Plasma and liver pharmacokinetics of the GalNAc-siRNA JNJ-73763989 in rAAV-HBV infected mice

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interfering RNA, $T_{\text{max}}$, time to maximum concentration; TMDD, target-mediated drug disposition; $V_c$, central volume; $V_L$, liver volume; $V_P$, peripheral volume

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ABSTRACT

JNJ-73763989 is a N-Acetylgalactosamine (GalNAc) conjugated short interfering RNA (siRNA) combination product, consisting of 2 triggers, in clinical development for chronic hepatitis B virus (HBV) infection treatment, inducing a selective degradation of all HBV mRNA transcripts. Our aim is to characterize the plasma and liver pharmacokinetics (PK) of JNJ-73763989 after intravenous (IV) and subcutaneous (SC) 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 IV or 1, 3 and 10 mg/kg SC. 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 (TMDD) including internalization to the liver compartment, linear elimination from plasma and liver, and first-order absorption following SC administration was suitable to describe both plasma and liver PK. After SC 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 (ASGPR) saturation. Increasing the dose decreased the relative liver uptake efficiency in mice for IV, and to a lower extent for SC administered JNJ-73763989. Lower dose levels administered SC 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 SC doses compared to higher IV 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 to develop 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. In 2019, WHO estimated 820,000 deaths annually among the 296 million people living with chronic HBV (World Health Organization, 2022).

As any other Hepadnavirus, HBV replicates via protein-primed reverse transcription of pregenomic RNA (pgRNA) (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 assembles into a mini-chromosome. This corresponds to a template for viral mRNA transcription, driving translation into viral proteins, including hepatitis B envelope antigen (HBeAg) and hepatitis B surface antigen (HBsAg) (Seeger 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 pgRNA, representing a replicative dead-end for the virus, it is expected to influence HBV replication, persistence and pathogenesis (Tu et al., 2017). The HBeAg 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 analogue therapies
for chronic HBV only aim for 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 successfully and specifically silence gene expression 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 (ORF) mRNA including transcripts derived from integrated DNA and cccDNA-derived transcripts, and JNJ-73763924, the X-trigger, targeting X ORF 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, suppression of viral protein expression is aimed at removing 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 triantennerary 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 (IV) administration compared to subcutaneous (SC) administration. After binding ASGPR, the GalNAc-conjugated siRNA is rapidly internalized via clathrin-mediated endocytosis (Akinc et al.,
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 IV and SC administration of both S- and X-trigger in recombinant adeno-associated (rAAV) HBV infected mice, aiming to understand the efficiency of JNJ-73763989 to enter the liver as a function of dosing regimen and route of administration.

**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 IV administration of rAAV-HBV virus (Beijing FivePlus, Molecular Medicine Institute, China) at a concentration of $1 \cdot 10^{11}$ viral genomes in 200 µL phosphate buffered saline (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 JNJ-73763989 IV or SC in a 2:1 S-to-X-trigger ratio at a total (S+X) dose 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 USDA 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 h (0.25 h for IV) and 1 h (0.75
8 h for IV) post-dose, and 75 μL of blood was sampled at 2 and 4 h 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. Samples were heat inactivated at 56 °C overnight and were stored at -80 °C until analysis.

All samples were analyzed for S- and X-trigger using hybridization-based liquid chromatography-fluorescence assay. The assay principle is described in Wang et al. 2016 (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 21000 ng/g for S-trigger and 10.0 ng/g to 10000 ng/g for X-trigger. To conduct the pharmacokinetic analysis, liver concentrations expressed in nmol·g⁻¹ were scaled to nmol·mL⁻¹ based on the calculated relative murine hepatocyte density of 1.088 mL·g⁻¹ (Sohlenius-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 to 10 mg/kg SC dose range and after a 10 mg/kg IV dose administration of JNJ-73763989. A two-compartment model for plasma with an 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 χ²-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) ≤ 50%), physiological relevance of parameter estimate, absence of correlations, and the principle of parsimony (Dykstra et al., 2015).

In this model, after SC 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:

$$\frac{dA_D}{dt} = -k_a \cdot A_D \text{ and } A_D(t = 0) = F \cdot \text{Dose}$$  \hspace{1cm} \text{Equation 1}

where $A_D$ represents the amount of S or X-trigger in the dosing compartment after SC 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 IV and SC administration of JNJ-73763989.

After IV bolus administration or SC 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, $k_{cp} = \frac{Q}{V_c}$ and $k_{pc} = \frac{Q}{V_p}$. 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 ($k_{int}$) as described in Equation 2.
\[ C_p + R \xrightarrow{k_{on}} RC \xrightarrow{k_{off}} C_L \]  

Equation 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 (QSS) approximation of the TMDD model (Yan et al., 2012; Koch et al., 2017). The QSS approximation assumes that the \(RC\) is at steady state, the complex internalization rate is not negligible compared to the dissociation rate and the drug binding to the transporter is balanced by the complex dissociation and internalization as shown in Equation 3:

\[ k_{on} \cdot C_p \cdot R - (k_{int} + k_{off}) \cdot RC = 0 \]  

Equation 3

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

\[ \frac{C_p \cdot R}{RC} = \frac{k_{int} + k_{off}}{k_{on}} = K_{SS} \]  

Equation 4

\[ C_p = \frac{1}{2} \left[ (C_{tot} - R_{tot} - K_{SS}) + \sqrt{(C_{tot} - R_{tot} - K_{SS})^2 + 4 \cdot K_{SS} \cdot C_{tot}} \right] \]  

Equation 5

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

Equation 6

\(C_{tot}\) represents the total (free and transporter-bound) S or X concentration and \(R_{tot}\) 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). \(k_{int}\) 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 \(k_{syn} = R_{tot} \cdot k_{int}\).
Both S- and X-trigger 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 $K_{SS,S}$ and $K_{SS,X}$ were estimated (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:

\[
\frac{dc_{PS}}{dt} = M_{11} \cdot G_1 + M_{12} \cdot G_2 + M_{13} \cdot G_3 \quad \text{Equation 7}
\]

\[
\frac{dc_{PX}}{dt} = M_{21} \cdot G_1 + M_{22} \cdot G_2 + M_{23} \cdot G_3 \quad \text{Equation 8}
\]

\[
\frac{dR}{dt} = M_{31} \cdot G_1 + M_{32} \cdot G_2 + M_{33} \cdot G_3 \quad \text{Equation 9}
\]

Equations 7 to 9 are the product of matrix (M) and vector (G) as shown by Koch et al (Koch et al., 2017) and reported in Equation 10:

\[
\begin{pmatrix}
\frac{dc_{PS}}{dt} \\
\frac{dc_{PX}}{dt} \\
\frac{dR}{dt}
\end{pmatrix}
= M \left( C_{PS}, C_{PX}, R \right) \cdot g \left( C_{PS}, C_{PX}, R \right) \quad \text{Equation 10}
\]

Where matrix $M \left( C_{PS}, C_{PX}, R \right)$ is characterized by:

\[
\frac{1}{D}
\begin{pmatrix}
D - R \left( R + C_{PX} + K_{SS,X} \right) & C_{PS}R & -C_{PS} \left( R + K_{SS,X} \right) \\
C_{PX}R & D - R \left( R + C_{PS} + K_{SS,S} \right) & -C_{PX} \left( R + K_{SS,S} \right) \\
-R \left( R + K_{SS,X} \right) & -R \left( R + K_{SS,S} \right) & D - C_{PS} \left( R + K_{SS,S} \right) - C_{PX} \left( R + K_{SS,S} \right)
\end{pmatrix}
\]

where D represents the determinant characterized by:

\[
D = R^2 + C_{PS}K_{SS,X} + C_{PX}K_{SS,S} + C_{PS}R + C_{PX}R + K_{SS,S}K_{SS,X} + K_{SS,S}R + K_{SS,X}R \quad \text{Equation 11}
\]

and vector $g \left( C_{PS}, C_{PX}, R \right)$ is characterized by:
Where the elimination rate constant, \( k_{el} \), defined as \( \frac{C_{lp}}{V_C} \), was defined for the S- and X-trigger as \( k_{el,P,S} \) and \( k_{el,P,X} \) respectively.

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

\[
\frac{dA_L}{dt} = k_{int} \cdot RC \cdot V_C - k_{el,L} \cdot A_L \tag{Equation 13}
\]

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

**Statistical model.** Between-subject variability (BSV) of the model parameters was assumed to be log-normally distributed. The individual parameter estimates (\( \theta_i \)) are defined according to Equation 14:

\[
\theta_i = \theta_{pop} \cdot e^{\eta_{\theta,i}} \tag{Equation 14}
\]

where \( \theta_{pop} \) is the typical population parameter, and \( \eta_{\theta,i} \) is assumed to be an independent and random normal individual deviation from \( \log(\theta_{pop}) \) with zero mean and a variance of \( \omega^2 \). Residual error was described by an additive error model in the log domain for both plasma and liver concentrations. For the \( j \)th observed concentration of the \( i \)th individual for the \( k \)th trigger, the relation for observation \( Y_{ijk} \) is described by Equation 15.

\[
\log(Y_{ijk}) = \log(c_{pred,ijk}) + \epsilon_{ijk} \tag{Equation 15}
\]

where \( c_{pred,ijk} \) is the predicted plasma or liver concentration for the \( j \)th concentration of the \( i \)th individual for the \( k \)th trigger, and \( \epsilon_{ijk} \) is assumed to be an independent and random normal variable representing the residual error for the log \( j \)th concentration of the \( i \)th individual for the \( k \)th trigger, with zero mean and a variance of \( \sigma_k^2 \).
**Model-based simulations.** Single dose deterministic simulations for IV and SC doses of 1, 3 and 10 mg kg\(^{-1}\) were performed to evaluate the influence of dose and administration route on the liver uptake of JNJ-73763989. Multiple dose deterministic simulations for SC monthly dose levels of 1, 3 and 10 mg kg\(^{-1}\) for dosing intervals ranging between 1 day 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.** Modelling and simulation analyses were conducted using non-linear mixed-effects modeling in NONMEM® version 7.4.0 (Icon Development Solutions, Ellicott City, MD, USA) 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(R) Fortran 64 Compiler Professional, Version 11.1. The first-order conditional estimation method (FOCE) was used. The exploratory and statistical analyses, diagnostic plots and post-processing 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 ([Supp. Fig. 1](#)), although liver JNJ-73763989 concentrations after 1 mg/kg SC 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 RSE were below 25%.
The absolute bioavailability (RSE%) 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 ΔOFV=0.65<3.84, at significance level 0.05 with df=1) and also the joint estimate was not statistically different from 100% (likelihood ratio test ΔOFV=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 SC absorption rate constant of 0.234 h⁻¹, corresponding to an absorption half-life of 2.73 h, was estimated for both S- and X-trigger. After IV dosing, the elimination from plasma was fast as indicated by the $k_{el}$ of S- and X-trigger, which were estimated at 5.52 h⁻¹ (plasma half-life of 0.123 h) and 10.1 h⁻¹ (plasma half-life of 0.0686 h), 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 h⁻¹ and 0.00188 h⁻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.

Plasma-to-liver transport was characterized by a TMDD model. $K_{SS}$ differed approximately 2-fold 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). Fig. 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-trigger if administered as a monotherapy. An increased relative occupancy can be observed at $C_{max}$ after 10 mg/kg IV (100%) compared to SC (43%). The relative contributions of S- and X-trigger towards saturating the transporter 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 allometric relationship on the clearance and volume of
distribution (see 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% coefficient of variation (CV)). Liver residual variability was relatively low (10.3% and 9.7% CV for S- and X-trigger, respectively) compared to plasma residual variability (29.1% and 38.3% CV for S- and X-trigger, respectively).

SC administered JNJ-73763989 liver uptake efficiency is increased compared to IV 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 IV 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 SC administration, respectively. Fig. 4 (lower panels) presents the actual dose that is reaching the liver, which increases upon increasing the dose, but this increase is relatively higher for SC compared to IV administration. The absolute drug amount reaching the liver after 3 mg/kg SC administration is greater than that after 10 mg/kg IV 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 SC administered JNJ-73763989 are shown in Figure 5. Simulations show less than dose proportional liver PK upon multiple dosing, 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 SS after 10 mg/kg monthly dosing increased from monthly to daily dosing (ca. 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.

**DISCUSSION**

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

Absorption. After SC 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 non-clinical species, McDougall et al. have suggested near-complete bioavailability of GalNAc-conjugated siRNA after subcutaneous administration (McDougall et al., 2022). The absorption rate constant (0.234 h⁻¹) after SC administration was substantially smaller than the elimination rate constant (5.52 h⁻¹ for S and 10.1 h⁻¹ for X-trigger), which translates into a terminal decline phase 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 SC dosing relative to IV dosing (Fig. 2).

Distribution. The estimated central volume of distribution ($V_c$), 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 IV or SC 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 non-linear liver distribution, driven by saturable ASGPR-mediated hepatocyte internalization, was characterized by competitive binding between S- and X-trigger (Fig. 3). The X-trigger affinity was found to be approximately 2-fold 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-trigger respectively, which are exceeded immediately after 10 mg/kg IV dosing, resulting in 100% relative occupancy at $C_{max}$ after IV. 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 $C_{max}$ after 10 mg/kg JNJ-73763989 IV administration exceeded $K_{SS,S}$ by 1443-fold and $K_{SS,X}$ by 721-fold, whereas $C_{max}$ after 10 mg/kg JNJ-73763989 SC administration did not
exceed $K_{SS,S}$ or $K_{SS,X}$ (less than 1-fold). As a consequence, relative occupancy at $T_{max}$ after a single 10 mg/kg SC dose of JNJ-73763989 was 53.9%. At plasma concentrations lower than 10.4 nM and 2.8 nM for S- and X-trigger respectively, less than 10% ASGPR occupancy was expected, and the liver uptake increased proportionally with plasma concentrations. Finally, the steady-state volume of distribution ($V_{SS} = V_c + V_p$) 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 non-specific linear process, which accounts for all possible mechanisms of JNJ-73763989 elimination, except the liver disposition. After IV administration of JNJ-73763989, the alpha and beta plasma half-lives for S-trigger were estimated to be 6.39 min and 64.2 min respectively, and 3.77 min and 59.4 min, respectively, for X-trigger. Liver half-life was 12.8 days and 15.3 days for S- and X-trigger, 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 SC administration, relative to IV administration at the same dose level, which is explained by the flip-flop phenomenon and its effect on transporter saturation. Moreover, JNJ-74763989 is administered as SC 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 IV administration of JNJ-73763989. This may lead to considerable dose wastage and a reduction of liver uptake efficiency relative to the SC 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 IV dosing compared to SC administration. Furthermore, the difference in the fraction of the dose that reaches the hepatocytes between IV and SC dosing decreased with dose (Fig. 4). Similar results were found by McDougall et al. and Ayyar et al., both reporting increased ASGPR saturation upon increasing dose (Ayyar et al., 2021; McDougall et al., 2022). 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. (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 $C_{avg}$ at SS is typically observed for less compared to 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 and 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 to combine 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 SC absorption in this mouse model allow to limit transporter saturation, thereby increasing relative drug amounts 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 SC in mice maximize liver uptake efficiency (in terms of relative drug amounts).
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AUTHOR CONTRIBUTIONS

Participated in research design: Goeyvaerts, Perez-Ruixo

Conducted in silico experiments: Sandra, T’jollyn, Goeyvaerts, Dosne, Vermeulen

Performed data analysis and interpretation of the results: Sandra, T’jollyn, Goeyvaerts, Dosne, Vermeulen, Perez-Ruixo

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

All authors reviewed the draft version and provided approval of the final version that was submitted for publication.
REFERENCES


FOOTNOTES

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Conflicts of Interest. Louis Sandra, Huybrecht T’jollyn, Nele Goeyvaerts, An Vermeulen, Anne-Gaëlle Dosne and Juan-José Pérez-Ruixo were employees of Janssen Research & Development at the time this analysis was conducted. Louis Sandra, Huybrecht T’jollyn, Nele Goeyvaerts, An Vermeulen, Anne-Gaëlle Dosne and Juan-José Pérez-Ruixo are shareholders of Johnson & Johnson.

Availability of data and material. The datasets generated and/or analyzed during the current study belong to Janssen Research & Development and are not publicly available.

FIGURE CAPTIONS

Fig. 1: Schematic representation of the JNJ-73763989 pharmacokinetic 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 SC or IV dosing. \( k_a \): SC absorption rate constant, \( CL_p \): plasma clearance, Q: intercompartmental flow, \( CL_L \): liver clearance, \( k_{int} \): liver internalization rate constant, \( k_{syn} \): zero-
order transporter synthesis rate, $k_{deg}$: first-order transporter degradation rate constant, $R$: receptor (ASGPR).

**Fig. 2:** JNJ-73763976 (S-trigger) and JNJ-73763924 (X-trigger) observed plasma and liver concentrations (circles) and corresponding population model predictions after IV and SC administration of JNJ-73763989. Dots and error bars represent mean observed data ($\pm$SD), full lines represent population model predictions. Black horizontal dotted line indicates $K_{SS,S}$ and $K_{SS,X}$, red horizontal dotted line indicates LLOQ. Plasma observations for IV 10 mg/kg, SC 3 mg/kg and SC 1 mg/kg were collected from $n=3$ mice per cohort. Plasma observations for SC 10 mg/kg were collected from $n=12$ mice per cohort for concentration-time points until 4 h after dosing and $n=6$ mice at 24 h after dosing. Liver observations were collected from $n=6$ mice. Relative standard error on the mean is approximately 10% across replicates.

**Fig. 3:** Model estimated relative transporter occupancy as a function of JNJ-73763976 (S-trigger) and JNJ-73763924 (X-trigger) concentration. (left) relative occupancy of JNJ-73763976 or JNJ-73763924 monotherapy compared to relative occupancy of JNJ-73763989 (JNJ-73763976 and JNJ-73763924 2:1 simultaneous administration) and (right) Relative contribution of JNJ-73763976 and JNJ-73763924 to the relative occupancy when administered simultaneously in a 2:1 ratio. Selected competitive binding concentrations represent biorelevant concentration ranges. Vertical dashed lines represent $C_{max}$ of JNJ-73763976 and JNJ-73763924 concentrations after 10 mg/kg SC administration. Relative occupancy was calculated using equations 1 to 5 in the supplementary materials (Supp. Formulae).

**Fig. 4:** Model estimated fraction of the administered dose (upper panels) and absolute dose amount (lower panels) reaching the liver after JNJ-73763989 (2:1 ratio JNJ-73763976 (S-trigger): JNJ-73763924 (X-trigger)) SC and IV 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.

Fig. 5: Simulated JNJ-73763976 (S-trigger; upper) and JNJ-73763924 (X trigger; lower) liver concentrations following multiple JNJ-73763989 total monthly doses of 1, 3 and 10 mg/kg SC administered at a monthly, biweekly, weekly and daily schedule. Full lines represent deterministic simulations, horizontal dotted lines represent liver $C_{avg}$ at SS.
**TABLES**

<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^<em>^, 168^</em>^, 504^<em>^, 1008^</em>^</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>

Table 1: Characteristics of the male rAAV-HBV infected mice study design.

[^#] Animals from study group 2 were block randomized into cohorts of n=6 animals and sacrificed at different time points to obtain semi-longitudinal liver PK data. ^* Sampling time point after which the animal was sacrificed, liver PK data only available at final timepoint. ^[^] 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. IV: intravenous, SC: subcutaneous.
Table 2: Population pharmacokinetic parameter estimates of JNJ-73763989.

<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>$mL \cdot h^{-1} \cdot 26.7 \ g^{-1}$</td>
<td>11.00 (5.2)</td>
</tr>
<tr>
<td>$CL_{p,x}$</td>
<td>$mL \cdot h^{-1} \cdot 26.7 \ g^{-1}$</td>
<td>20.12 (2.6)</td>
</tr>
<tr>
<td>$V_c$</td>
<td>$mL \cdot 26.7 \ g^{-1}$</td>
<td>1.992 (12.7)</td>
</tr>
<tr>
<td>$Q$</td>
<td>$mL \cdot h^{-1} \cdot 26.7 \ g^{-1}$</td>
<td>1.726 (24.8)</td>
</tr>
<tr>
<td>$V_p$</td>
<td>$mL \cdot 26.7 \ g^{-1}$</td>
<td>2.252 (17.4)</td>
</tr>
<tr>
<td>$CL_{LS}$</td>
<td>$mL \cdot h^{-1} \cdot 26.7 \ g^{-1}$</td>
<td>0.00196 (5.3)</td>
</tr>
<tr>
<td>$CL_{LX}$</td>
<td>$mL \cdot h^{-1} \cdot 26.7 \ g^{-1}$</td>
<td>0.00164 (1.6)</td>
</tr>
<tr>
<td>$V_L$</td>
<td>$mL \cdot 26.7 \ g^{-1}$</td>
<td>0.8711 (9.6)</td>
</tr>
<tr>
<td>$K_{SSS}$</td>
<td>$nM$</td>
<td>143.2 (16.0)</td>
</tr>
<tr>
<td>$K_{SSX}$</td>
<td>$nM$</td>
<td>73.7 (14.8)</td>
</tr>
<tr>
<td>$k_{int}$</td>
<td>$h^{-1}$</td>
<td>1.925 (14.0)</td>
</tr>
<tr>
<td>$R_{tot}$</td>
<td>$nM$</td>
<td>647 (fixed, Bon et al.)</td>
</tr>
<tr>
<td>$\omega_{V_L}$</td>
<td>%</td>
<td>33.00 (14.7)</td>
</tr>
<tr>
<td>$\sigma_{CP,S}$</td>
<td>%</td>
<td>29.11 (7.9)</td>
</tr>
<tr>
<td>$\sigma_{CP,X}$</td>
<td>%</td>
<td>38.30 (8.5)</td>
</tr>
<tr>
<td>$\sigma_{CL,S}$</td>
<td>%</td>
<td>10.32 (15.9)</td>
</tr>
<tr>
<td>$\sigma_{CL,X}$</td>
<td>%</td>
<td>9.67 (15.4)</td>
</tr>
</tbody>
</table>
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\(^{-1}\). F: absolute bioavailability, \(k_{a}\): SC absorption rate constant, \(CL_{p}\): plasma clearance, \(V_{c}\): volume of central compartment, Q: intercompartmental flow, \(V_{p}\): volume of peripheral compartment, \(CL_{L}\): liver clearance, \(V_{L}\): volume of liver compartment, \(K_{SS}\): steady-state constant, \(k_{int}\): liver internalization rate constant, \(R_{tot}\): total ASGPR concentration, \(\omega\): inter-individual variability on the CV scale, \(\sigma\): residual unexplained variability on the CV scale.
Table 3: Number of doses needed to achieve 90% of steady state (SS), average liver concentrations at SS and liver accumulation ratio after multiple SC dosing of JNJ-73763989, stratified by dosing interval and total monthly dose.

<table>
<thead>
<tr>
<th>Total monthly dose level (mg/kg)</th>
<th>Dosing Interval (days)</th>
<th>JNJ-73763976 (S-trigger)</th>
<th>JNJ-73763924 (X-trigger)</th>
<th>JNJ-73763924 (X-trigger)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Doses until 90% SS reached</td>
<td>$C_{avg}$ at SS (nmol/g liver)</td>
<td>Dose normalized $C_{avg}$ at SS (nmol/g liver/mg/kg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>28</td>
<td>2</td>
<td>577.69</td>
<td>577.69</td>
</tr>
<tr>
<td>1</td>
<td>14</td>
<td>4</td>
<td>584.54</td>
<td>584.54</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>7</td>
<td>587.56</td>
<td>587.56</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>43</td>
<td>576.22</td>
<td>576.22</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
<td>2</td>
<td>1668.37</td>
<td>556.12</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>4</td>
<td>1721.31</td>
<td>573.77</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>7</td>
<td>1746.25</td>
<td>582.08</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>43</td>
<td>1725.66</td>
<td>575.2</td>
</tr>
<tr>
<td>10</td>
<td>28</td>
<td>2</td>
<td>4811.18</td>
<td>481.12</td>
</tr>
<tr>
<td>10</td>
<td>14</td>
<td>4</td>
<td>5359.02</td>
<td>535.90</td>
</tr>
<tr>
<td>10</td>
<td>7</td>
<td>7</td>
<td>5635.27</td>
<td>563.53</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>43</td>
<td>5727.12</td>
<td>572.71</td>
</tr>
</tbody>
</table>
All mice were SC dosed with JNJ-73763989 with a 2:1 S:X ratio. Total monthly dose amounts administered are constant (e.g. doses administered for the dosing interval of 2 weeks were half monthly doses). Doses until 90% SS reached was determined based on when 90% $C_{avg}$ is reached after initial dosing and the liver accumulation ratio at SS was determined by $C_{avg}$ at SS divided by the $C_{avg}$ after initial dosing. Simulations were carried out over a period of 1 year.
Fig. 1

```
IN_{SC,S}   SC depot   IN_{IV,S}   k_a   IN_{IV,X}   C_P,S
          k_a   Q   C_L_P,S     k_{syn}   RC_S   k_{int}   C_L,S
SC depot  Periphery  SC depot  Periphery  C_P,X  k_a   IN_{IV,X}   CL_{P,X}
          k_a   Q   C_L_{P,X}   k_{deg}   RC_X   k_{int}   C_L,X

Sampling compartment
Non-sampling compartment

Free concentration
C + Free concentration
R ↔ Complex
RC
```

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Fig. 3

Plasma concentration, nM

Relative transporter occupancy, %

Plasma concentration, nM

Relative transporter occupancy, %

- JNJ-73763924
- JNJ-73763989
- JNJ-73763976
Fig. 5
Article Title: Plasma and liver pharmacokinetics of the GalNAc-siRNA JNJ-73763989 in rAAV-HBV infected mice

Authors: Louis Sandra1,2, Huybrecht T’jollyn1, Nele Goeyvaerts1, An Vermeulen1,2, Anne-Gaëlle Dosne1, Juan-Jose Perez-Ruixo1

Journal: The Journal of Pharmacology and Experimental Therapeutics

Manuscript #: JPET-AR-2022-001229
**SUPPLEMENTAL MATERIAL**

**Supp. Fig. 1**: Goodness-of-fit (GOF) plots of the final structural model:

- **Plasma**
- **Liver**

CWRES: conditionally weighted residuals, DV: dependent variable, IPRED: individual prediction, PRED: population prediction, TSLD: time since last dose
**Supp. Formulae:** Formulae to calculate relative transporter occupancy (RO).

Equation 1: RO of S trigger alone

\[ RO_{S\text{alone}} = \frac{C_{P,S}}{C_{P,S} + K_{SS,S}} \]

Equation 2: RO of X trigger alone

\[ RO_{X\text{alone}} = \frac{C_{P,X}}{C_{P,X} + K_{SS,X}} \]

Equation 3: RO of S trigger in presence of X trigger (calculated via the empirical \( C_{P,S} \) vs \( C_{P,X} \) correlation)

\[ RO_{S\text{in presence of } X} = \frac{C_{P,S}}{C_{P,S} + K_{SS,S} \cdot \left(1 + \frac{C_{P,X}}{K_{SS,X}}\right)} \]

Equation 4: RO of X trigger in presence of S trigger (calculated via the empirical \( C_{P,S} \) vs \( C_{P,X} \) correlation)

\[ RO_{X\text{in presence of } S} = \frac{C_{P,X}}{C_{P,X} + K_{SS,X} \cdot \left(1 + \frac{C_{P,S}}{K_{SS,S}}\right)} \]

Equation 5: JNJ-73763989 RO

\[ RO_{JNJ-73763989} = \frac{C_{P,S}}{C_{P,S} + K_{SS,S} \cdot \left(1 + \frac{C_{P,X}}{K_{SS,X}}\right)} + \frac{C_{P,X}}{C_{P,X} + K_{SS,X} \cdot \left(1 + \frac{C_{P,S}}{K_{SS,S}}\right)} \]