Plasma and Liver Pharmacokinetics of the N-Acetylgalactosamine Short Interfering RNA JNJ-73763989 in Recombinant Adeno-Associated–Hepatitis B Virus–Infected Mice

Louis Sandra, Huybrecht T’jollyn, Nele Goeyvaerts, An Vermeulen, Anne-Gaëlle Dosne, and Juan-Jose Perez-Ruixo

Janssen Research and Development, Beerse, Belgium (L.S., H.T., N.G., A.V., A.-G.D., J.-J.P.-R.) and Laboratory of Medical Biochemistry and Clinical Analysis, Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium (L.S., A.V.)

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ABSTRACT

JNJ-73763989 is an N-acetylgalactosamine conjugated short interfering RNA combination product consisting of two triggers in clinical development for chronic hepatitis B virus (HBV) infection treatment that induces a selective degradation of all HBV mRNA transcripts. Our aim is to characterize the plasma and liver pharmacokinetics (PK) of JNJ-73763989 after intravenous and subcutaneous administration in recombinant adeno-associated (rAAV) HBV infected mice. Forty-two male rAAV-HBV infected C57Bl/6 mice received JNJ-73763989 doses of 10 mg/kg i.v. or 1, 3 and 10 mg/kg s.c. Plasma and liver concentrations were analyzed simultaneously using nonlinear mixed-effects modeling with the NONMEM 7.4. A population PK model consisting of a two-compartment disposition model with transporter-mediated drug disposition, including internalization to the liver mixed-effects modeling with the NONMEM 7.4. A population PK model consisting of a two-compartment disposition model with transporter-mediated drug disposition, including internalization to the liver compartment, linear elimination from plasma and liver, and first-order absorption following subcutaneous administration, was suitable to describe both plasma and liver PK. After subcutaneous dosing, absolute bioavailability was complete and flip-flop kinetics were observed. JNJ-73763989 distributes from plasma to liver via transporter-mediated liver internalization in less than 24 hours, with sustained (>42 days) liver exposure. The saturation of transporter-mediated liver internalization was hypothesized to be due to asialoglycoprotein receptor saturation. Increasing the dose decreased the relative liver uptake efficiency in mice for intravenously and, to a lesser extent, subcutaneously administered JNJ-73763989. Lower dose levels administered subcutaneously in mice can maximize the proportion of the dose reaching the liver.

SIGNIFICANCE STATEMENT

Pharmacokinetic modeling of JNJ-73763989 liver and plasma concentration-time data in mice indicated that the proportion of JNJ-73763989 reaching the liver may be increased by administering lower subcutaneous doses compared to higher intravenous doses. Model-based simulations can be applied to optimize the dose and regimen combination.

Introduction

Hepatitis B Virus (HBV), a hepatotropic partially double-stranded DNA Orthohepadnavirus and member of the Hepadnaviridae family, causes an infection that attacks the liver and induces both acute and chronic liver disease. Acute HBV infection occurs after contact with body fluids of an infected host. Approximately 2%–6% of adults with acute HBV will develop chronic HBV infection, whereas patients exposed to HBV in early childhood have a 90% chance of developing chronic HBV. Chronic HBV infection remains a major global public health problem since patients may develop liver cirrhosis and/or hepatocellular carcinoma and are at high risk of death. The World Health Organization (2022) estimated that there were 820,000 deaths among the 296 million people living with chronic HBV in 2019.

As any other Hepadnavirus, HBV replicates via protein-primed reverse transcription of pregenomic RNA (Summers and Mason, 1982). Upon infection, circular, partially double-stranded HBV DNA is converted in the nucleus of the hepatocytes to a covalently closed circular DNA (cccDNA) that

ABBREVIATIONS: ASGPR, asialoglycoprotein receptor; cccDNA, covalently closed circular DNA; C_{Ll}, liver concentration; C_{Lr}, liver elimination clearance; C_{P}, plasma concentration; C_{tot}, total plasma concentration; F, absolute bioavailability; GalNAc, N-acetylgalactosamine; HBeAg, hepatitis B envelope antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; k_{a}, first-order absorption rate; k_{el}, elimination rate; k_{int}, liver internalization rate; k_{dis}, complex dissociation rate; k_{off}, free drug and receptor association rate; K_{SS}, steady-state constant; OFV, objective function value; PK, pharmacokinetics; Q, intercompartmental flow; rAAV, recombinant adeno-associated virus; RAI, RNA interference; RSE, relative standard error; R_{tot}, total receptor concentration; sRNA, short interfering RNA; T_{max}, time to maximum concentration; TMDD, target-mediated drug disposition; V_{C}, central volume of distribution; V_{L}, liver volume; V_{p}, peripheral volume.

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assembles into a minichromosome. This corresponds to a template for viral mRNA transcription, driving translation into viral proteins, including hepatitis B envelope antigen (HBsAg) and hepatitis B surface antigen (HBsAg) (Seegert and Mason, 2015). Additionally, HBV DNA integration in the host DNA occurs via DNA repair pathways, starting in early HBV infection (Zhao et al., 2020). While integrated HBV DNA is unable to produce pregenomic RNA, representing a replicative dead end for the virus, it is expected to influence HBV replication, persistence, and pathogenesis (Tu et al., 2017). The HBsAg-positive chronic infection stage is characterized by a high HBV replication rate and high viral protein load, which results in elevated levels of HBV DNA, HBeAg, and HBsAg in serum. This leads to progressive exhaustion and hyporesponsiveness of HBV-specific CD4+ and CD8+ T cells, deteriorating the host’s immune system because of its ineffective antigen processing capacity and the complex interplay of suppressive cytokines and various other immunomodulators (Fisicaro et al., 2020; Yang et al., 2021).

Despite the availability of several therapeutic options, including a preventive vaccine, finding a cure for chronic HBV remains challenging. The available interferon and nucleoside/nucleotide analog therapies for chronic HBV only provide a functional cure, defined as sustained HBsAg loss and HBV DNA suppression when off treatment (Ning et al., 2019). Functional cure is associated with improved clinical outcomes, suggesting prolonged patient survival (Ahn et al., 2005). However, functional cure is rarely achieved using modern antiviral treatment, as it does not eliminate the risk of resurgence of the viral infection because of the nuclear persisting cccDNA (Loglio and Lampertico, 2020).

RNA interference (RNAi), the endogenous pathway used by short interfering RNA (siRNA) therapeutics, can be used as a tool to silence gene expression successfully and specifically by degrading specific mRNA sequences complementary to the siRNA therapeutic and reducing target protein expression (Fire et al., 1998). JNJ-73763989, a siRNA combination product in clinical development for chronic HBV treatment, induced a selective degradation of all HBV mRNA transcripts and consists of two triggers: JNJ-73763976, the S-trigger, targeting all S open reading frame mRNA, including transcripts derived from integrated DNA and cccDNA-derived transcripts, and JNJ-73763924, the X-trigger, targeting X open reading frame mRNA present in all cccDNA-derived transcripts (Gane et al., 2019b). Subsequently, the degradation of viral mRNA from all sources leads to decreasing levels of circulating HBV-related proteins, including HBsAg, HBeAg, and HBV DNA. Ultimately, the goal of suppressing viral protein expression is to remove the tolerogenic effects of high antigen load, which in turn might allow immune rejuvenation, thereby potentially increasing the likelihood to obtain chronic HBV functional cure.

In JNJ-73763989, both the S- and X-triggers are siRNAs conjugated with triantennary N-acetylgalactosamine (GalNAc), facilitating hepatic delivery. GalNAc conjugates show a high affinity for the asialoglycoprotein receptor (ASGPR), predominantly expressed on the plasma membrane of hepatocytes. However, this selective shuttle for liver delivery is saturable and may lead to reduced liver entry at high doses (Bon et al., 2017). Furthermore, the relative occupancy of the ASGPR may be impacted by the choice of administration route due to typically higher concentrations shortly after intravenous administration compared with subcutaneous administration. After binding ASGPR, the GalNAc-conjugated siRNA is rapidly internalized via clathrin-mediated endocytosis (Akinc et al., 2010). While the exact mechanism of escape across the endosomal lipid bilayer membrane remains unknown, enough siRNAs enter the cytoplasm to induce robust responses in vivo (Springer and Dowdy, 2018).

Our goal was to characterize the pharmacokinetics (PK) of JNJ-73763989 in plasma and liver after intravenous and subcutaneous administration of both S- and X-triggers in recombinant adeno-associated (rAAV) HBV–infected mice to understand how efficiently JNJ-73763989 enters the liver as a function of dosing regimen and route of administration.

Materials and Methods

Study Design and Bioanalysis Methods. Data from a preclinical in vivo study including 42 rAAV-HBV–infected C57Bl/6 male mice were used. Animals, approximately 6 weeks old, with a body weight ranging from 24 to 30 g, were used to establish the rAAV-HBV infection by intravenous administration of rAAV-HBV virus (Beijing Five-Plus, Molecular Medicine Institute; China) at a concentration of 1 × 1011 viral genomes in 200 μL PBS 60 days prior to JNJ-73763989 dosing. Infection was considered stable over time as monitored by measuring HBsAg in the vehicle cohort. Mice were block randomized to intravenous or subcutaneous administration of JNJ-73763989 in a 2:1 S-to-X-trigger ratio at total (S+X) doses of 1, 3, and 10 mg/kg. JNJ-73763989 dosing formulations were freshly prepared by dissolving JNJ-73763989 in PBS (at pH 7.4). Dosing formulations were kept in the refrigerator, protected from light, and were stable for 26 days. All mice had continuous access to water and food ad libitum, and were treated humanely, i.e., in accordance with the European Council Directive of November 24, 1986 (86/609/EEG) or the United States Department of Agriculture Animal Welfare Act (9 CFR, Parts 1, 2, and 3) and the conditions specified in the Guide for Care and Use of Laboratory Animals (Clark et al., 1997).

Detailed information about the study design can be found in Table 1. Sparse venous blood sampling was performed via the saphenous vein. A 25-μL blood sample was taken at 0.5 hours (0.25 hour for intravenous) and 1 hour (0.75 hour for intravenous) postdose, and 75 μL of blood were sampled at 2 and 4 hours after dosing. Blood samples were centrifuged within 1 hour after sampling, and plasma was stored at −80°C within 1 hour after centrifugation. Blood and plasma samples were always protected from direct exposure to UV light to avoid RNA self-cleavage (Ariza-Mateos et al., 2012).

At study endpoints, mice were sacrificed via decapitation after euthanasia. Blood and tissues were harvested between 1 and 42 days after initial dosing. The liver tissues were individually homogenized in homogenization buffer (1/9 w/v) using Precellys homogenizer beads at high speed for 30 seconds. Cytotoxicity was determined using an in vitro cellular viability assay (Page et al., 2010).

All samples were analyzed for S- and X-triggers using hybridization-based liquid chromatography-fluorescence assay. The assay principle is described by Wang and Ji (2016). The linear range of quantification in plasma was 2.10 ng/mL to 2100 ng/mL for S-trigger and 1.00 ng/mL to 1000 ng/mL for X-trigger, whereas the linear range for quantification in liver homogenate was 21.0 ng/g to 21,000 ng/g for S-trigger and 10.0 ng/g to 10,000 ng/g for X-trigger. To conduct the pharmacokinetic analysis, liver concentrations expressed in nmol x g−1 were scaled to nmol x mL−1 based on the calculated relative murine hepatocyte density of 1.085 mL x g−1 (Solhenius-Sternbeck, 2006; Morales-Navarrete et al., 2015).

Modeling Analysis. The structural PK model of JNJ-73763989 used for the PK data analysis is schematically presented in Fig. 1. A nonlinear mixed-effects population PK modeling approach was used to jointly describe the plasma and liver concentration-time data across a 1–10 mg/kg s.c. dose range and a 10 mg/kg i.v. dose administration of JNJ-73763989. A two-compartment model for plasma with an
TABLE 1
Characteristics of the male rAAV-HBV-infected mice study design
All individual groups (n = 6) were divided into two cohorts (n = 3) for blood sampling within the first 4 blood sampling occasions (within 4 hours after dosing). The first cohort underwent blood sampling at the first and the third sampling timepoints, the second cohort underwent blood sampling at the second and the fourth sampling timepoints.

<table>
<thead>
<tr>
<th>Study Group</th>
<th>N</th>
<th>Route of Administration</th>
<th>JNJ-73763989 Dose (mg/kg)</th>
<th>PK Sampling Regimen (Hours Since Dosing)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>IV</td>
<td>10</td>
<td>0.25, 0.75, 2, 4, and 168*</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>SC</td>
<td>10</td>
<td>0.5, 1, 2, 4, 24*, 168*, 504*, and 1008*</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>SC</td>
<td>3</td>
<td>0.5, 1, 2, 4, and 1008*</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>SC</td>
<td>1</td>
<td>0.5, 1, 2, 4, and 1008*</td>
</tr>
</tbody>
</table>

IV, intravenous; SC, subcutaneous.

Animals from study group 2 were block randomized into cohorts of n = 6 animals and sacrificed at different time points to obtain semilongitudinal liver PK data.

Additional liver compartment, including the competition for the transporter-mediated drug disposition (TMDD) from plasma to liver, was selected to describe JNJ-73763989 PK. Both triggers were described by the same structural model, with potential differences in parameter estimates, which was judged based on visual inspection of diagnostic plots and on reduction of the objective function value (OFV) in line with $\chi^2$-test (3.84 reduction for 1 degree of freedom and $P = 0.05$). The final model was selected based on lowest OFV, adequate goodness-of-fit and visual predictive check, acceptable parameter uncertainty [e.g., percentual relative standard error (RSE) $\leq 50\%$], physiologic relevance of parameter estimate, absence of correlations, and the principle of parsimony (Dykstra et al., 2015).

In this model, after subcutaneous dosing, a linear absorption for S- and X-trigger, characterized by the first-order absorption rate constant ($k_a$), was assumed and described as follows:

$$\frac{dA_D}{dt} = -k_a \cdot A_D + A_D(t=0)=F \cdot Dose \quad (1)$$

Where $A_D$ represents the amount of S- or X-trigger in the dosing compartment after subcutaneous JNJ-73763989 administration. Absolute bioavailability ($F$) was estimated (and constrained to be between 0 and 1 using a logit transformation) for both triggers by simultaneous analysis of plasma and liver concentrations after intravenous and subcutaneous administration of JNJ-73763989.

After intravenous bolus administration or subcutaneous absorption, plasma concentration of S- and X-triggers were present in the central compartment, with a central volume of distribution ($V_C$). The distribution from the central compartment to the peripheral compartment was characterized by the intercompartmental flow ($Q$) and peripheral volume of distribution ($V_P$), which define the transfer rate constant from central to peripheral and vice versa, $k_{P,C} = \frac{Q}{V_c}$ and $k_{C,P} = \frac{Q}{V_p}$. All volumes and clearances were allometrically scaled with allometric exponents of 0.75 for clearances and 1 for volumes.

The plasma concentrations ($C_P$) of S- and X-triggers in the central compartment were eliminated via a linear pathway, quantified by plasma elimination clearances ($CL_P$) or by binding to the transporter, represented by $R$ in the model schematic of Fig. 1. Plasma S- and X-triggers bind to the transporter ($R$), forming the transporter-siRNA complex ($RC$) according to the second-order association rate ($k_{on}$) and first-order dissociation rate constant ($k_{off}$). Once the $RC$ is formed, it is internalized into the liver, according to a first-order process represented by a liver internalization rate constant ($K_{SS}$) as described in eq. 2.

$$C_P \xrightarrow{k_{on}} RC \xrightarrow{k_{off}} C_L \quad (2)$$

Once internalized in the liver, both triggers are distributed in a liver volume ($V_L$), and the corresponding liver concentrations ($C_L$) are eliminated via a linear pathway, quantified by a liver elimination clearance ($CL_L$). The binding of JNJ-73763989 to the transporter was characterized by the quasi-steady-state approximation of the TMDD model (Yan et al., 2012; Koch et al., 2017). The quasi-steady-state approximation assumes that the $RC$ is at steady state, the complex internalization rate is not negligible compared with the dissociation rate, and the drug binding to the transporter is balanced by the complex dissociation and internalization as shown in eq. 3.

$$k_{on} \cdot C_P \cdot R - (k_{int} + k_{off}) \cdot RC = 0 \quad (3)$$

The steady-state constant ($K_{SS}$) was estimated as shown in eqs. 4-6.

$$\frac{C_P \cdot R}{RC} = \frac{k_{int} + k_{off}}{k_{on}} = K_{SS} \quad (4)$$

![Fig. 1. Schematic representation of the JNJ-73763989 PK model. Suffixes S and X represent either JNJ-73763976 (S-trigger) or JNJ-73763924 (X-trigger). $C_P$ and $C_L$ represent the concentrations ($C$) in plasma and liver, respectively. Compartments in blue represent sampling compartments. IN represents subcutaneous or intravenous dosing.](image-url)
\[ C_P = \frac{1}{2} \left( C_{tot} - R_{tot} - K_{SS} \right) + \sqrt{\left( C_{tot} - R_{tot} - K_{SS} \right)^2 + 4 \cdot K_{SS} \cdot C_{tot}} \]  

(5)

\[ RC = \frac{R_{tot} \cdot C_P}{K_{SS} + C_P} \]  

(6)

\[ C_{tot} \] represents the total (free and transporter-bound) S or X concentration and total receptor concentration \( (R_{tot}) \) represents the total transporter concentration. Provided the binding properties of GalNAc conjugates to ASGPR, \( R_{tot} \) was assumed to be equal to the murine hepatic total ASGPR concentration of 647 nM reported by Bon et al. and was fixed in the model (Bon et al., 2017), \( K_{tot} \) was assumed to be consistent between both triggers and equal the transporter degradation rate, \( K_{deg} \). Assuming \( R_{tot} \) is constant over time and \( K_{int} = K_{deg} \), this resulted in \( K_{int} = R_{tot} \cdot K_{deg} \).

Both S- and X-triggers were assumed to bind to the same ASGPR binding site. Therefore, competitive binding between the two triggers was accounted for as previously described in literature, and \( K_{SS} \) was estimated for the S- and X-triggers as previously described in literature (Koch et al., 2017). The ordinary differential equations describing the S- and X-trigger concentrations in plasma as well as the unbound transporter concentrations were as follows:

\[ \frac{dC_{P,S}}{dt} = M_{11} \cdot G_1 + M_{12} \cdot G_2 + M_{13} \cdot G_3 \]  

(7)

\[ \frac{dC_{P,X}}{dt} = M_{21} \cdot G_1 + M_{22} \cdot G_2 + M_{23} \cdot G_3 \]  

(8)

\[ \frac{dR}{dt} = M_{11} \cdot G_1 + M_{12} \cdot G_2 + M_{13} \cdot G_3 \]  

(9)

Eqs. 7–9 are the product of matrix \( M \) and vector \( G \) as shown by Koch et al. (2017) and reported in eq. 10:

\[ \begin{pmatrix} \frac{dC_{P,S}}{dt} \\ \frac{dC_{P,X}}{dt} \\ \frac{dR}{dt} \end{pmatrix} = M \begin{pmatrix} C_{P,S} \\ C_{P,X} \\ R \end{pmatrix} \cdot g \left( \begin{pmatrix} C_{P,S} \\ C_{P,X} \\ R \end{pmatrix} \right) \]  

(10)

Where matrix \( M \left( C_{P,S}, C_{P,X}, R \right) \) is characterized by:

\[ \begin{pmatrix} D - RA \\ C_{P,X}R \\ D - RB \end{pmatrix} = D - RA \\ C_{P,X}R \\ D - RB \\ -RA' \ -RB' \ -DB \ -CA' \ -CA' \ -CB' \]  

(11)

Where \( D \) represents the determinant characterized by:

\[ D = R^2 + C_{P,S}K_{SS,X} + C_{P,X}K_{SS,S} + C_{P,S}R + C_{P,X}R + K_{SS,S}K_{SS,X} + K_{SS,S}R + K_{SS,X}R \]

\[ A, B, A', B' \]

represent:

\[ A = R + C_{P,X} + K_{SS,X} \]

\[ B = R + C_{P,S} + K_{SS,S} \]

\[ A' = R + K_{SS,X} \]

\[ B' = R + K_{SS,S} \]

and vector \( g \left( C_{P,S}, C_{P,X}, R \right) \) is characterized by:

\[ \begin{pmatrix} k_a \frac{C_{P,S}}{V_C} - \left( k_{u,P,S} + k_{C,S} \right) C_{P,S} - k_{P,C,S} - k_{u,C,S} \frac{C_{P,S}}{K_{SS,S}} \frac{C_{P,S}}{C_{P,X}} \\ k_a \frac{C_{P,X}}{V_C} - \left( k_{u,P,X} + k_{C,X} \right) C_{P,X} - k_{P,C,X} - k_{u,C,X} \frac{C_{P,X}}{K_{SS,X}} \frac{C_{P,X}}{C_{P,S}} \\ k_{syn} - k_{deg}R - k_{tot} \frac{C_{P,S}}{K_{SS,S}} - k_{tot} \frac{C_{P,X}}{K_{SS,X}} \end{pmatrix} \]  

(13)

Where the elimination rate constant, \( k_u \), defined as \( \frac{C_P}{t} \), was defined for the S- and X-triggers as \( k_{u,P,S} \) and \( k_{u,P,X} \) respectively.

After liver internalization, liver disposition is characterized by (using eq. 4):

\[ \frac{dA_L}{dt} = k_{in} \cdot RC \cdot V_C - k_{d,L} \cdot A_L \]  

(14)

\( A_L \) represents S- or X-trigger liver amounts and \( k_{d,L} \) represents the first-order liver elimination rate.

**Statistical Model.** Between-subject variability of the model parameters was assumed to be log-normally distributed. The individual parameter estimates (\( \theta_i \)) are defined according to eq. 15:

\[ \theta_i = \theta_{pop} \cdot e^{\epsilon_{ij}} \]  

(15)

where \( \theta_{pop} \) is the typical population parameter, and \( \epsilon_{ij} \) is assumed to be an independent and random normal variable representing the residual error for the log \( \theta_i \) concentration of the \( i^{th} \) individual for the \( j^{th} \) trigger, with zero mean and a variance of \( \sigma_{ij}^2 \).

**Model-Based Simulations.** Single-dose deterministic simulations for intravenous and subcutaneous doses of 1, 3, and 10 mg kg\(^{-1}\) were performed to evaluate the influence of dose and administration route on liver uptake of JNJ-73763989. Multiple-dose deterministic simulations for subcutaneous monthly dose levels of 1, 3, and 10 mg kg\(^{-1}\) for dosing intervals ranging from 1 to 28 days over a period of 1 year were performed to evaluate the influence of ASGPR saturation and to select the dosing regimen that maximizes JNJ-73763989 liver uptake. For multiple-dose deterministic simulations, the total dose amount administered per month was maintained constant across all dosing intervals (e.g., for a dosing interval of 2 weeks, the amount administered per dosing occasion is equal to the total monthly amount divided by 2).

**Software.** Modeling and simulation analyses were conducted using nonlinear mixed-effects modeling in NONMEM version 7.4.0 (Icon Development Solutions; Ellicott City, MD) in a validated environment. HP3 GxP, based on Good Automated Manufacturing Practice and in accordance with 21 CFR Part 11 and good clinical practice regulations. The Fortran compiler applied was Intel Fortran 64 Compiler Professional, Version 11.1. The first-order conditional estimation method was used. The exploratory and statistical analyses, diagnostic plots and postprocessing of NONMEM results were carried out in R version 3.4.1 (R Foundation for Statistical Computing; Vienna, Austria).

**Results.**

The final analysis dataset consisted of 262 concentration-time observations collected from 42 male rAAV-HBV infected mice, of which 178 observations originated from plasma and 84 observations from liver. The two-compartment PK model, including TMDD from plasma to liver, as described in the methods section, adequately described the JNJ-73763989 disposition, as can be observed in Fig. 2 (Supplemental Fig. 1), although liver JNJ-73763989 concentrations after 1 mg/kg s.c. administration were slightly overpredicted. \( CL_P, CL_L \), and \( K_{SS} \) were found to be trigger-specific. All other parameters were consistent between both triggers. Table 2 displays the parameter estimates of the final population PK model. Both fixed and random effects were estimated with adequate precision as RSEs were below 25%.
The absolute bioavailability was estimated to be 99.99% (RSE = 87.87%) for S-trigger and 98.43% (RSE = 23.19%) for X-trigger. However, the absolute bioavailability was not found to be different between the two triggers (likelihood ratio test \( \Delta \text{OFV} = 0.65 < 3.84 \), at significance level 0.05 with df = 1) and the joint estimate was not statistically different from 100% (likelihood ratio test \( \Delta \text{OFV} = 1.92 < 5.99 \), at significance level 0.05 with df = 2). Therefore, the absolute bioavailability was considered to be 100% for both S- and X-trigger. A subcutaneous absorption rate constant of 0.234 hour\(^{-1}\), corresponding to an absorption half-life of 2.73 hour, was estimated for both S- and X-trigger. After intravenous dosing, the elimination from plasma was fast, as indicated by the \( k_d \) of S- and X-trigger, which were estimated at 5.52 hour\(^{-1}\) (plasma half-life of 0.123 hour) and 10.1 hour\(^{-1}\) (plasma half-life of 0.0686 hour), respectively. JNJ-73763989 showed rapid distribution from plasma to liver with sustained liver exposure due to a slow liver elimination. The liver elimination rate constants for S- and X-trigger (0.00225 hour\(^{-1}\) and 0.00188 hour\(^{-1}\), respectively) were found to be markedly lower than the corresponding plasma elimination rate constants, with S- and X-trigger liver half-life of 12.8 days and 15.3 days, respectively.
TABLE 2
Population pharmacokinetic parameter estimates of JNJ-73763989
All volumes and clearances were allometrically scaled and centered around the mean mouse body weight of 26.7 g. Liver volumes were scaled based on the calculated liver density of 1.088 mL g$^{-1}$.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Parameter estimate (RSE%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>%</td>
<td>100 (fixed)</td>
</tr>
<tr>
<td>$k_a$</td>
<td>h$^{-1}$</td>
<td>0.2341 (2.2)</td>
</tr>
<tr>
<td>$CL_{p,S}$</td>
<td>mL h$^{-1}$ 26.7 g$^{-1}$</td>
<td>11.00 (5.2)</td>
</tr>
<tr>
<td>$CL_{p,X}$</td>
<td>mL h$^{-1}$ 26.7 g$^{-1}$</td>
<td>20.12 (2.6)</td>
</tr>
<tr>
<td>$V_C$</td>
<td>mL 26.7 g$^{-1}$</td>
<td>1.992 (12.7)</td>
</tr>
<tr>
<td>Q</td>
<td>mL h$^{-1}$ 26.7 g$^{-1}$</td>
<td>1.726 (24.8)</td>
</tr>
<tr>
<td>$V_p$</td>
<td>mL 26.7 g$^{-1}$</td>
<td>2.252 (17.4)</td>
</tr>
<tr>
<td>$CL_{S,S}$</td>
<td>mL h$^{-1}$ 26.7 g$^{-1}$</td>
<td>0.00196 (5.3)</td>
</tr>
<tr>
<td>$CL_{S,X}$</td>
<td>mL h$^{-1}$ 26.7 g$^{-1}$</td>
<td>0.00164 (1.6)</td>
</tr>
<tr>
<td>$K_{SS,S,nM}$</td>
<td>nM</td>
<td>0.8711 (9.6)</td>
</tr>
<tr>
<td>$K_{SS,S,nM}$</td>
<td>nM</td>
<td>73.7 (14.8)</td>
</tr>
<tr>
<td>$h_{int}$</td>
<td>h$^{-1}$</td>
<td>1.925 (14.0)</td>
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<tr>
<td>$R_{eid}$</td>
<td>nM</td>
<td>647 (fixed, Bon et al.)</td>
</tr>
<tr>
<td>$\omega_{V,S}$</td>
<td>%</td>
<td>33.00 (14.7)</td>
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<td>$\sigma_{C,S}$</td>
<td>%</td>
<td>29.11 (7.9)</td>
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<td>$\sigma_{C,S}$</td>
<td>%</td>
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<td>$\sigma_{C,X}$</td>
<td>%</td>
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</tbody>
</table>

$\omega_i$, inter-individual variability on the CV scale; $\sigma$, residual unexplained variability on the CV scale.

Plasma-to-liver transport was characterized by a TMDD model. $K_{SS}$ differed approximately twofold between the triggers, where X-trigger showed a higher affinity for ASGPR ($K_{SS,S}$ = 143.2 nM and $K_{SS,X}$ = 73.7 nM). Figure 3 (left) shows the relative occupancy of the transporter as a function of increasing trigger concentrations, showing the differences in transporter affinity for S- and X-triggers if administered as a monotherapy. An increased relative occupancy can be observed at $C_{max}$ after 10 mg/kg i.v. (100%) compared with s.c. (43%). The relative contributions of S- and X-triggers to transporter saturation can be observed in Fig. 3 (right). A constant ratio of 1.89 between the S-trigger relative occupancy in presence of X-trigger as well as the X-trigger relative occupancy in presence of S-trigger can be observed. This ratio is close to the dosing ratio of 2:1 (S-to-X), reflecting the interplay between the estimated difference in affinity and the plasma concentration ratios.

Body weight was found to contribute to the variability observed in JNJ-73763989 plasma and liver PK. The inclusion of this covariate effect as an allometric relationship on the clearance and volume of distribution (see Materials and Methods section) significantly improved the model fit ($\Delta$OFV = −16.02). Between-subject variability was only found significant for the liver volume of distribution ($\omega_{V,L}$ 33.0% CV). Liver residual variability was relatively low (10.3% and 9.7% CV for S- and X-trigger, respectively) compared with plasma residual variability (29.1% and 38.3% CV for S- and X-trigger, respectively).

Subcutaneously administered JNJ-73763989 liver uptake efficiency is increased compared with intravenously administered drug as illustrated in Fig. 4 (upper panels). Simulations indicated that the relative liver uptake for 1 and 10 mg/kg JNJ-73763989 decreased from 46.0% to 16.2% for S-trigger and from 45.9% to 10.7% for X-trigger after intravenous administration, compared with a decrease from 60.1% to 50.1% for S-trigger and from 61.5% to 51.4% for X-trigger after subcutaneous administration, respectively. Fig. 4 (lower panels) presents the actual dose that is reaching the liver, which increases with the dose, but this increase is relatively higher for subcutaneous compared...
with intravenous administration. The absolute drug amount reaching the liver after 3 mg/kg s.c. administration is greater than that after 10 mg/kg i.v. administration for both triggers.

Model-based simulations of the mouse liver concentration-time after multiple dose regimens of monthly 1, 3, and 10 mg/kg JNJ-73763989 are shown in Fig. 5. Simulations show less than dose-proportional liver PK upon multiple doses, especially with higher doses associated with longer intervals in this simulation setup. Interestingly, for the total doses currently investigated in this mouse model (Table 3), the liver $C_{avg}$ at steady state after 10-mg/kg monthly dosing increased from monthly to daily dosing (~20%), whereas for the two lower dose (1 and 3 mg/kg), an increase (from monthly to weekly dosing) is followed by a slight decrease (from weekly to daily dosing). This observation is due to different saturation effects across different dose levels.

**Fig. 4.** Model estimated fraction of the administered dose (A) and absolute dose amount (B) reaching the liver after JNJ-73763989 (2:1 ratio JNJ-73763976 (S-trigger): JNJ-73763924 (X-trigger)) subcutaneous and intravenous administration of 1 mg/kg (~0.67 mg/kg JNJ-73763976, 0.33 mg/kg JNJ-73763924 (X-trigger)), 3 mg/kg (~2 mg/kg JNJ-73763976, 1 mg/kg JNJ-73763924), or 10 mg/kg (~6.67 mg/kg JNJ-73763976, 3.33 mg/kg JNJ-73763924). Dashed line: 100% for upper panels, identity line in lower panels.

**Discussion**

A two-compartment plasma PK model with TMDD describing liver internalization, linear distribution to a nonspecific peripheral compartment, linear elimination from plasma and liver, with first-order absorption following subcutaneous administration was suitable to describe the time course of S- and X-trigger concentrations (including between-subject variability) after both intravenous and subcutaneous
administration of JNJ-73763989 in male rAAV-HBV infected mice.

**Absorption.** After subcutaneous administration, the absolute bioavailability for both triggers was found to be complete. Although some variability seems to be associated with the assessment of the bioavailability in nonclinical species, McDougall et al. (2022) have suggested near-complete bioavailability of GalNAc-conjugated siRNA after subcutaneous administration. The absorption rate constant (0.234 hour⁻¹) after subcutaneous administration was substantially smaller than the elimination rate constant (5.52 hour⁻¹) after intravenous administration. Consequently, the slow absorption rate translated into a prolonged terminal half-life in plasma governed by the slow absorption process. Due to the so-called "flip-flop" kinetics, the slow absorption rate translated into a prolonged terminal half-life after subcutaneous dosing relative to intravenous dosing (Fig. 2).

**Distribution.** The estimated $V_C$, 1.99 mL for a 26.7-g mouse, was very similar to the mouse plasma volume (around 1.56 mL according to FELASA guidelines). Following intravenous or subcutaneous administration, both S- and X-triggers distributed rapidly to the liver. Furthermore, the model predicted a liver weight of 0.80 g, consistent with the liver weight observed in C57BL/6 mice (3% of the total body weight) (Kushida et al., 2011). The nonlinear liver distribution, driven by saturable ASGPR-mediated hepatocyte internalization, was characterized by competitive binding between S- and X-triggers (Fig. 3). The X-trigger affinity was found to be approximately twofold higher relative to S-trigger affinity for the transporter.

Based on Fig. 3 (left panel), it was determined that >90% relative occupancy is achieved at plasma concentrations greater than 843 nM and 230 nM for S- and X-triggers, respectively, which are exceeded immediately after 10 mg/kg i.v. dosing, resulting in 100% relative occupancy at $C_{\text{max}}$ after dose. During the period where plasma concentrations are above those values, transport into the hepatocyte would be at close to maximum capacity, thus limiting the efficiency of liver transport due to transporter saturation. The $C_{\text{max}}$ after 10 mg/kg JNJ-73763989 i.v. administration exceeded $K_{\text{SSS}}$ by 1443-fold and $K_{\text{SSS}}$ by 721-fold, whereas $C_{\text{max}}$ after 10 mg/kg s.c. JNJ-73763989 administration did not exceed $K_{\text{SSS}}$ or $K_{\text{SSS}}$ (less than 1-fold). Consequently, relative occupancy at $T_{\text{max}}$ after a single 10 mg/kg s.c. dose of JNJ-73763989 was 53.9%. At plasma concentrations lower than 10.4 nM and 2.8 nM for S- and X-triggers, respectively, less than 10% ASGPR occupancy was expected, and the liver uptake increased proportionally with plasma concentrations. Finally, the steady-state volume of distribution ($V_{\text{SS}} = V_C + V_p$) was approximately 4.244 mL, suggesting that JNJ-73763989 distributes into the extravascular water.

**Elimination.** The elimination (clearance) of JNJ-73763989 from plasma was relatively fast and was quantified through a nonspecific linear process, which accounts for all possible mechanisms of JNJ-73763989 elimination except the liver disposition. After intravenous administration of JNJ-73763989, the $\alpha$ and $\beta$ plasma half-lives for S-trigger were estimated to be 6.39 minutes and 64.2 minutes, respectively, and 3.77 minutes and 59.4 minutes, respectively, for X-trigger. Liver half-life was 12.8 days and 15.3 days for S- and X-triggers, respectively, which is substantially longer than the plasma half-life.

The JNJ-73763989 PK features described above have important consequences in defining the optimal route of administration, dose level, and dosing regimen, as described below.

**Route of Administration.** The results of the mice study confirmed higher JNJ-73763989 liver exposure after subcutaneous administration, relative to intravenous administration at the
TABLE 3

<table>
<thead>
<tr>
<th>Total Monthly Dose</th>
<th>Dosing Interval (days)</th>
<th>Total Doses</th>
<th>Dose Normalized Cavg at SS (nmol/g liver/mg/kg)</th>
<th>Dose Normalized Cavg at SS (nmol/g liver)</th>
<th>Liver Accumulation at SS (Cavg at SS divided by the Cavg at initial dosing)</th>
<th>Liver Accumulation Ratio at SS</th>
<th>Doses Until 90% SS Reached</th>
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same dose level, which is explained by the flip-flop phenomenon and its effect on transporter saturation. Moreover, JNJ-74763989 is administered as subcutaneous injections in clinical phase studies, enhancing liver uptake (Gane et al., 2019a).

The limited number of ASGPR can be readily saturated with the high plasma concentrations achieved after intravenous administration of JNJ-73763989. This may lead to considerable dose wastage and a reduction of liver uptake efficiency relative to the subcutaneous route, whereas the prolonged absorption provides relatively lower plasma concentrations leading to reduced ASGPR saturation but longer-lasting liver penetration (Fig. 4).

**Dose Level.** Given the ASGPR-mediated disposition, the increase in JNJ-73763989 liver exposure with dose became less than dose-proportional, as shown in Fig. 4. This effect is clearly more prominent following intravenous dosing compared with subcutaneous administration. Furthermore, the difference in the fraction of the dose that reaches the hepatocytes between intravenous and subcutaneous dosing decreased with dose (Fig. 4). Similar results were found by McDougall et al. (2022) and Ayyar et al. (2021), both reporting increased ASGPR saturation upon increasing dose. This behavior is typically associated with a less than dose-proportional increase in liver exposure and more than dose-proportional increase in plasma exposure. Interestingly, this finding of the percentual dose recovery in the liver increasing in a less than dose-proportional manner is also consistent with that of McDougall et al., (2022).

**Dosing Regimen.** Repeated dosing according to the simulated dosing regimens leads to JNJ-73763989 liver accumulation in mice. Since higher doses lead to increased transporter saturation, lower Cavg at steady state is typically observed for less frequent dosing compared with more frequent dosing for a given total monthly dose. Moreover, longer dosing intervals lead to more fluctuation in the time course (peak-to-trough) of JNJ-73763989 liver concentrations. In this context, daily dosing will lead to smaller fluctuations in the JNJ-73763989 liver concentration-time profile but slightly larger liver accumulation than weekly, biweekly, or monthly dosing (Fig. 5; Table 3). Interestingly, with the simulated multiple dosing regimen, daily dosing leads to accumulation of JNJ-73763989 in plasma, subsequently leading to a less efficient liver uptake, as expected in the absence of plasma accumulation. Consequently, weekly dosing of JNJ-73763989 in mice may allow combination of the smallest liver concentration fluctuations with negligible plasma accumulation.

In conclusion, JNJ-73763989 is a GalNAc-siRNA combination product consisting of S- and X-triggers, saturating the transporter at sufficiently high concentrations, which is limiting liver uptake. Complete bioavailability and slower plasma kinetics after subcutaneous absorption in this mouse model allow limitation of transporter saturation, thereby increasing relative drug volumes reaching the liver. Model-based simulations can aid in the optimization of dose and regimen combination in case there is transporter saturation. Our findings suggest lower dose levels administered subcutaneously in mice maximize liver uptake efficiency in terms of relative drug amounts.

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**Authorship Contributions**

**Participated in research design:** Goeyvaerts, Perez-Ruixo.

**Conducted experiments:** Sandra, Tjollyn, Goeyvaerts, Vermeulen, Dosne.

**Performed data analysis:** Sandra, Tjollyn, Goeyvaerts, Vermeulen, Dosne, Perez-Ruixo.

**Wrote or contributed to the writing of the manuscript:** Sandra, Perez-Ruixo.

**References**


Address correspondence to: Huybrecht Tjollyn. E-mail address: htjollyn@its.jnj.com

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