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Analysis of C4 Concentrations to Predict Impact of Patient-Reported Diarrhea Associated With the Ileal Bile Acid Transporter Inhibitor Linerixibat

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ABSTRACT

Linerixibat, an ileal bile acid transporter (IBAT) inhibitor, is being evaluated for the treatment of pruritus in primary biliary cholangitis (PBC). Diarrhea is commonly reported with this drug class as IBAT inhibition redirects bile acids (BA) to the colon. Serum 7-alpha-hydroxy-4-cholesten-3-one (C4) measurement is a validated method to identify BA diarrhea. To inform dose selection, we characterized the relationship between linerixibat dose, C4 levels, and patient-reported bother on the gastrointestinal symptom rating scale (GSRS) diarrhea question. A kinetic-pharmacodynamic model was developed using data from five Phase 1/2 trials, to describe the effect of linerixibat dose (1–180 mg) and regimen (once/twice daily) on C4 concentrations over time. GSRS data from patients with PBC and pruritus in the Phase 2b GLIMMER study (NCT02966834) were used to develop a proportional odds model to predict the probability of a score of 1–7 (no–very severe discomfort) to the question "Have you been bothered by diarrhea during the past week?" in relation to linerixibat dose. The two models were linked to describe the linerixibat dose-C4-diarrhea bother relationship. Models were validated using graphical and numerical assessment and visual predictive checks. Linerixibat caused dose-dependent increases in C4 until saturation (~180 mg total daily dose). Increased C4 concentrations trended with increased GSRS diarrhea scores. Simulations demonstrated increases in moderate-to-very severe (≥ 4) diarrhea scores with increasing linerixibat dose. Increases in patient-reported diarrhea scores were linerixibat dose-dependent. Selecting an optimal dose that maximizes linerixibat's ability to improve pruritus while minimizing patient-reported diarrhea bother is important to support treatment adherence.

1 | Introduction

Cholestatic pruritus affects up to 81% of patients with primary biliary cholangitis (PBC) over the course of this rare liver disease and substantially impairs quality of life [1–4]. There are no therapies specifically licensed globally to treat pruritus in patients with PBC [5, 6]. Treatment guidelines recommend a stepwise approach, with bile acid binding resins, such as cholestyramine, recommended as the first-line treatment option for pruritus in patients with PBC [5, 7–10]. However, cholestyramine and other guideline-recommended pruritus therapies have little evidence to support their use and are often poorly tolerated by patients [7, 8, 11–13]. Thus, pruritus is often undertreated [2, 4, 14], and new therapies are needed to address itch in patients with cholestatic liver disease.

Linerixibat, a selective, small molecule inhibitor of the ileal bile acid transporter (IBAT), reduced pruritus without causing

Summary

- What is the current knowledge on the topic?
  - Linerixibat is an ileal bile acid transporter (IBAT) inhibitor currently under investigation for the treatment of patients with primary biliary cholangitis (PBC) experiencing cholestatic pruritus. Diarrhea is a commonly reported adverse event following treatment with IBAT inhibitors due to an excess of bile acids reaching the colon. Linerixibat increases serum 7-alpha-hydroxy-4-cholesten-3-one (C4) concentration and bile acid diarrhea can be identified by measuring serum C4 concentration.
- What question did this study address?
  - This study aimed to characterize the relationship between linerixibat dose, C4 levels, and patient-reported diarrhea bother to inform linerixibat dose selection.
- What does this study add to our knowledge?
  - Linerixibat treatment led to dose-dependent increases in C4 concentrations that predicted an increased probability of moderate-to-very severe diarrhea bother, according to the gastrointestinal symptom rating scale diarrhea question, a patient-reported outcome measure.
- How might this change drug discovery, development, and/or therapeutics?
  - This study highlights the utility of using modeling approaches to combine biomarker data with patient-reported outcome measures to provide information on dose selection and tolerability.

serious adverse events (AEs) in a Phase 2a study (NCT01899703) and in the Phase 2b GLIMMER trial (NCT02966834) of patients with PBC and pruritus [15–17]. A Phase 3 trial (GLISTEN, NCT04950127) evaluating linerixibat for the treatment of pruritus in patients with PBC is currently ongoing [18]. IBAT is responsible for enterohepatic circulation of bile acids (BAs) and reabsorbs the majority of BAs excreted into the small intestine, recirculating them to the liver via the portal vein [19]. Inhibition of IBAT interrupts ileal reabsorption of BAs, leading to an excess in the colon [20, 21]. When BAs reach the colon and associated gut microbiota, they interact with the G-protein coupled BA receptor 1, increasing colonic motility, transit, visceral sensation, fluid secretion, and mucosal permeability. The resulting BA diarrhea is a commonly reported AE with IBAT inhibitors, including linerixibat, volixibat, maralixibat, and odevixibat [17, 20, 22–24].

The reduction in BA absorption resulting from IBAT inhibition leads to increased concentrations of plasma/serum 7-alpha-hydroxy-4-cholesten-3-one (C4) [15, 22–24]. This metabolic intermediate in the synthesis of BAs from hepatic cholesterol has previously been proposed as a diagnostic biomarker for BA diarrhea [21]. Measurement of serum C4 is a validated method to detect BA diarrhea, and a C4 cutoff of 52.5 ng/mL has been reported to diagnose BA diarrhea with a specificity of 83% [21].

Characterizing the relationship between linerixibat dose, C4 levels, and patient-reported bother on the gastrointestinal symptom rating scale (GSRS) diarrhea question is useful for dose selection with the intention to maximize the pharmacodynamic (PD) effect of linerixibat while minimizing intolerability due to diarrhea. As IBAT inhibitors act locally in the distal ileum of the gastrointestinal tract and are minimally absorbed into the systemic circulation, plasma drug concentrations are not useful for traditional exposure-response analysis [25]. Kinetic-pharmacodynamic (k-PD) modeling is one effective approach for examining dose-biomarker relationships for drugs where plasma pharmacokinetic (PK) data are unavailable [26]. In this study, we employed a k-PD model to describe the relationship between linerixibat dose and serum C4 concentration based on data from GLIMMER and Phase 1/Phase 2a studies. Using patient-reported data collected in GLIMMER from the GSRS, a proportional odds (PO) model was developed to predict the probability of a score of 1–7 (no–very severe discomfort) to the question "Have you been bothered by diarrhea during the past week?" in relation to linerixibat dose. As the GSRS assesses a patient's experience and discomfort with treatment-associated side effects [27], GSRS findings can be used to provide insights into treatment tolerability [16, 28]. The k-PD and PO models were linked to form the k-PD-PO model, which describes the relationship between linerixibat, C4 concentration, and patient-reported bother due to diarrhea, in order to infer patient tolerability associated with different doses of linerixibat. The dose–response model was used to elucidate the relationship between linerixibat dose and bother due to diarrhea and the potential to use C4 as a surrogate of diarrhea intensity.

2 | Methods

2.1 | Dose-C4 Model Development

2.1.1 | Study Population and C4 Measurement

The linerixibat dose-C4 relationship utilized data from Phase 1 studies (NCT01416324, NCT01607385, and NCT02801981) in which serum C4 levels were sampled in healthy participants receiving different linerixibat doses (range: 1–180 mg) administered as once- (QD) or twice-daily (BID) regimens (Table S1) [29]. In addition, C4 was evaluated in patients with PBC in two Phase 2 studies of linerixibat 90 mg BID (NCT01899703) [17] or linerixibat 20–180 mg QD or 40–90 mg BID (GLIMMER) [15]. Serum C4 was measured using a liquid chromatography with tandem mass spectrometry (LC–MS/MS) assay with a lower limit of quantitation of 2 ng/mL and an upper limit of quantitation of 200 ng/mL at Covance (Princeton, NJ, USA) and Q^2 Solutions (Ithaca, NY, USA). See the Methods S1 for full details on C4 measurement.

2.1.2 | Structural Model Development for Dose-C4 Analysis

Various diurnal models to fit the C4 data over time were evaluated, and a dual cosine input was used in the indirect response model. See Methods S1 for further details. Other drug effect models were also evaluated including a linear effect model. However, when trying to describe the drug effect as a linear

relationship, there was an increase in the objective function (OFV) value.

2.1.3 | Dose-C4 k-PD Model Covariates

Covariates including age, body weight, body mass index, baseline fibroblast growth factor 19 concentration, participant status (healthy versus patients with PBC), and race were evaluated to assess the impact of key demographics and other characteristics on drug exposure in participants. Covariates were added to the structural parameters with the stepwise covariate modeling approach (SCM) to test the covariate–parameter relationship. This technique tests different covariate–parameter relationships (linear, exponential, power) in a forward fashion ($p < 0.05$ and ΔOFV:3.84, df = 1) to build up the full model, which in turn are evaluated in the backward elimination step ($p < 0.01$ and ΔOFV:6.63). Only runs with successful minimization were used in selecting covariates to add/remove in each step.

2.1.4 | Dose-C4 k-PD Model Validation

Model validation was performed using graphical and numerical assessment including goodness-of-fit and visual predictive checks (VPCs). Plots of observed data versus population prediction (PRED) and individual prediction (IPRED) were examined for adequate fit. Numerical validation was determined on the basis of successful numerical convergence, acceptable parameter precision (relative standard error [RSE] < 50%), and biological plausibility of parameter values. VPCs (1000 replications) were performed with the base and final population k-PD model parameters to compare the distribution of simulated population k-PD data to the observed data.

2.1.5 | Dose-C4 k-PD Model Simulations

Based on the final k-PD model that was developed, simulations were performed to explore the effect of the GLIMMER dose regimens of linerixibat (20 mg, 90 mg, 180 mg QD; 40 mg, 90 mg BID; or placebo) on C4 concentration over time.

2.2 | k-PD-PO Joint Model for Linerixibat Dose-C4-Diarrhea Bother

GSRS data from patients with PBC and pruritus were obtained from GLIMMER [15]. The GSRS questionnaire is a validated, interview-based rating scale for evaluating gastrointestinal symptoms [27]. It contains 15 categories and uses a seven-point Likert-type scale ranging from 1 (absence of troublesome symptoms) to 7 (very troublesome symptoms) [27, 30]. In this analysis, the GSRS diarrhea category was assessed. See Methods S1 for further details on the GSRS items. GSRS diarrhea data were collected weekly, from Day 1 to Week 20, using an electronic diary. Participants were asked the following question (Question 11) as part of the GSRS measure: "Have you been bothered by diarrhea during the past week?". Responses were collected on a scale from 1 (*no discomfort at all*) to 7 (*very severe discomfort*).

2.2.1 | Structural Model Development for Dose-C4-Diarrhea Bother Analysis

To characterize the relationship between linerixibat dose, C4 concentration, and patient bother according to the GSRS diarrhea question, the k-PD model was linked to the longitudinal PO model utilizing the individual PK parameters with standard errors (IPPSE) method. To propagate the PK (C4) information to the PD (GSRS score) estimation step, an IPPSE method with the individual biomarker PK parameter estimates and their uncertainty (SE) was used.

2.2.2 | Dose-C4-Diarrhea Bother Model Covariate Analysis

Scientific rationale, graphical and statistical approaches were used to identify which covariates would be examined on the probability of GSRS diarrhea score and to assess the mathematical relationship.

2.2.3 | Dose-C4-Diarrhea Bother Model Validation

The model was validated using VPCs.

2.2.4 | Dose-C4-Diarrhea Bother Model Simulations

Simulations were performed for 1000 subjects treated with linerixibat 20 mg, 90 mg, 180 mg QD, or 40 mg, 90 mg BID, or placebo. The demographics of participants in GLIMMER were used for all simulations for the longitudinal PO model.

2.3 | Software

All data analysis was performed using NONMEM software, Version 7.3 (ICON Development Solutions). The PsN (version 4.5.1) tool kit and R (version 3.2.5) were used for automating and controlling the runs, data visualization, and graphical analysis (goodness-of-fit graphics, including pcVPCs and bootstrap analysis), respectively. Pirana (version 2.9.7, build 20170815) was used to keep track of run records and results [31].

3 | Results

3.1 | Dose-C4 Analysis

3.1.1 | Dataset, Demographics, and Baseline Characteristics

A summary of baseline demographics for studies included in the analysis is shown in Table 1. Across all studies, 63% (161/255) of patients were female and the geometric mean (CVb%) age ranged from 31 (35%) to 55 (21%) years. A total of 3292 C4 concentration values obtained from 255 participants (86 healthy volunteers and 169 patients with PBC) were included in the biomarker analysis (Table 1). For participants with measurable C4 concentration included in the analysis, the baseline geometric mean (CVb%) C4

TABLE 1 | Demographics, clinical status, and number of participants with measurable C4 concentration contributing to the biomarker analysis.

	NCT01416324 (*N* = 14)[a]	NCT01607385 (*N* = 56)[a]	NCT02801981 [29] (*N* = 16)[a]	NCT01899703 [17] (*N* = 22)[a]	NCT02966834 (*N* = 147)[a]
Status (PBC patient/HV)	HV	HV	HV	PBC	PBC
Female, *n* (%)	3 (21)	1 (2)	0	19 (86)	138 (94)
Body weight, geometric mean (CVb%), kg	79.6 (16.0)	82.2 (14.8)	62.8 (8.1)	71.7 (18.1)	68.2 (23.4)
Age, geometric mean (CVb%), years	31 (35)	38 (28)	33 (26)	52 (21)	55 (21)
Number of measurable C4 concentrations included in analysis, *n* (%)	559 (98)	1127 (96)	766 (100)	160 (85)	680 (100)
Baseline C4 concentration, geometric mean (CVb%), ng/mL[b]	10.6 (55.0)	7.4 (79.0)	12.5 (98.5)	8.5 (159.8)	14.1 (106.4)

Abbreviations: C4, 7-alpha-hydroxy-4-cholesten-3-one; CVb, between participant coefficient of variation; HV, healthy volunteer; kg, kilogram; PBC, primary biliary cholangitis.
[a]*N* numbers refer to number of participants enrolled.
[b]Mean (SD) derived only from patients with measurable C4 concentration as shown in the row above.

concentration was 10.6 ng/mL (55.0%), 7.4 (79.0%), 12.5 (98.5%), 8.5 (159.8%), and 14.1 (106.4%) in the five studies included.

3.1.2 | k-PD Model Development to Describe Linerixibat Dose-C4 Relationship

A population k-PD indirect response model with a stimulatory component on C4 production was employed to describe the effect of linerixibat on C4 concentrations over time. To account for the diurnal variation in C4 concentrations, different periodical functions were tested. A dual cosine function was shown to best describe the nychthemeral variation in serum C4 concentration. The period values of 8 and 12 h best described the daily rhythm-adjusted mean for C4. For the drug effect component of the model, the linerixibat dose rate from the virtual PK compartment was used as the input to the indirect response model and was best described with an E_{max} model as stimulation on the zero-order rate constant for synthesis (K_{in}) of C4. A combined residual error model was used, and all model parameters were estimated with acceptable precision (< 30% RSE for fixed effects and ≤ 35% RSE for random effects). The parameter estimates from the base model are listed in Table 2.

3.1.3 | Dose-C4 k-PD Model Covariates

The indirect response model with linerixibat effect described by an E_{max} model was selected as the final population k-PD model. A schematic representation of the k-PD model used to describe the relationship between linerixibat dose, C4 concentration, and patient-reported diarrhea is shown in Figure 1. The goodness-of-fit plots for the final model suggested that in general there was a good agreement between the observed and model predicted C4 concentration (Figure 2).

3.1.4 | Dose-C4 k-PD Model Validation

The condition number was 13.34, indicating that there was good stability in the parameter estimates. VPCs confirmed that the model adequately captured the observed C4 concentration over time and trends across different doses within the 5th and 95th percentiles of the simulated values (Figure S1).

3.1.5 | Dose-C4 k-PD Model Simulations

The final k-PD model showed a saturable linerixibat dose–response relationship (i.e., E_{max}) with C4 concentration. The two linerixibat BID dose regimens (40 and 90 mg) led to an increase in C4 of approximately 24% compared to the change from baseline for a similar daily QD dose (i.e., 90 mg BID versus 180 mg QD; Figure 3). The median (90% prediction interval) of C4 concentrations for each dose was as follows: placebo, 16.7 (5.08, 41.1); linerixibat 20 mg QD, 27.3 (8.21, 82.8); linerixibat 40 mg BID, 39.7 (12.4, 120); linerixibat 90 mg QD, 36.2 (11.1, 110); linerixibat 90 mg BID, 44.2 (14.4, 131); linerixibat 180 mg QD, 40.9 (12.8, 121).

3.2 | Development of a k-PD-PO Model Linking Linerixibat Dose-C4-Diarrhea Bother

To characterize the relationship between linerixibat dose and bother due to diarrhea, we developed a longitudinal PO model to predict the probability of a given score to the GSRS Question

TABLE 2 | Final parameter estimates of the population k-PD model for C4.

Parameter	Model parameter estimates (% RSE)[a]	% CV or SD for IIV (% RSE) [% shrinkage]
C4 model fixed effects		
Baseline (ng/mL)	11.9 (5.58)	89.2 (8.97) [6]
K_{out} (h^{-1})	0.135 (6.19)	—
$Amplitude_1$ (ng/mL)	0.268 (21.3)	—
$Acrophase_1$ (h)	5.77 (0.996)	—
Effect of baseline C4 on amplitude (ng/mL)	−0.0130 (61.7)	—
$Amplitude_2$ (ng/mL)	0.0227 (38.7)	—
$Acrophase_2$ (h)	21.7 (3.86)	—
KDE (h^{-1})	0.110 FIX[b]	—
E_{max}	5.55 (10.4)	55.8 (28.1) [46]
EDK_{50} (mg/h)	3.03 (23.5)	358 (27.5) [55]
PO model for tolerability fixed effects		
Intercept, $m=2$	−4.05 (8%)	5.44 (13) [6]
Intercept, $m=3$	1.26 (3%)	—
Intercept, $m=4$	0.983 (4%)	—
Intercept, $m=5$	1.15 (5%)	—
Intercept, $m=6$	1.11 (6%)	—
Intercept, $m=7$	1.53 (7%)	—
E_{max}	11.6 (4%)	—
EC_{50} (ng/mL)	42.9 (10%)	—
Residual variability for C4 model		
Additive error (SD)	0.000492 FIX[b]	—
Proportional error (CV%)	44.8 (2.05)	—

Abbreviations: C4, 7-alpha-hydroxy-4-cholesten-3-one; CV, coefficient of variation; EC_{50}, half maximal effective concentration; EDK_{50}, apparent *in vivo* potency of the drug; h, hours; IIV, inter-individual variability; KDE, rate constant of first-order elimination of the drug from the virtual compartment; K_{out}, rate constant of C4 elimination; PO, proportional odds, RSE, relative standard error; SE, standard error; SD, standard deviation.
[a]RSE (%) is calculated as SE/Estimate × 100; %CV is calculated as $[\exp(\text{variance})-1]^{1/2} \times 100$ in case of an exponential error model or $(\text{variance})^{1/2}$ in case of additive error model.
[b]FIX indicates the parameter for this value was fixed rather than estimated.

11 "Have you been bothered by diarrhea during the past week?", which was collected as a patient-reported outcome in GLIMMER, in relation to linerixibat dose. The final model development dataset consisted of 147 participants from GLIMMER, with 713 observations during the 4-week initial placebo run-in period and 1640 observations from the 12-week treatment period. The observed proportion of participants with given GSRS scores according to dose and treatment phase is shown in Figure S2. Compared with placebo, linerixibat treatment was associated with an increased proportion of participants reporting higher scores on the diarrhea question. VPCs confirmed that the final PO model adequately captured the probability of a certain C4 score; the median of the observed data generally fell within the respective 95% confidence intervals of the simulated data for all C4 concentrations (Figure S3).

3.3 | Dose-C4-Diarrhea Bother Model Observations and Simulations

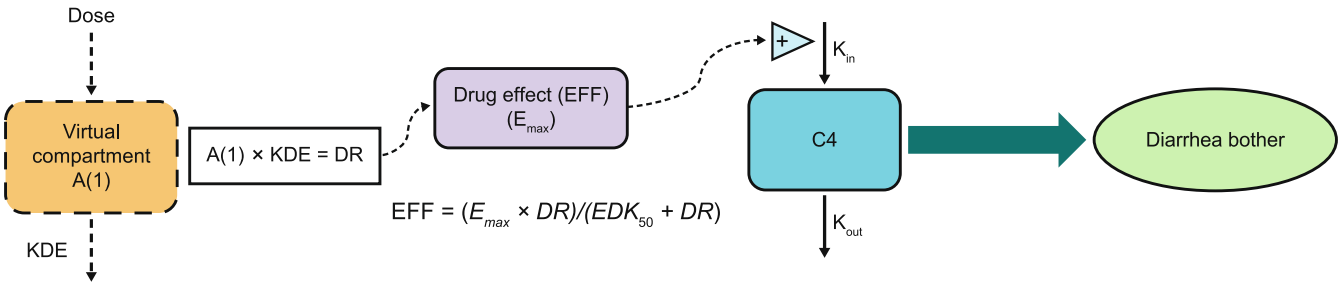
Increased C4 concentrations trended with increased patient-reported diarrhea bother scores in the GSRS tool. C4 concentrations ≥52 ng/mL (the cutoff previously reported to predict BA diarrhea with a specificity of 83%) [21] were associated with a 62% increased probability of moderate-to-very severe bother compared with C4 concentrations <14 ng/mL, as observed in participants in the placebo arm. C4 concentrations predicted an increased probability of moderate-to-very severe bother due to diarrhea (Figure 4 and Figure S4).

Simulations showed increases of 9%, 17%, 20%, 19%, and 23% in moderate-to-very severe diarrhea bother scores (≥4) for the 20 mg, 90 mg and 180 mg QD, and 40 mg and 90 mg BID doses, respectively, after the first week of linerixibat dosing. This was consistent with the rank order of on-treatment diarrhea AEs by treatment arm in GLIMMER (placebo: 11%; 20 mg QD: 38%; 90 mg QD: 65%; 180 mg QD: 67%; 40 mg BID: 52%; 90 mg BID: 68%) [15].

4 | Discussion

The dose-C4 k-PD model showed that linerixibat led to a rapid, dose-dependent increase in C4, a marker of BA synthesis. The increase in C4 was not continual but reached a steady-state as observed in the single fasting sample, consistent with that observed in the Phase 2b GLIMMER study [15]. Linerixibat treatment inhibits BA absorption, leading to an accumulation of BAs in the ileum and a downstream compensatory increase in C4 [20]. BAs are released from the gall bladder after meal-inducing contraction [32]. Linerixibat is therefore expected to have a greater effect on BAs and C4 if taken prior to gall bladder contraction after both morning and evening meals as opposed to only in the morning, when linerixibat, as a competitive reversible IBAT inhibitor, has likely been cleared from the small intestine prior to the dinner-induced gall bladder contraction, given gut transit times of a small molecule drug. The findings from the study align with this hypothesis; linerixibat BID dosing resulted in a higher C4 concentration when compared with a similar QD total daily dose of linerixibat dosed only in the morning. Linerixibat treatment also results in an excess of BAs reaching the colon, causing increased gut motility and diarrhea [17, 20]. Consistent with the observed data, initiation of linerixibat resulted in a dose-dependent increase in the proportion of patients reporting higher scores for the GSRS diarrhea bother question. Increased C4 concentrations correlated with increased diarrhea bother scores, regardless of linerixibat dose.

The dose-C4 k-PD model accurately described the effect of linerixibat on C4 concentrations over time using a simplified empirical approach. Similar models using data from the



$$\frac{d\,(C4)}{dt} = K_{in} \times \left(1 + AMP * \cos\left((Time - T_{peak}) \times \left(\frac{2\pi}{8h}\right)\right) + AMP_2 * \cos\left((Time - T_{peak2}) \times \left(\frac{2\pi}{12h}\right)\right)\right) \times (1 + EFF) - K_{out} \times C4$$

FIGURE 1 | Population k-PD model used to describe the relationship between linerixibat dose, C4 concentration and patient-reported diarrhea bother. AMP, amplitude; C4, 7-alpha-hydroxy-4-cholesten-3-one; EDK_{50}, apparent *in vivo* potency of the drug; h, hours; KDE, rate constant of first-order elimination of the drug from the virtual compartment, K_{in}, rate constant of C4 synthesis; K_{out}, rate constant of C4 elimination; k-PD, kinetic-pharmacodynamic; T_{peak}, time to acrophase.

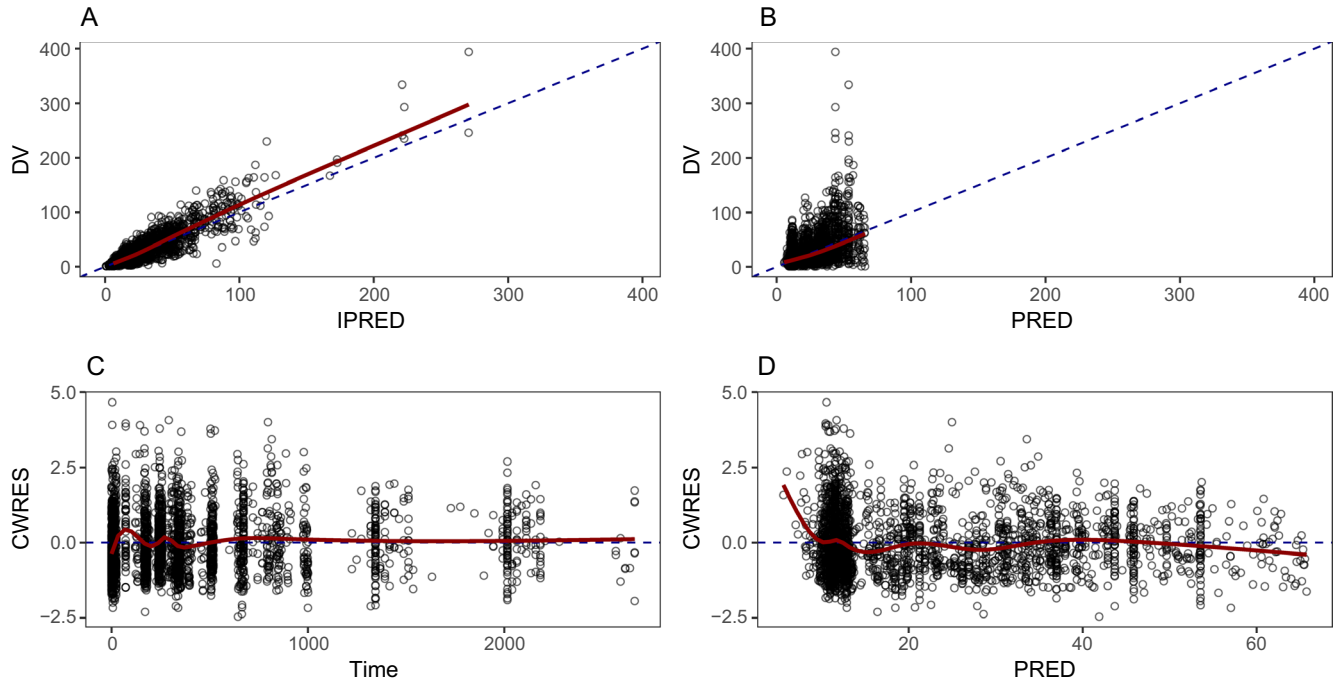


FIGURE 2 | Goodness-of-fit plots for the final population k-PD model of linerixibat dose-C4 concentration. Circles represent the DV, IPRED, PRED, and CWRES. The solid line represents the line of unity, and the dashed blue line represents the trend line for the corresponding data. C4, 7-alpha-hydroxy-4-cholesten-3-one; CWRES, conditional weighted residuals; DV, observed C4 concentration; IPRED, individual predictions; k-PD, kinetic-pharmacodynamic; PRED, population predictions.

GLIMMER study have previously been used to describe the effect of linerixibat dose on pruritus assessed on a numerical rating scale and on total serum BA concentrations [33]. Taken together, these findings highlight that modeling PD biomarkers is an effective method to optimize the dose of minimally absorbed drugs. C4 concentrations are known to vary diurnally due to the circadian system of cholesterol homeostasis and BA synthesis [34–36]. The dose-C4 k-PD model accounted for this using a dual cosine function on the synthesis rate. The relationship between linerixibat and C4 was best described with an E_{max} model, and a dose-dependent increase that appeared to plateau at approximately 180 mg total daily dose was evident. During model development, the KDE parameter estimate was similar to the value estimated in a prior k-PD model for linerixibat [33]. Therefore, to ensure successful minimization of the maximum likelihood algorithm, and to improve model stability, the KDE was fixed. In addition, a combined residual error with a fixed additive error was used to capture both proportional and additive residual variabilities and ensure minimization, as has been described elsewhere [37]. Exploration of the relationship between covariates and model parameters did not indicate a strong relationship between any of the continuous covariates and the model parameters. The C4 concentration at randomization had the only moderate correlation coefficient greater than 0.5. This moderate correlation was with the baseline C4 concentration parameter, a ratio of K_{in} over K_{out} in the

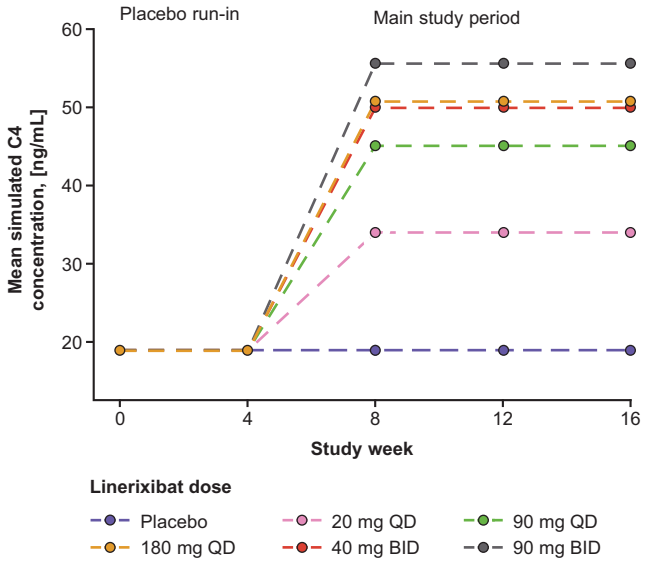


FIGURE 3 | Simulated mean C4 trough concentrations (fasted morning sample) over time for linerixibat dosing regimens and timepoints assessed in the Phase 2b GLIMMER study. The simulated C4 concentrations represented in the figure are fasting morning serum C4 samples. BID, twice daily; C4, 7-alpha-hydroxy-4-cholesten-3-one; QD, once daily.

indirect response model. While the SCM resulted in a significant change in the OFV analysis of goodness-of-fit plots, it did not result in model improvement, and therefore, this covariate was not included in the final model.

Fasting serum C4 is a direct measure of hepatic BA synthesis and is significantly correlated with fecal loss of BAs [38]. Vijayvargiya et al. demonstrated that a fasting serum cutoff of > 52.5 ng/mL of C4 could be used to predict BA diarrhea with a specificity of 83% [21]. In this study, model predictions for the upper 20% of fasting serum C4 concentrations were associated with a 62% increased probability of moderate-to-very severe bother according to the GSRS diarrhea question, compared with C4 concentrations < 14 ng/mL, as observed in participants in the placebo arm. The linerixibat dose-C4 relationship suggested that a dose of 40 mg BID results in an average fasting serum C4 concentration below this threshold. This was the dose selected for the Phase 3 GLISTEN trial, and its appropriateness is further supported by the adherence observed in the GLIMMER study; only one patient (4%) in the 40 mg BID group permanently discontinued treatment due to diarrhea, whereas three patients (14%) permanently discontinued treatment in the linerixibat 90 mg BID group and five patients (19%) in the linerixibat 180 mg BID group [15].

This analysis of GSRS data provides important insights into patient-reported diarrhea bother associated with IBAT inhibitors such as linerixibat, which may shed light on patient tolerability. Of note, the GSRS scores do not correlate directly with the reported AE of diarrhea, which may warrant further examination of the grouping of GSRS scores and how they can be better translated with the clinician-reported AEs. Furthermore, while these patient-reported outcome data do not directly assess treatment tolerability, which can be highly subjective and differ from person to person, they do describe patient bother and discomfort associated with diarrhea and therefore yield potential insights on patients' perspective of the impact to their quality of life [27]. Previous studies have also used the GSRS to investigate tolerance as it provides a rich dataset for understanding patient experiences of important gastrointestinal symptoms associated with treatment, including diarrhea [16, 28, 39]. Importantly, C4 has the potential to be used to titrate the dose of linerixibat to decrease the proportion of higher GSRS diarrhea severity scores. While the GSRS questionnaire may not prove useful in other indications, for example, chronic constipation, this framework of linking dose with serum C4 concentration and bowel movements could be helpful in identifying an appropriate dose of treatments such as elobixibat [40].

Interestingly, none of the covariates that were screened improved the base model fits or helped explain the variability in serum C4 concentrations or diarrhea severity scores. One potential reason is that approximately 80% of the C4 data were collected in tightly controlled Phase 1 and 2a studies in which the meals, meal composition, and mealtimes were controlled. Since C4 is a by-product of cholesterol metabolism intake, a meal has the potential to have a large impact; however, this dataset did not account for difference in meal composition. Importantly, the models did not identify race as a significant covariate despite the dataset including 38 Japanese versus 103 Caucasian participants. While expected based on a previous analysis [33] and the consistent efficacy and safety findings between the Japanese and overall populations in GLIMMER [41], it demonstrates that there is no difference in the effect of linerixibat on C4 or on patient-reported diarrhea bother in Japanese versus Western participants.

There are some limitations that need to be considered when interpreting the results of this study. Firstly, the link between the diarrhea bother question scores and what is acceptable to the patient from a tolerability perspective, such that they would want to reduce their dose or discontinue the drug, is unclear. Secondly, this model did not account for difference in meal composition despite the potential impact on C4 concentrations. However, in the Phase 1 and 2a studies, patients were domiciled in the clinic and provided standardized meals at controlled time points relative to dose administration and blood sampling. Thirdly, the inter-individual variability of EDK_{50} is quite high in this study, suggesting considerable differences in patient responses, which will impact linerixibat potency on changes in serum. One reason is this model compartment of drug in the GI tract is a virtual compartment, not supported by data but indirectly estimated based on linerixibat effect on C4 across many different studies with varying designs and patient populations.

In conclusion, linerixibat led to dose-dependent increases in serum C4 concentrations with BID regimens, which were greater than those observed with QD regimens. The average trough concentrations with linerixibat 40 mg BID, which is the dose used in the Phase 3 GLISTEN study, are below the threshold associated with BA diarrhea. C4 concentrations predicted an increased probability of moderate-to-very severe bother according to the GSRS diarrhea question. The increase in the diarrhea bother scores highlights the importance of dose evaluation for optimal dose selection, as demonstrated in GLIMMER, and the trade-off between maximizing the PD effect of linerixibat versus the increase in scores

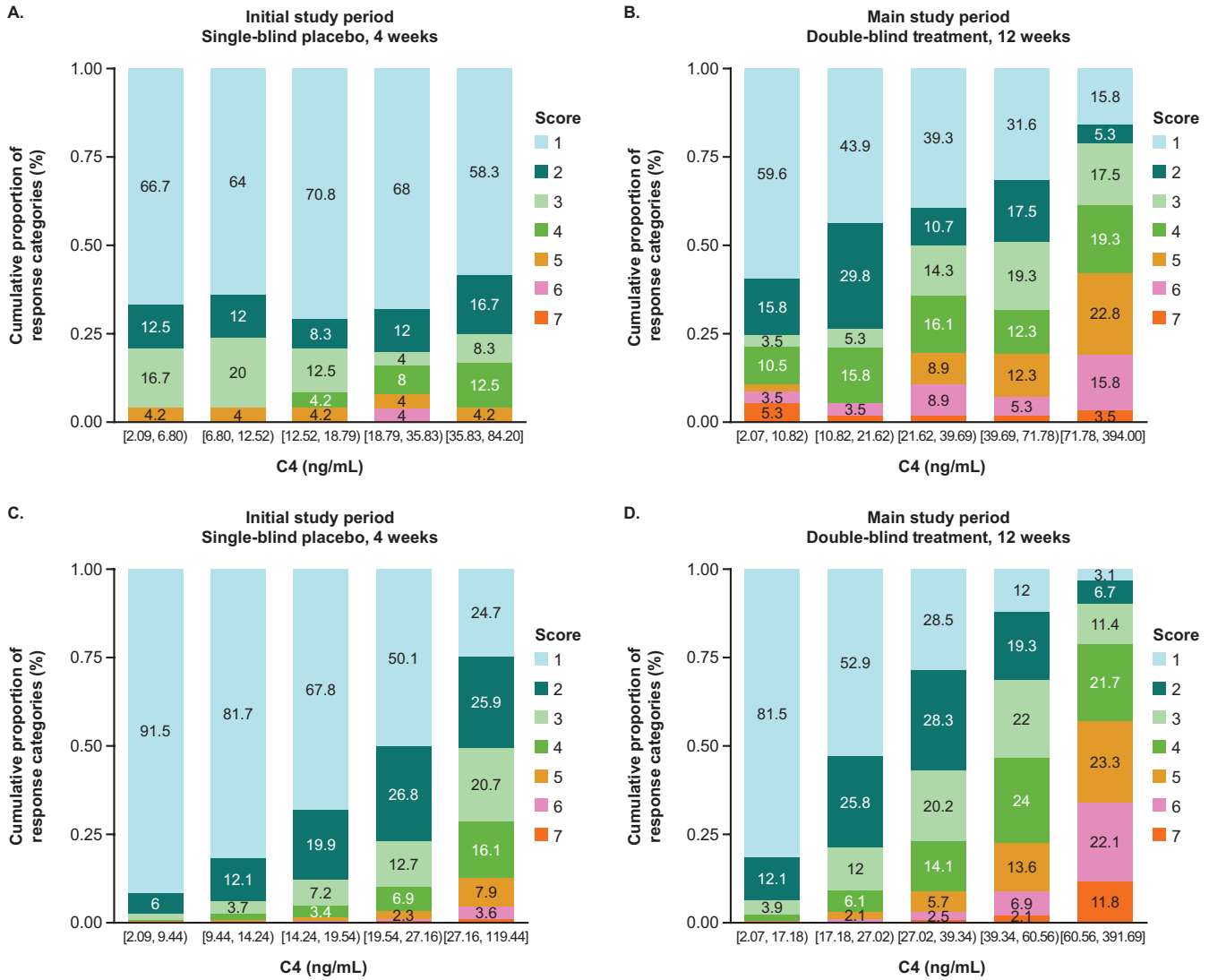


FIGURE 4 | Observed (A, B) and simulated (C, D) GSRS diarrhea question* scores stratified by serum C4 quartiles. *GSRS diarrhea Question 11: "Have you been bothered by diarrhea during the past week?" Score based on level of discomfort: 1 = none; 2 = minor; 3 = mild; 4 = moderate; 5 = moderately severe; 6 = severe; 7 = very severe. At baseline (A and C) and during active treatment period (B and D). Percentages lower than 2% are not displayed in the plots. C4, 7-alpha-hydroxy-4-cholesten-3-one; GSRS, gastrointestinal symptom rating scale.

for the GSRS diarrhea question. This analysis underlines that k-PD-PO modeling is a useful approach to examine dose-biomarker relationships and inform dose selection and drug tolerability.

Author Contributions

All authors wrote the manuscript. F.C., R.M., and B.S. designed the research. F.C., R.M., J.C., and B.S. performed the research. F.C., R.M., B.S., J.C., and A.R.S. analyzed the data.

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Conflicts of Interest

F.C., R.M., A.R.S., J.C., and B.S. are employed by GSK and hold financial equities in GSK.

Data Availability Statement

For requests for access to anonymized subject level data, please contact corresponding author.

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Supporting Information

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