints. These differences were explained by endpoint-specific concentration–effect relationships. After the final dose, nonlinear pharmacodynamics prolonged HBsAg and HBV DNA inhibition despite continuously declining liver exposure. Sensitivity analysis identified hepatic elimination and liver distribution as the major determinants of persistence and showed that pharmacodynamic potency becomes increasingly important near the response threshold. A key contribution is that longitudinal whole-animal efficacy data were converted into a quantitative, model-derived in vivo dose–response relationship for three distinct HBV endpoints, providing information that cannot be obtained from a conventional in vitro concentration–response experiment alone. Together, these results provide an interpretable applied-mathematics framework for quantifying dose effects, exposure–response relationships, endpoint-specific antiviral activity, and persistence in HBV siRNA therapy.

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Data availability

No new data were generated or analyzed in support of this research.

Declaration of competing interest

The authors declare that they have no known competing financial interests.

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