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ARTICLE

# PK/PD modeling of FXI antisense oligonucleotides to bridge the dose-FXI activity relation from healthy volunteers to end-stage renal disease patients

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**Abstract**

IONIS-FXI$_{RX}$ (BAY2306001) is an antisense oligonucleotide that inhibits the synthesis of coagulation factor XI (FXI) and has been investigated in healthy volunteers and patients with end-stage renal disease (ESRD). FXI-LICA (BAY2976217) shares the same RNA sequence as IONIS-FXI$_{RX}$ but contains a GalNAc-conjugation that facilitates asialoglycoprotein receptor (ASGPR)-mediated uptake into hepatocytes. FXI-LICA has been studied in healthy volunteers and is currently investigated in patients with ESRD on hemodialysis. We present a model-informed bridging approach that facilitates the extrapolation of the dose-exposure-FXI relationship from IONIS-FXI$_{RX}$ to FXI-LICA in patients with ESRD and, thus, supports the selection of FX-LICA doses being investigated in patients with ESRD. A two-compartment pharmacokinetic (PK) model, with mixed first- and zero-order subcutaneous absorption and first-order elimination, was combined with an indirect response model for the inhibitory effect on the FXI synthesis rate via an effect compartment. This PK/pharmacodynamic model adequately described the median trends, as well as the interindividual variabilities for plasma drug concentration and FXI activity in healthy volunteers of IONIS-FXI$_{RX}$ and FXI-LICA, and in patients with ESRD of IONIS-FXI$_{RX}$. The model was then used to predict dose-dependent steady-state FXI activity following repeat once-monthly doses of FXI-LICA in a virtual ESRD patient population. Under the assumption of similar ASGPR expression in patients with ESRD and healthy volunteers, doses of 40 mg, 80 mg, and 120 mg FXI-LICA are expected to cover the target range of clinical interest for steady-state FXI activity in the phase IIb study of FXI-LICA in patients with ESRD undergoing hemodialysis.

**Study Highlights**

**WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?**

IONIS-FXI$_{RX}$ is an antisense oligonucleotide inhibiting the synthesis of coagulation factor XI (FXI) that was clinically studied in healthy volunteers, patients who underwent total knee arthroplasty, and patients with end-stage renal disease (ESRD). FXI-LICA shares the same RNA sequence as IONIS-FXI$_{RX}$ but contains a GalNAc-conjugation. FXI-LICA was recently studied in healthy volunteers.

**WHAT QUESTION DID THIS STUDY ADDRESS?**

We present a model-informed bridging approach to extrapolate the dose-exposure-FXI relationship from IONIS-FXI$_{RX}$ to FXI-LICA to support dosing decisions for FXI-LICA in a phase IIb study in patients with ESRD on hemodialysis.

**WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?**

Doses of 40 mg, 80 mg, and 120 mg were confirmed as dose levels to be tested in the phase IIb study of FXI-LICA in patients with ESRD undergoing hemodialysis.

**HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?**

This analysis serves as an example for a model-informed bridging approach that can speed up the clinical development process.

## INTRODUCTION

Coagulation factor XI (FXI) is a promising target for development of a new generation of anticoagulants with superior properties. Inhibiting FXI promises strong antithrombotic efficacy in combination with reduced bleeding risk.[1] Clinical and epidemiologic data from patients with constitutively low FXI levels provide evidence for reduced thromboembolic risk in combination with no or only minimally increased bleeding risk.[2-4] Today, the value of FXI as a therapeutic target is being supported by novel clinical data from anti-FXI therapeutics under development.[5]

Antisense oligonucleotides (ASOs) are synthetized strings of nucleic acids that bind specifically to complementary intracellular RNA produced by a particular gene, thereby preventing its translation to the specific protein. Other applications of ASOs include splice modulation by blocking the binding sites of pre-mRNA splicing factors.

IONIS-FXI$_{RX}$ (BAY2306001, former name ISIS-416858) is a 20-mer 2′-MOE chimeric ASO inhibitor of the synthesis of FXI in the liver that was the first drug candidate demonstrating that a reduction in FXI activity leads to a significantly reduced risk of postoperative venous thromboembolism in comparison to standard of care in patients who underwent total knee arthroplasty (TKA).[5] In this proof-of-concept study, two dose levels of IONIS-FXI$_{RX}$ (200 mg and 300 mg administered once-weekly by subcutaneous administration) were compared to enoxaparin. The 200 mg dose regimen was noninferior, and the 300 mg dose regimen was superior to enoxaparin for the prevention of venous thromboembolism, and the rate of major or clinically relevant nonmajor bleeding was numerically lower with the 300 mg dose regimen of IONIS-FXI$_{RX}$ than with enoxaparin. In addition, a phase II study with IONIS-FXI$_{RX}$ was conducted in patients with end-stage renal disease (ESRD) requiring hemodialysis.[6] IONIS-FXI$_{RX}$ doses of 200 mg and 300 mg once-weekly significantly reduced clotting in the dialyzer and venous chamber compared to placebo and were not associated with major or clinically relevant nonmajor bleeding events or with any drug-related serious adverse events.[6]

FXI-LICA (BAY2976217, former name ION-957943) is a generation 2.0 triantennary *N*-acetyl galactosamine (GalNAc) conjugated 2′-MOE chimeric ASO inhibitor of FXI that shares the same RNA sequence as IONIS-FXI$_{RX}$. The GalNAc conjugation facilitates asialoglycoprotein receptor (ASGPR)-mediated uptake[7] thereby maximizing drug delivery to the hepatocytes, which are the target cells for inhibition of FXI synthesis. The GalNAc-conjugation has a strong influence on the pharmacokinetic (PK) and pharmacodynamic (PD) allowing for lower doses and less frequent dosing (once-monthly vs. once-weekly) to yield comparable pharmacological activity as unconjugated ASOs. The effective doses for 50% of the population (ED50s) for hepatocyte-targeting GalNAc-conjugated ASOs are reported to be in the range between 4 and 10 mg/week, up to 20–30-fold more potent than the corresponding unconjugated ASOs.[8] The PK/PD, safety, and tolerability of FXI-LICA have recently been studied in healthy volunteers (HVs).[9] FXI-LICA is currently being investigated in a phase IIb dose finding study in patients with ESRD on hemodialysis (NCT04534114).

The available clinical data of the unconjugated IONIS-FXI$_{RX}$ in the same patient population in combination with

PK/PD data obtained in the two phase I studies with IONIS-$FXI_{RX}$ and FXI-LICA provides an excellent basis for a model-informed bridging analysis. The aim of this study is to extrapolate the dose-exposure-FXI relationship from the unconjugated to the GalNAc-conjugated FXI-antisense oligonucleotide inhibitor and to support the dosing decisions for FXI-LICA in the phase IIb study in patients with ESRD on hemodialysis.

## METHODS

The bridging concept to extrapolate the dose-exposure-FXI relationship from IONIS-$FXI_{RX}$ to FXI-LICA in patients with ESRD is outlined in Figure 1. The concept integrates the currently available clinical data of the two ASOs in HVs and patients with ESRD. The compound-related differences (derived from HV data of both ASOs) as well as differences between HVs and patients with ESRD (obtained from IONIS-$FXI_{RX}$, unpublished data) are translated and applied to predict the dose-FXI activity relationship of FXI-LICA in patients with ESRD, as described in detail below. Technical aspects of the modeling procedures and PK/PD model diagnostics are described in the Supplementary Information.

### Data used for PK/PD modeling

#### IONIS-$FXI_{RX}$ PK/PD data in healthy volunteers (study ASO-CS1)

Study ASO-CS1 was a double-blind, placebo-controlled, dose-escalation, phase I study to assess the safety, tolerability, and PK of single and multiple doses of IONIS-$FXI_{RX}$, administered subcutaneously to HVs. IONIS-$FXI_{RX}$ plasma concentrations and FXI activity data were available from a total of 88 subjects receiving either single or multiple doses of IONIS-$FXI_{RX}$ in the range between 50 mg and 400 mg (22 subjects received placebo). The subjects in the multiple dose arm received three doses of IONIS-$FXI_{RX}$ during week 1 followed by once-weekly administration during weeks 2–6 (in total, 8 doses). This study demonstrated that IONIS-$FXI_{RX}$ produced a sustained reduction in FXI activity in healthy subjects in a dose- and concentration-dependent manner.[10]

#### IONIS-$FXI_{RX}$ PK/PD data in patients with ESRD (study ASO-CS4)

Study ASO-CS4 was a phase II, randomized, double-blind, placebo-controlled study of IONIS-$FXI_{RX}$, administered

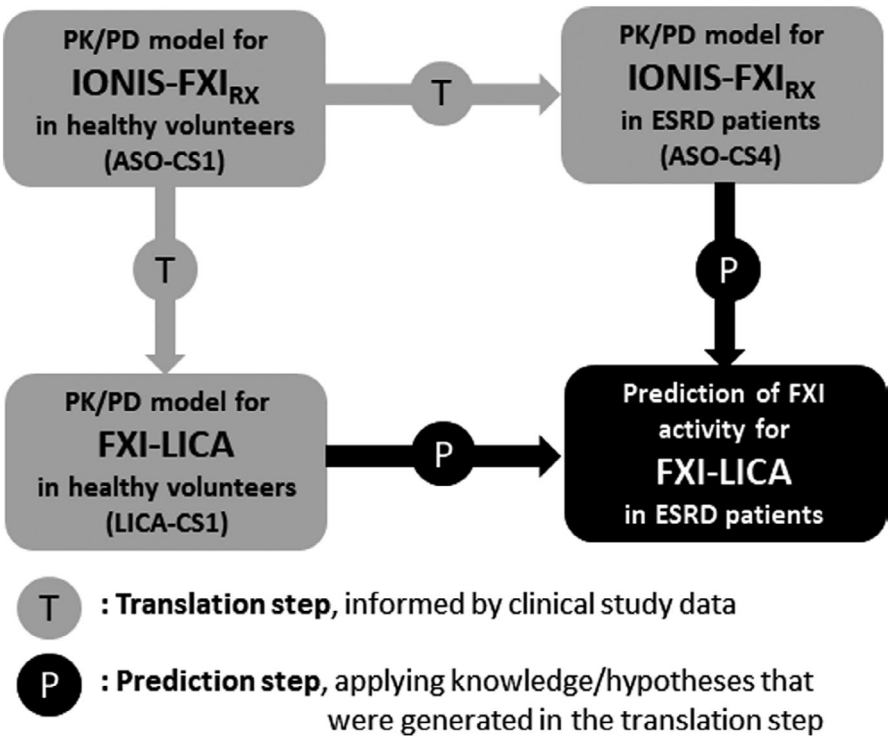


**FIGURE 1** Schematic diagram of the bridging concept to extrapolate the dose-exposure-FXI relationship from IONIS-$FXI_{RX}$ to FXI-LICA and from healthy volunteers to in patients with ESRD. ESRD, end-stage renal disease; FXI, coagulation factor XI; PD, pharmacodynamic; PK, pharmacokinetic

subcutaneously to patients with ESRD on hemodialysis. In total, 49 patients with ESRD provided PK and PD data after repeated doses every 28 days of either 200 mg or 300 mg IONIS-$FXI_{RX}$ over a period of up to 12 weeks (13 patients with ESRD received placebo). Details of this study are provided separately.[6] IONIS-$FXI_{RX}$ showed a dose- and concentration-dependent sustained reduction in FXI activity. To determine the effect of dialysis on IONIS-$FXI_{RX}$ PK, a PK-cohort in study ASO-CS4 received one 300 mg dose of IONIS-$FXI_{RX}$ immediately after dialysis and again 28 days later immediately before dialysis. It was shown that hemodialysis did not alter the PK or PD of IONIS-$FXI_{RX}$.

### FXI-LICA PK/PD data in healthy volunteers (study LICA-CS1)

Study LICA-CS1,[9] the first-in-human study of FXI-LICA, was a double-blind, placebo-controlled, dose-escalation phase I study to assess the safety, tolerability, PK, and PD of single and multiple doses of FXI-LICA. In total, 66 HVs were randomized, 48 of them received FXI-LICA subcutaneously either as single doses of 40, 60, 80, or 120 mg, or as once-weekly doses of 10, 20, or 30 mg for six doses, or 80 mg every 4 weeks for four doses. After subcutaneous absorption, FXI-LICA peak plasma levels were observed within a few hours after dosing and declined thereafter in a multiphasic fashion with an initial, relatively fast disposition phase that dominated the plasma clearance followed by a slower elimination phase.[9] Exposure increased with dose across the studied dose range of 10–120 mg and FXI activity was significantly reduced in all dose groups compared to placebo in a dose-dependent manner.[9]

A summary table describing the composition of the PK/PD dataset, including information about PK/PD sampling times, is provided in the Supplementary Information Table S1.

## PK/PD model development

Starting point for PK/PD modeling was a previously developed PK/PD model by Yu et al.[10] to describe PK and FXI activity of IONIS-$FXI_{RX}$ in HVs after subcutaneous administration. The plasma PK of IONIS-$FXI_{RX}$ was described by a two-compartment model (volumes V2 and V3, intercompartmental clearance Q) with first-order absorption (kA) and first-order elimination from the central compartment (CL). Plasma FXI activities were fitted to an indirect response model linked to the central compartment with a drug inhibitory effect on the zero-order rate constant for FXI synthesis.[10]

This PK/PD model was extended by integrating IONIS-$FXI_{RX}$ data from patients with ESRD (study ASO-CS4), as well as FXI-LICA phase I data after ascending single and multiple subcutaneous doses to HVs (study LICA-CS1). The PK/PD model was structurally modified in two ways (Figure 2). First, a zero-order absorption process (fraction F2, duration D2) was included in parallel to the first-order absorption process (fraction of the dose F1; F1 + F2 = 100%) and, second, a theoretical effect compartment was linked to the central compartment via a first-order rate constant (keo). The effect compartment was introduced to mechanistically separate the compartment in which the two ASOs are acting (i.e., the liver) from the central compartment and motivated by the additional receptor-mediated hepatic uptake mechanism that is available for FXI-LICA. An additional factor (keo_pat) that modulates the concentration in this effect compartment was introduced to estimate a potential effect of ESRD on the intracellular uptake of IONIS-$FXI_{RX}$. The inhibition of FXI-synthesis (kin) was described via an indirect response model as previously reported,[10] but the inhibitory concentration (IC50) was now related to the theoretical effect compartment concentration rather than the concentration in the central compartment.

The PK part of the model was separately fitted to the PK data of IONIS-$FXI_{RX}$ and FXI-LICA. Interindividual variability (IIV) was included on kA, V2, V3, CL, Q, and F1 in the PK model for IONIS-$FXI_{RX}$. Correlations between IIV on CL, IIV on Q, and IIV on V3 were observed and an implementation of the OMEGA BLOCK in NONMEM was tested but not considered in the final model due to resulting poor relative standard errors (RSEs). In the PK model for FXI-LICA, IIV was estimated for kA, V2, V3, CL, and Q and correlations between IIV on CL, IIV on V2, and IIV on Q were taken into account in the final PK model through the implementation of the OMEGA BLOCK. An exploratory covariate analysis was performed that focused on the influence of body weight and in particular on the influence of ESRD on all parameters (except kA) in the IONIS-$FXI_{RX}$ PK model. Given that only a relatively small amount of phase I data was available for FXI-LICA, a thorough investigation of the influence of potentially relevant covariates for PK and PD of the GalNAc-conjugated ASO was beyond the scope of this study. A full covariate analysis will be conducted at a later stage when the phase II data for FXI-LICA in patients with ESRD is also available.

The indirect response model was simultaneously fitted to pooled FXI activity data of IONIS-$FXI_{RX}$ and FXI-LICA, allowing to assess potential differences in the PK/PD relationship between HVs and patients with ESRD (informed by IONIS-$FXI_{RX}$). This ensured the same baseline FXI activity, keo, and kout for both ASOs, because these are considered to be compound-independent parameters. IC50 values were estimated per compound and reflect different potencies

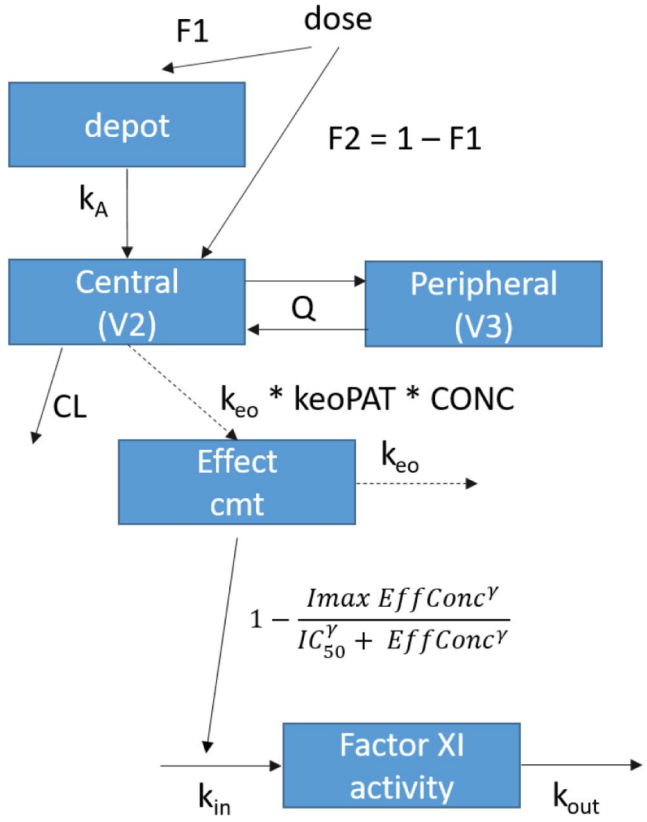


**FIGURE 2** Structure of the PK/PD model for subcutaneously administered IONIS-FXI$_{RX}$ and FXI-LICA

and differences in the uptake in hepatocytes of both ASOs due to the GalNAc-conjugation of FXI-LICA. IIV was included on the baseline FXI activity, IC50, keo, and kout. A proportional error model was used to describe the residual unexplained variability in FXI activity data from the three different studies.

## PK/PD simulations for FXI-LICA in patients with ESRD

Simulations were performed to predict the time courses of FXI activity and the average steady-state FXI activity over one dosing interval in patients with ESRD receiving once-monthly subcutaneous doses of FXI-LICA. These simulations provided the basis for the selection of doses that are being tested in the phase IIb study of FXI-LICA in patients with ESRD. Covariates identified as statistically significant in the exploratory covariate analysis for IONIS-FXI$_{RX}$ were also considered for FXI-LICA. In particular, the influence of ESRD on the PK and PD of IONIS-FXI$_{RX}$ that was identified by the PK/PD model was carried forth to the PK/PD simulations for FXI-LICA. This approach is based on the assumption that the receptor-mediated uptake process is not affected by the presence of ESRD and that the difference between HVs and patients with ESRD is expected to be the same for both compounds. Further, the body weight range in the simulated patients with ESRD treated with FXI-LICA is expected to be the same as the one observed in patients with ESRD who received IONIS-FXI$_{RX}$.

Eight hundred virtual patients with ESRD were simulated with body weights that were randomly sampled from a uniform distribution reflecting the body weight distribution in patients with ESRD in study ASO-CS4 (between 48.5 kg and 164 kg).

To explore the potential influence of changes in the subcutaneous FXI-LICA administration on PK and PD, the sensitivity of the FXI-LICA PK and PD at steady-state with respect to the fractional contributions of the two parallel subcutaneous absorption pathways (F1) was analyzed. Two extreme scenarios were simulated assuming that the whole dose (here: 80 mg once-monthly) was completely absorbed either via the zero-order or the first-order route. These scenarios were then compared to the reference scenario based on the F1 estimated by the PK/PD model for FXI-LICA.

# RESULTS

## PK/PD model for IONIS-FXI$_{RX}$ and FXI-LICA

The structural PK/PD model depicted in Figure 2 was able to accurately describe the plasma concentration-time profiles and the resulting time course of FXI activity for both ASOs, IONIS-FXI$_{RX}$ and FXI-LICA. Goodness-of-fit (GOF) plots and visual predictive checks (VPCs) that demonstrate model adequacy and predictive performance in HVs and patients with ESRD are shown in the Supplementary Information. Supplementary Information Tables S2–S4 summarize the PK and PD model parameters obtained for IONIS-FXI$_{RX}$ and FXI-LICA. All parameters were estimated with adequate precision (RSE < 50%). The inclusion of the mixed first- and zero-order absorption pathways considerably improved the model fit for both FXI-LICA (drop in objective function value [OFV]: −57.4 points) and IONIS-FXI$_{RX}$ (drop in OFV: −53.3 points) by reducing a bias in the absorption phase that was apparent when subcutaneous absorption was assumed to follow a first-order kinetic (data not shown). Approximately 69% (95% confidence interval [CI]: 56%–79%) and 62% (95% CI: 52%–71%) of the dose contributing to systemic exposure of IONIS-FXI$_{RX}$ and FXI-LICA were absorbed following first-order absorption. The remaining dose was absorbed via zero-order absorption with an estimated duration of ~ 4.9 h (95% CI: 4.5 h–5.2 h) and 9.4 h (95% CI: 9.0 h–9.8 h), respectively.

Plasma clearance of IONIS-FXI$_{RX}$ in HVs was estimated to be 3.12 L/h (95% CI: 2.86 L/h–3.38 L/h). Compared to HVs, the CL of patients with ESRD was significantly reduced by a factor of ~ 53% (95% CI: 45%–61%). ESRD also had a statistically significant effect on V3, and body weight

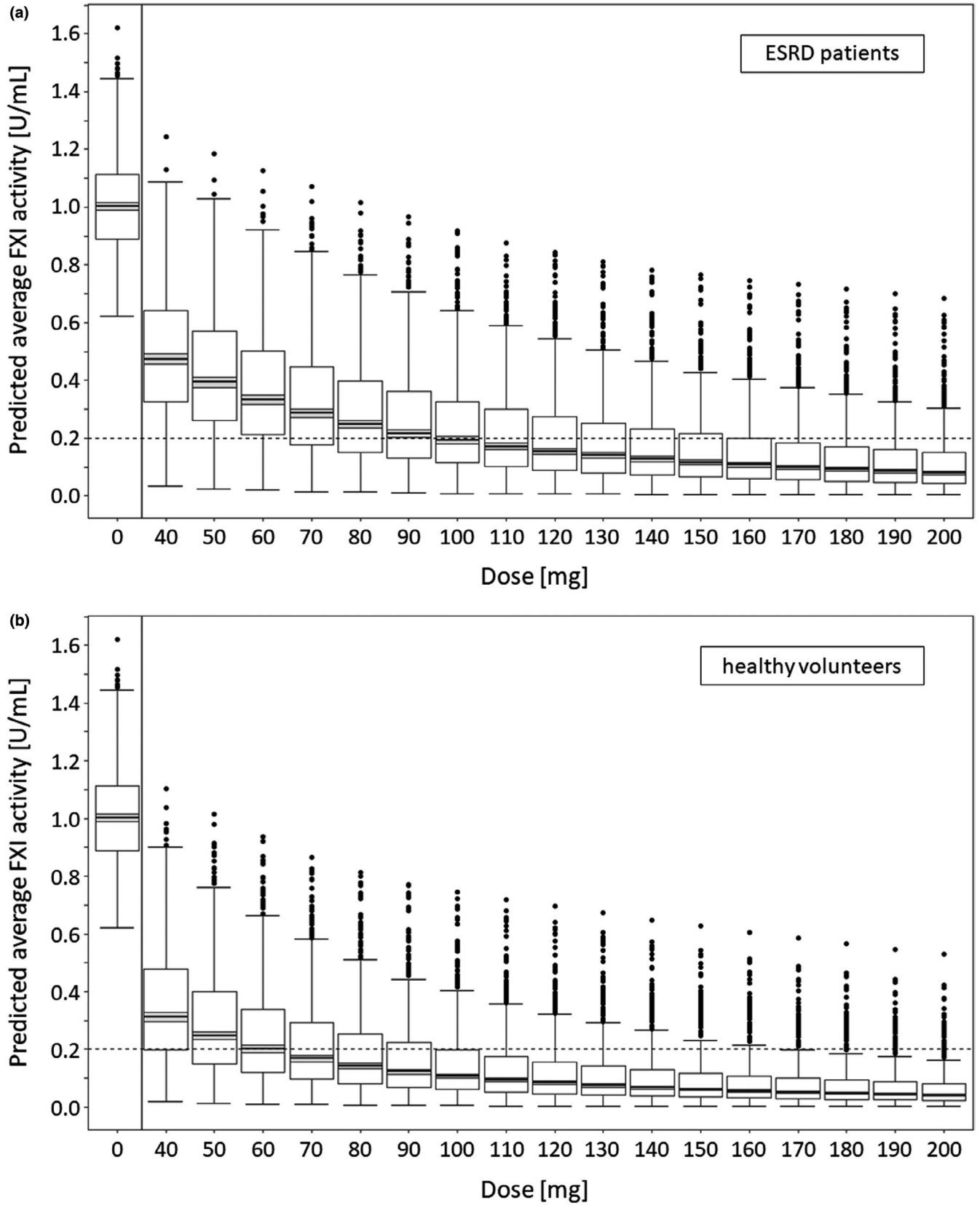


**FIGURE 3** Predicted distribution of average steady-state FXI activity after once-monthly subcutaneous administration of FXI-LICA to (a) patients with ESRD and (b) healthy volunteers ($N = 800$ each). The dotted line indicates a FXI activity of 0.2 U/ml as observed in the 300 mg cohort of the IONIS-$FXI_{RX}$ TKA study for comparison

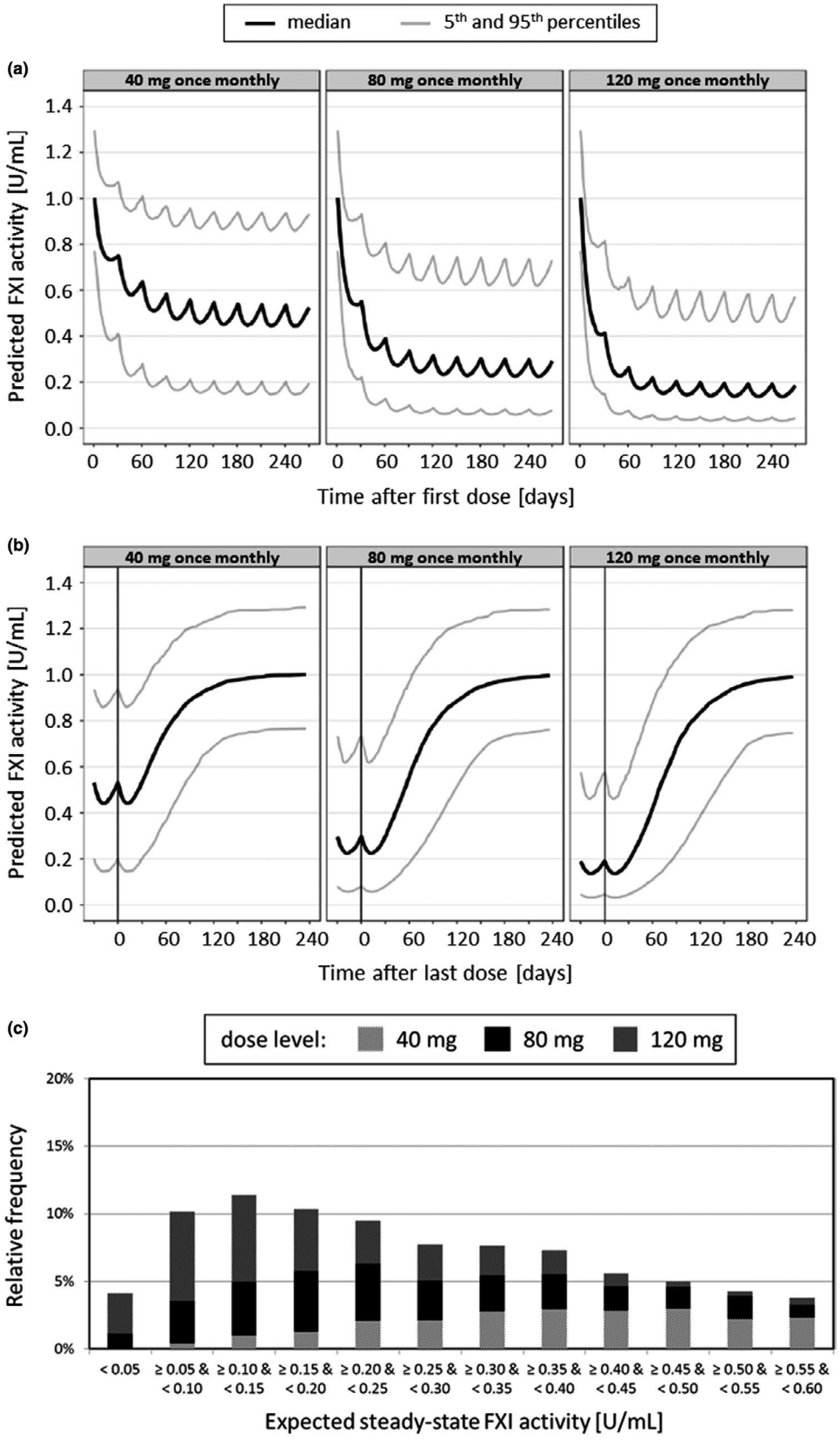


**FIGURE 4** (a) Model-predicted time courses (median and 5% to 95% prediction interval) of FXI-activity in patients with ESRD following repeated once-monthly subcutaneous dosing of FXI-LICA and (b) after stopping FXI-LICA dosing for dose levels of 40 mg, 80 mg, and 120 mg; (c) resulting distribution of expected average FXI activities at steady-state per dose level assuming a 1:1:1 randomization (40 mg dose: light gray, 80 mg dose: black, 120 mg dose: dark gray)

had a statistically significant effect on V2. Low correlations between random-effect parameters were found except for the correlation between IIV on Q and IIV on V3 ($R^2 = 0.6$). Its inclusion in the OMEGA BLOCK, however, led to an unstable model and was thus not kept in the final model. The random-effects were reasonably well centered around zero with shrinkage lower than 30% for all parameters of IONIS-$FXI_{RX}$, except for F1 (41%).

For FXI-LICA, plasma clearance was estimated to be 12.8 L/h in HVs (95% CI: 11.4 L/h–14.2 L/h). Correlations between IIV on CL, IIV on V2, and IIV on Q were around 0.6 and 0.7 and considered in the final PK model for FXI-LICA through the implementation of the OMEGA BLOCK in NONMEM. The random-effects were well-centered around zero with shrinkage lower than 6% for CL, V2, Q, and V3, and equal to 17% for kA.

The rate constant keo that was estimated globally for both ASOs to be 0.00115 1/h (95% CI: 0.00102 1/h–0.00219 1/h). The keo_pat was estimated to be 0.329 (95% CI: 0.242–0.415), resulting in approximately one-third lower concentration in the effect compartment in patients with ESRD compared to HVs. The IC50-values in the theoretical effect compartment were estimated to be 167 ng/ml (95% CI: 139 ng/ml–195 ng/ml) for IONIS-$FXI_{RX}$ and 2.59 ng/ml (95% CI: 2.18 ng/ml–3.00 ng/ml) for FXI-LICA. Low correlations between the random-effect parameters were obtained. The estimates for IIV were well-centered around zero with shrinkage lower than 12% for baseline FXI activity and IC50, and slightly above 30% for kout and keo (i.e., 37% and 32%, respectively). The final PK/PD model was dose-linear within the observed dose ranges for both IONIS-$FXI_{RX}$ and FXI-LICA.

## Prediction of FXI activity in patients with ESRD after FXI-LICA administration

Figure 3a shows the distribution of average (defined as time-average over one dosing-interval) steady-state FXI activity after once-monthly subcutaneous administration of FXI-LICA doses between 40 and 200 mg to patients with ESRD as predicted by the PK/PD model. The population median FXI activity at steady-state after FXI-LICA doses of 40 mg, 80 mg, and 120 mg in patients with ESRD were 0.47 U/ml (95% CI: 0.16 U/ml–0.875 U/ml), 0.25 U/ml (95% CI: 0.06 U/ml–0.64 U/ml), and 0.15 U/ml (95% CI: 0.035 U/ml–0.48 U/ml), respectively, with a considerable IIV. For comparison, the corresponding distributions of average steady-state FXI activity for HVs are shown in Figure 3b. The median FXI activity and IIV are slightly lower than predicted for patients with ESRD at the same dose level.

Based on clinical considerations (outlined below), these three dose levels were selected to be tested in the clinical phase IIb study. Figure 4 shows the model-predicted time courses of FXI-activity in patients with ESRD following once-monthly subcutaneous administration (a) and after stopping FXI-LICA dosing (b) at the selected dose levels. According to Figure 4a, steady-state conditions are reached after approximately four to five doses. It can be seen from Figure 4b that FXI activities return to their baseline values within ~ 5 months after the last dose of FXI-LICA. Figure 4c shows the predicted distribution of FXI activities at steady-state in patients with ESRD binned into 0.05 U/ml intervals. The staggered bars indicate the expected contributions by the three selected dose levels assuming a 1:1:1 randomization. The resulting distribution of steady-state FXI activities is relatively even across the FXI bins with a moderate peak in the 0.10–0.15 U/ml range. Approximately one of three patients with ESRD is expected to have average FXI activity at steady-state below 0.2 U/ml and one of seven patients below 0.1 U/ml at steady-state. FXI activities below 0.1 U/ml are predominately expected in the 120 mg and 80 mg dose groups, as indicated by the colored bars in Figure 4c.

## Sensitivity analysis with respect to mixed subcutaneous absorption pathways

The results of the sensitivity analysis with respect to the relative contributions of the two parallel subcutaneous absorption pathways of FXI-LICA are shown in Figure 5. The median steady-state maximum concentration ($C_{max}$) of FXI-LICA was increased by a factor of 1.33, if the dose was assumed to be completely absorbed via the first-order route (F1 = 100%, green) and decreased by a factor of 0.95, if absorption occurred completely via the zero-order route (F1 = 0%, blue) compared to the reference scenario (F1 = 62%, red). These factors were small in relation to the IIV in the steady-state $C_{max}$, as indicated by the largely overlapping boxes and whiskers in Figure 5 (left). The average FXI activity at steady-state is practically identical across the three scenarios Figure 5 (right), indicating that the PD effect is insensitive to variations of the fractional contributions of the two identified absorption pathways.

# DISCUSSION

The presented model-informed bridging approach combines PK/PD knowledge that was generated in two phase I studies of FXI-LICA and IONIS-$FXI_{RX}$ and one phase II study with IONIS-$FXI_{RX}$ in patients with ESRD to support the selection of clinical doses of FXI-LICA to be tested in a phase IIb study in the same patient population. The main assumption of this bridging approach is that the receptor-mediated uptake process that is the key difference between the GalNAc-conjugated FXI-LICA and the unconjugated IONIS-$FXI_{RX}$

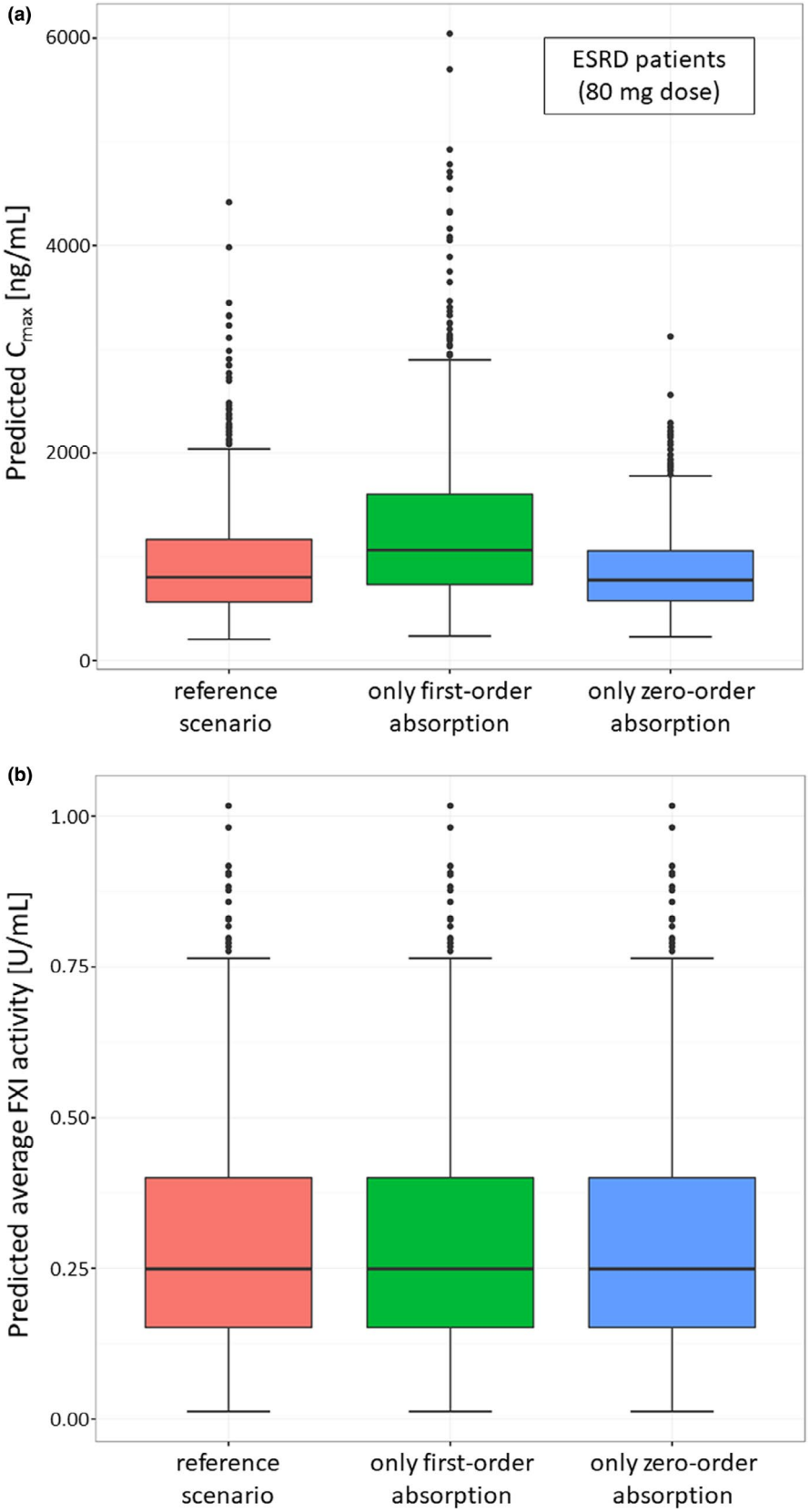


**FIGURE 5** Sensitivity analysis with respect to the relative contributions of the first- and zero-order absorption pathways. (a) maximum concentration and (b) average FXI activity at steady-state following once monthly administration of 80 mg FXI-LICA)

is not affected by the presence of ESRD. At high FXI-LICA plasma concentrations, the receptor-mediated uptake can become saturated and potentially limit the rate and extent of FXI-LICA uptake into the liver. In an in vivo mouse model, Bon et al.[11] showed that hepatic uptake of an ASGPR-specific antibody was saturable at plasma concentrations above ~ 0.5 μmol/L. Wang et al. reported an under-dosed proportional increase in liver exposure of monkeys receiving GalNAc conjugated ASOs over a wide dose range (1–40 mg/kg) suggesting that liver uptake may reach saturation at 3 mg/kg and higher doses.[8] Patients with acute or chronic liver diseases, as well as patients with hepatocellular carcinoma (HCC), were shown to have reduced ASGPR levels that correlate with the severity of the liver disease[12,13] or grade of the HCC.[14] In patients with severe hepatocellular damage, ASGPR concentrations were ~ 30% of those observed in normal controls (0.241 μmol/L vs. 0.792 μmol/L).[12] To our knowledge, no quantitative information about alterations of ASGPR expression in patients with ESRD are available to date, but given the moderate modulations of ASGPR expression reported for severe liver diseases, it seems reasonable to assume that the receptor-mediated hepatic uptake of FXI-LICA will not be different in patients with ESRD compared to HVs.

The pooled PK/PD database from the two ASOs was used to inform a population PK/PD model that adequately described the median trends as well as the IIVs for plasma concentration and FXI activity across dose groups and studies for IONIS-$FXI_{RX}$ and FXI-LICA. The model parameter estimates for IONIS-$FXI_{RX}$ in HVs were very well in line with the parameters estimated in the initial model by Yu et al.[10] (Supplementary Information Table S5). The IC50 estimates cannot be directly compared because this parameter is linked to the effect compartment concentration in the current model and to the concentration of the central compartment in the initial model.[10] The PK and PD parameter estimates for both ASOs (Supplementary Information Tables S2–S4) reflect the differences in their dose-exposure and PK/PD relationships. The first-order subcutaneous absorption rate kA is ~ 4.5-times greater and the zero-order absorption duration (D2) is ~ 1.9-times longer for FXI-LICA than for IONIS-$FXI_{RX}$, whereas the fractions of the dose that are absorbed via the first-order process are similar (~ 62% for FXI-LICA and 69% for IONIS-$FXI_{RX}$). The plasma clearance estimated for HVs is ~ 4.1-times greater, and the central and peripheral volumes are 5.1-times and 5.6-times smaller for FXI-LICA compared to IONIS-$FXI_{RX}$. The PK/PD model also identified differences in the dose-exposure and PK/PD relationship of IONIS-$FXI_{RX}$ between HVs and patients with ESRD. In patients with ESRD, plasma clearance and the peripheral volume of IONIS-$FXI_{RX}$ were reduced by factors of 53% and 38%, respectively. In addition, a factor of approximately one-third on the effect site concentration was estimated for patients with ESRD based on IONIS-$FXI_{RX}$ data.

Both ASOs have in common that the reduction in FXI activity is delayed and sustained when compared to the PK time scale. The delay in FXI reduction is due to the turnover of FXI that has a half-life of ~ 48 h in the circulation.[15,16] FXI-LICA is absorbed within a few hours after subcutaneous administration, but the maximal FXI reduction is observed ~ 14 days after dosing—corresponding to approximately seven half-lives of FXI—regardless of the dose.[9] In the PK/PD model, the delayed PD effect is expressed by the rate constant keo that describes the uptake into and pharmacological action in the theoretical effect compartment. Because concentration measurements are only available in the central compartment (plasma) but not in the effect compartment (liver), the model parameters of the effect compartment need to be interpreted cautiously. The estimated value for keo is ~ 750-times smaller than kA of FXI-LICA, demonstrating that the time scales for absorption from the subcutaneous depot and the reduction of FXI activity are largely decoupled. At a given plasma concentration, keo and IC50 together determine the apparent potency. To assess the difference in apparent potency of the two FXI ASOs, doses and dosing regimens that lead to a similar extent of FXI reduction were compared. Repeated subcutaneous doses of 40 mg/month of FXI-LICA will lead to a comparable extent of FXI reduction in steady-state as 200 mg/week of IONIS-$FXI_{RX}$ in patients with ESRD, demonstrating an ~ 20-fold higher potency of FXI-LICA compared to IONIS-$FXI_{RX}$. This increase in potency is well in line with data reported for other pairs of hepatocyte-targeting GalNAc-conjugated and unconjugated ASOs.[8]

The introduction of an additional zero-order absorption route considerably improved the PK model fit. Parallel first- and zero-order subcutaneous absorption pathways are not uncommon for larger molecules[17] and have, for example, been observed for therapeutic proteins, such as human erythropoietin[18] or interferon-alpha.[19] The two pathways are usually associated with drug deposition and subsequent absorption into the systemic circulation from different subcutaneous compartments. For large molecules, the first-order process is often attributed to lymphatic absorption, whereas the zero-order process is interpreted as absorption through the blood capillaries,[17] but the physiological interpretation of such kinetic observations is problematic and experimental proofs for the proposed attributions to either lymphatic or capillary uptake are lacking to date. It is also known that the subcutaneous injection site, as well as the needle length and injection technique (e.g., skin pinching), can affect the location of the deposited drug and, in turn, influence the PK of large therapeutic proteins.[20–22] The currently available PK data for IONIS-$FXI_{RX}$ and FXI-LICA was not sufficient to

quantitatively investigate any dependencies of the subcutaneous absorption parameters with regard to the exact injection site or technique, because the information about different injection sites was sparse. This will be further assessed based on when the PK and PD data of the ongoing phase II study becomes available. Here, a sensitivity analysis was performed with respect to the relative contributions of the two absorption pathways that demonstrated that the extent of FXI reduction at steady-state is not affected when the administered dose is exclusively absorbed via the first- or zero-order pathway. The relative effects on $C_{max}$ of FXI-LICA were much smaller than the IIV indicating, overall, that the PK and PD of FXI-LICA are insensitive against variations in the contributions of the two absorption pathways.

Finally, the PK/PD model was used to predict the average steady-state FXI activity in dependence of dose following repeated once-monthly doses of FXI-LICA in a virtual ESRD patient population (Figure 3a). Compared to HVs (Figure 3b), the predicted FXI activity is slightly higher when the influence of ESRD on the PK and PD observed for IONIS-$FXI_{RX}$ is considered also for FXI-LICA. These simulations supported the selection of doses being tested in the phase IIb study of FXI-LICA in patients with ESRD. The FXI target range was defined under consideration of literature data reporting relationships between reduced FXI levels and the occurrence of thromboembolic events. A large observation cohort including 10,193 adults in Israel demonstrated that FXI activity of 30–50% was associated with reduced cardiovascular event rates in a population with genetically lowered FXI levels when compared to the group of patients with greater than 50% of FXI levels.[4] Salomon et al. reported that relative FXI levels below 15% were associated with a lower incidence of ischemic stroke and deep vein thrombosis in patients with severe FXI deficiency.[2,3] It is assumed that lowering of FXI levels into the range described above will lead to clinical efficacy in the sense of preventing thromboembolic events in patients with ESRD. This target range is also supported by the TKA study of IONIS-$FXI_{RX}$. At the time of surgery, the mean FXI activities were 0.38 U/ml in the 200 mg group, and 0.20 U/ml in the 300 mg group.[5]

In summary, a model-informed bridging concept was applied to translate the dose-exposure-FXI relationship from the unconjugated IONIS-$FXI_{RX}$ to the GalNAc-conjugated FXI-LICA in patients with ESRD. Based on the presented considerations, 40 mg, 80 mg, and 120 mg were confirmed as dose levels to be tested in the phase IIb study of FXI-LICA in patients with ESRD. The expected distribution of average FXI activities at steady-state is broad and, if confirmed in the clinical study, would allow for a subsequent quantitative investigation of the relationship between FXI activities and the clinical safety and efficacy end points in patients with ESRD.

## CONFLICT OF INTEREST

S.W., A.S., M.J., and A.W.A.L. are employees of and potential share owners of Bayer AG. El.M. and N.S. are employees of LAP&P and were paid consultants for Bayer during the conduct of the analysis. M.L. is a former employee of Bayer AG. C.B., E.M., R.Z.Y., Y.W., S.W.J., R.G., and S.B. are employees and stock holders of Ionis.

## AUTHOR CONTRIBUTIONS

S.W., El.M., N.S., A.S., M.J., M.L., and A.W.A.L. wrote the manuscript. All authors designed and performed the research. S.W., El.M., N.S., A.S., R.Z.Y., and Y.W. analyzed the data.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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