Human Pharmacokinetics of LYS006, an Oral Leukotriene A4 Hydrolase Inhibitor Displaying Target-Mediated Drug Disposition

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List of Abbreviations

ADME, absorption, distribution, metabolism, and excretion; Ae, amount of drug excreted into urine; AUC, area under the concentration-time curve; AUC\text{last}, area under the concentration-time curve from time zero to the time of the last quantifiable concentration; AUC\text{tau,ss}, area under the concentration-time curve from time zero to the end of the dosing interval tau at steady state; AUC\text{0–t}, area under the concentration-time curve from time zero to time “t” where t is a defined time-point after administration; BCRP, breast cancer resistance protein; b.i.d., twice daily; CI, confidence interval; CL/F, apparent systemic clearance from plasma following extravascular administration; CLr, renal clearance from plasma; CLr,f, renal filtration clearance; Cmax, observed maximum concentration following drug administration; Cmax,ss, observed maximum concentration following drug administration at steady state; CV\%, percentage coefficient of variation; DDI, drug-drug interaction; FE, food effect; FIH, first-in-human; GFR, glomerular filtration rate; HEK293, human embryonic kidney cell lines; HS, hidradenitis suppurativa; Km, intrinsic transporter affinity of Michaelis-Mention constant; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LLOQ, lower limit of quantification; LTA4H, leukotriene A4 hydrolase; LTB4, leukotriene B4; LXA4, lipoxin A4; MAD, multiple ascending dose; MDCKII, Madin-Darby canine kidney strain II cells; MRP2, multidrug resistance-associated protein 2; MRP4, multidrug resistance-associated protein 4; NASH, nonalcoholic steatohepatitis; P-gp, p-glycoprotein; PK, pharmacokinetic; q.d., once daily; Racc, accumulation ratio calculated using AUC values obtained from a dosing interval; SAD, single ascending dose; SD, standard deviation; T1/2, Terminal half-life; Tmax, time to reach maximum concentration after drug administration; Tmax,ss, time to reach maximum concentration after drug administration at steady state;
TMDD, target-mediated drug disposition; UC, ulcerative colitis; Vmax, maximal transporter velocity; Vss/F, apparent volume of distribution at steady state; βL, βU, lower and upper limits of the 90% CI for the slope of the power equation
ABSTRACT

LYS006 is a potent leukotriene A4 hydrolase inhibitor currently in clinical development for long-term treatment of various neutrophil-driven inflammatory conditions. Here, we present pharmacokinetics from the first-in-human study with complementary metabolism and transporter profiling data. The randomized first-in-human study included nine cohorts receiving 5–2*100 mg of LYS006 or placebo, a crossover food-effect part, and a multiple-dose part consisting of two fasted (5 mg and 15 mg q.d.) and three fed cohorts (20–80 mg b.i.d.) of LYS006 or placebo. LYS006 and metabolites were assessed in plasma and urine, and transporters involved in LYS006 disposition were analyzed in vitro. Systemic plasma exposure increased with dose; steady-state exposure was dose proportional up to 40 mg b.i.d. Steady state was achieved after ~3 days with mean accumulation of 2.1-fold for 5 mg q.d. and ≤1.4-fold for all higher doses. Despite limited accumulation, a long terminal T1/2 was observed. The long T1/2 and saturable binding to blood cells, which causes a highly nonlinear blood-to-plasma distribution, reflect a strong impact of target binding on drug distribution at lower concentrations. Skin biopsy and blister fluid concentration data indicated saturable binding in the former but not the latter, suggesting saturable binding in tissues beyond blood. Major excretion of LYS006 (~90% of dose) through urine at steady state triggered renal transporter investigations that identified LYS006 as substrate of OAT3, OAT4, BCRP, and MRP4. Seven metabolites were identified in human plasma and urine, comprising only 4% of the dose recovered in urine at steady state.

Keywords: leukotriene A4 hydrolase inhibitor, first-in-human, pharmacokinetics, metabolism, target-mediated drug disposition, renal transporter
SIGNIFICANCE STATEMENT

Pharmacokinetic data from a first-in-human study combined with in vitro work support dose and regimen selection for patient studies with LYS006 and provide guidance on drug interaction investigations and other clinical pharmacology work needed for further development. Mass balance information at steady state without the use of a radiolabel, skin concentrations, and identification of the major clearance pathway, as well as the transporters driving elimination, make this a particularly conclusive early study despite nonlinear pharmacokinetics impacted by target binding.
INTRODUCTION

LYS006 is a novel, highly potent, and competitive inhibitor of leukotriene A4 hydrolase (LTA4H) that inhibits the biosynthesis of the pro-inflammatory leukotriene B4 (LTB4) (Markert et al., 2021; Röhn et al., 2021), which is crucial in recruiting and activating neutrophils (Numao et al., 2017). LTA4H inhibitors also increase the anti-inflammatory, resolution-enhancing lipoxin A4 (Markert et al., 2021; Rao et al., 2007). Changing the LTB4 and lipoxin A4 balance is expected to be relevant in conditions involving neutrophil-driven inflammation such as inflammatory acne, hidradenitis suppurativa (HS), ulcerative colitis (UC), and nonalcoholic steatohepatitis (NASH). Several LTA4H inhibitors, including DG-051 (Sandanayaka et al., 2010), JNJ-40929837 (Barchuk et al., 2014), Acebilustat (Elborn et al., 2018), and BI-691751 (clinicaltrial.gov), have been investigated. However, none of these candidates have progressed yet beyond Phase 2 clinical trials potentially due to lack of efficacy, toxicity, or adverse events. Compound-specific issues and/or choice of target indication could be the reason behind the failure of first-generation LTA4H inhibitors (Röhn et al., 2021). LYS006 is a new-generation selective LTA4H inhibitor, and with its promising preclinical profile, it shows potential to be the best-in-class therapeutic candidate for inflammatory diseases such as acne, HS, UC, and NASH (Penno et al., 2020; Markert et al., 2021). Proof-of-concept studies are underway to investigate the efficacy and safety of LYS006 in these indications (ClinicalTrials.gov Identifiers NCT03497897, NCT03827798, NCT04074590, and NEXSCOT-NCT04147195).

Preclinical investigations found a nonlinear pharmacokinetic (PK) profile of LYS006 across species (mouse, dog, and rat) with a suggestion of target-mediated drug disposition (TMDD), reflected by a pronounced concentration-dependent blood-to-
plasma distribution, likely due to saturation of LTA4H in blood cells (Markert et al., 2021). At high blood concentrations, close to and above target saturation (>250 nM; 100 ng/mL), LYS006 was predominantly found in the plasma of mouse, human (in vitro), dog, and rat; however, blood-to-plasma ratios increased with decreasing concentrations. Absence of concentration-dependent blood-to-plasma distribution of LYS006 in a LTA4H-knockout mouse reaffirmed that target binding affected the distribution of LYS006 (Markert et al., 2021).

Although TMDD is more often associated with high-molecular-weight drugs such as antibodies, it has also been observed in low-molecular-weight drugs (An, 2020) if those act on highly specific targets and when sensitive bioanalytical methods are used (An, 2017; van Waterschoot et al., 2018). A specific target site–binding PK model is useful to explain such complex nonlinearity in the PK of a drug. In this context, the model explaining the TMDD of draflazine resulting from the capacity-limited high-affinity binding of draflazine to the nucleoside transporter on the erythrocytes is notable (Snoeck et al., 1996; Snoeck et al., 1999) and has some similarity to what we observe for LYS006.

To support further clinical development, this first-in-human (FIH) study was conducted to investigate the PK and safety of LYS006 following single and multiple ascending doses in healthy volunteers. Considering the complexity of the expected TMDD, serial blood as well as plasma samples were analyzed in this study. Herein we report the clinical PK profile of LYS006, including metabolite identification and semi-quantification, as well as complementary in vitro work identifying transporters critical for the renal excretion of LYS006.
MATERIALS AND METHODS

Study Design

This was a single-center, randomized, participant-blinded and investigator partially blinded, placebo-controlled, sequential parallel-group FIH study in 121 healthy adults. The study protocol was reviewed by an independent ethics committee (Commissie voor Medische Ethiek, ZNA Konigin Paola Kinderziekenhuis, B-2020 Antwerpen, Belgium), and the study was conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent before any assessments were performed.

The study consisted of three parts: Part 1 (single ascending dose [SAD]), Part 2 (open-label, crossover food effect [FE]), and Part 3 (multiple ascending dose [MAD]). Participants were randomized using a validated system that automated the random assignment of treatment numbers in the specified ratio. The study comprised a 28-day screening period and a baseline period, followed by a treatment period and a follow-up period, which included an end-of-study evaluation. An overview of the study design is detailed in Fig. 1.

**Single Ascending Dose.** The SAD arm included nine cohorts, each with eight participants (except for cohort 7 where n=7 and cohort 9 where n=6). Each cohort was randomized 6:2 to receive either LYS006 or placebo, administered orally under fasting conditions. The first cohort received a dose of 10 mg, which was approximately 2-fold below the maximal recommended starting dose. The latter was derived by converting the no-observed-adverse-effect-level in the most sensitive toxicology species to a human equivalent dose and applying a 10-fold safety margin (CDER, 2005). A single 10-mg dose was predicted to be subtherapeutic based on
dog pharmacology data and a prediction of human pharmacokinetics. Since exposure at 10 mg was higher than predicted, the dose was decreased to 5 mg in the second cohort before escalation to higher dose levels. The goal of the escalation in SAD was to establish the safety at predicted therapeutic and somewhat supratherapeutic exposure to support multiple-dose administration in this study as well as in future patient trials. In cohorts 1–6, escalating single doses up to 70 mg were administered. To increase the daily dose and exposure in SAD but limit $C_{\text{max}}$, (which was considered safety relevant), cohorts 7–9 received two doses of 70, 100, and 40 mg, respectively, on a single day separated by 12 hours. In the first cohort, two sentinel participants (one LYS006-dosed, one placebo-dosed) were dosed first at the same time. In cohort 9 of the SAD arm, patients received 40 mg of LYS006 twice daily (for one day) separated by 12 hours under limited and controlled hydration to assess urine concentrations of LYS006 at lower urine flow.

**Food Effect.** The FE arm with a moderate fat meal had a two-way crossover, single-dose study design, which included two cohorts, each with six participants who were randomized 1:1 to receive 10 mg of LYS006 in one of two sequences: fasted then fed and fed then fasted. Both treatment periods were separated by a washout period of at least 14 days. The FE arm started once the safety from the first 72 hours of the 30-mg dose level in SAD was confirmed as satisfactory in all participants in the cohort and PK data were available.

**Multiple Ascending Dose.** The MAD arm included five cohorts, each with eight participants. Each cohort was randomized 6:2 to receive either LYS006 (5–80 mg) or placebo. LYS006 was administered orally, either once daily (q.d.; cohorts 1 and 2) or twice daily (b.i.d.; cohorts 3–5). There were 12 dosing days in total. On day 12, only the morning dose was administered in the b.i.d. cohorts. Cohorts 1 and 2 were
dosed under fasting conditions and cohorts 3–5 under fed conditions. In each cohort, a sentinel group of two participants (one LYS006-dosed, one placebo-dosed) was dosed at the same time.

**Objectives**

The objectives of this study were to assess the plasma, urine, skin and blister fluid, and blood PK of LYS006 in healthy participants. The time points of PK sample collection for SAD, FE, and MAD studies are detailed in **Supp. Tables S1–3**. Complementary work was performed to assess metabolites of LYS006 in plasma and urine, as well as in vitro investigation of the transporters involved in LYS006 disposition.

**Key Inclusion and Exclusion Criteria**

Healthy men and women aged 18–45 years, weighing ≥50 kg with a body mass index (BMI) in the range of 18–30 kg/m², with vital signs within the normal ranges, and with the ability to provide written informed consent were eligible to participate. Participants were excluded if they were using other investigational drugs, had a history of clinically significant electrocardiogram abnormalities, or had known presence of long QT syndrome. Pregnant women, smokers, and/or those with a history of drug/unhealthy alcohol use were also excluded. A comprehensive list of inclusion and exclusion criteria can be found in **Supp. Table S4**.

**Pharmacokinetic Analyses**

PK samples were collected at pre-defined time points (detailed in **Supp. Tables S1–3**). LYS006 concentrations were determined in plasma and urine by a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. The lower limits of quantification (LLOQ) were 0.200 ng/mL and 5.00 ng/mL for plasma and urine,
respectively. In addition, LYS006 concentrations were determined in the blood, skin, and blister fluid samples by qualified LC-MS/MS methods, with LLOQs of 0.200 ng/mL, 2.00 ng/g, and 0.200 ng/mL, respectively (a detailed description of LYS006 quantification can be found in Supp. Methods S3). Concentrations below the LLOQ were treated as zero in summary statistics and for PK parameter calculations.

**Urine PK.** The amount of LYS006 (Ae) and fraction of dose excreted into urine pools was determined from the urine concentration and volume-time data. (The amount of LYS006 in each collected time pool was calculated as (urine volume)×(urine concentration). For each participant, the amounts from all collected pools were added to obtain the Ae for this participant. Fraction of dose excreted (%) was calculated as (Ae/Dose)×100. The renal clearance (CLr) was determined based on drug exposure (AUC) and Ae available for the same time period (Ae(t1-t2)/AUC(t1-t2)). Time periods based on collected urine pools were 0–2, 2–4, 4–8, 8–12, 12–24, and 24 hours, at times >24 hours. Based on the observed concentration dependency of CLr, combining data across times representing a wide range of plasma concentrations (e.g., AUC(τau)) was considered inadequate. In SAD, corresponding urine pools were collected up to 72 hours after dosing. In MAD, urine samples were collected over one dosing interval at steady state on day 12.

**Skin Exposure.** Blister fluid (induced 24 hours earlier by topical application of cantharidin) (Day et al., 2001) and skin tissue (skin punch biopsies) were analyzed in the 15-mg q.d. and the 20-, 40-, and 80-mg b.i.d. treatment groups of the MAD part, with samples collected pre-dose on Day 9 (blister fluid) and Day 10 (skin tissue). Samples were also collected in the placebo group to maintain blinding. In SAD, only skin blister fluid was assessed in the LYS006 30-mg treatment group, with samples collected at 24 hours. To obtain the ratios to blood or plasma concentrations, the
concentration values obtained in skin tissue or blister fluid (Supp. Methods S3) were divided by the blood or plasma concentrations from the samples collected at the same sampling time and participants assuming that 1 g of skin is equivalent to 1 mL of plasma or blood.

**Plasma and Blood PK.** Parameters determined included $T_{\text{max}}$ (time to reach maximum concentration after drug administration), $T_{\text{max,ss}}$ (time to reach maximum concentration after drug administration at steady state), $T_{1/2}$ (terminal half-life), $C_{\text{max}}$ (observed maximum concentration following drug administration), $C_{\text{max,ss}}$ (observed maximum concentration following drug administration at steady state), $AUC_{\text{last}}$ (area under the concentration-time curve from time zero to the time of the last quantifiable concentration), $AUC_{\text{tau,ss}}$ (area under the concentration-time curve from time zero to the end of the dosing interval $\tau$ at steady state), $AUC_{0-t}$ (area under the concentration-time curve from time zero to time “$t$,“ where $t$ is a defined time point after administration), $R_{\text{acc}}$ (accumulation ratio calculated using AUC values obtained from a dosing interval), $V_{\text{ss}}/F$ (apparent volume of distribution at steady state), and $CL/F$ (apparent systemic clearance from plasma following extravascular administration). LYS006 PK parameters were determined using the actual recorded sampling times and noncompartmental methods using Phoenix WinNonlin (Version 6.4, Certara, Princeton, NJ, US) on the plasma, urine, and blood concentration data.

**Metabolite Analysis and Drug Disposition**

**Preparation of Plasma and Urine Pools for Metabolite Analysis.** Human plasma and urine samples were obtained at Day 12 (steady state) from six participants from the MAD cohort dosed at 15 mg q.d. with LYS006. Plasma aliquots were pooled across participants over a time range of 0–24 hours in a manner designed to mimic
the AUC of components (Hamilton et al., 1981). Urine aliquots were pooled across subjects and collection time periods to obtain a representative Ae over a time range of 0–24 hours.

**Plasma and Urine Sample Extraction for Metabolite Profiling.** A subfraction of the above plasma pools was prepared by protein precipitation and extraction with two volumes of acetonitrile (Fisher Chemical, Loughborough, UK) containing internal standard (defluorinated drug-related compound). The mixture was vortexed for 30 seconds using a Vortex-Genie® 2 (Scientific Industries, Bohemia, NY, US), placed in an ultrasonic bath (Branson Ultrasonic, Danbury, CT, US) for 5 minutes, and centrifuged (centrifuge Multifuge X3R, Thermo Fisher Scientific, Germany) at 10,000×g for 10 minutes at 10°C. The supernatant was collected, and the resulting pellet was reconstituted in half volume of water (Optima™ LC/MS Grade; Fisher Chemical, Loughborough, UK) and extracted again with one volume of acetonitrile containing internal standard following the same procedure. Both supernatants were combined and evaporated to dryness under a stream of nitrogen using a Techne Sample Concentrator (Techne, Cambridge, UK). The residue was reconstituted with a mixture of water/acetonitrile (3/1, v/v) and sonicated in a water bath for 5 minutes; then water was added to reach the original plasma volume. Finally, the reconstituted sample was centrifuged at 10,000×g for 10 minutes at 10°C. The clear supernatant was injected into the UHPLC-MS/MS system (Waters) for analysis. Urine pools were directly injected into the UHPLC-MS/MS system for analysis.

**Metabolite Structure Characterization.** After sample extraction and LC-MS/MS analysis (see Supp. Methods S1.1), metabolite structures were assigned based on exact mass measurements (see Supp. Table S11) and MS/MS fragmentation experiments, and comparison of LC-MS/MS data with reference standards (M3 and
M4). Metabolites M1, M3, M4, and M6 were semi-quantified using synthetic standards or \[^3\text{H}\]-radiolabeled standards from absorption, distribution, metabolism, and excretion (ADME) study of rat. For further details on metabolite semi-quantification, refer to **Supp. Methods (S1.2 and S1.3)**.

**Mass Balance Calculation.** Mass balance was estimated as the percentage of dose excreted in urine as LYS006 or metabolites over one steady-state dosing interval, based on combined data from the validated LC-MS quantification of LYS006 and exploratory semi-quantification of metabolites using the methods described above. Amount of LYS006 or metabolites was determined based on the concentration measured in the sample and the total volume of urine collected during the dosing interval. The percent of dose excreted by metabolites was calculated based on the amount of metabolite excreted in molar units and the dose in molar units.

**Statistical Analysis of Clinical Data**

Statistical analyses were performed with SAS 9.4 (SAS Institute Inc, Cary, NC, US). The PK analysis set included all participants with at least one available valid PK concentration measurement, who received any study drug, and who experienced no protocol deviations with relevant impact on PK data. The cohort sizes in SAD and MAD were not chosen based on statistical considerations. The cohort sizes in FE were powered to reject a food effect of ≥2-fold change in systemic exposure with food. If the FE was <25% of increase, the statistical power to reject a 2-fold increase was 87%, assuming an intra-participant CV of exposure of 40%. Descriptive summary statistics of PK parameters were provided by treatment and visit/sampling time point. PK parameters included mean, geometric mean, standard deviation, coefficient of variation, median, minimum, and maximum. In addition to PK summary statistics, the dose exposure relationship for relevant PK parameters from plasma
(AUC\textsubscript{last} [SAD only], AUC\textsubscript{0–24h} [SAD only], C\textsubscript{max,ss} [MAD only], AUC\textsubscript{tau,ss} [MAD only], and C\textsubscript{max} [SAD only]) was analyzed with the following power model:

\[
\ln(PK) = m + b \cdot \ln(Dose) + \text{error} \quad (\text{Eq. 1}).
\]

In FE, the PK parameters AUC\textsubscript{0–12h}, AUC\textsubscript{0–24h}, AUC\textsubscript{0–240h}, and C\textsubscript{max} were log transformed and analyzed with an analysis of variance model containing fixed effects for sequence, treatment, period, and participant nested within sequence. FE was then estimated by ratios of geometric means of PK parameters on fed and fasted conditions along with 90% confidence intervals (CIs) (back transformed from log scale). Only participants with evaluable data for both periods (fed and fasted) were included. In MAD, some doses were given in the fasted state and some in the fed state. The FE indicated the effect of feeding status on PK parameters; thus, it was important to allow for this effect in the analysis of dose proportionality in MAD. The final model fitted was one with different intercepts for fed and fasted status but a common slope for dose:

\[
\ln(PK) = m + \text{food effect} + b \cdot \ln(Dose) + \text{error} \quad (\text{Eq. 2}).
\]

Owing to the impact of feeding status on C\textsubscript{max,ss}, Eq. 2 was also used to perform the analysis in MAD on that parameter. An estimator for the slope (b), including the 90% CI, was provided based on the log-transformed observations. This estimator and CI were then “back transformed” to the original scale. The assessment of dose proportionality was based on this CI (location and width). An additional analysis of AUC\textsubscript{tau,ss} in MAD was carried out by omitting the top dose level. PK dose proportionality was considered across the whole dose range if the 90% CI (β\textsubscript{L}, β\textsubscript{U}) for the slope β\textsubscript{j} was completely contained within a prespecified critical region (b\textsubscript{L}, b\textsubscript{U}), where the two limits b\textsubscript{L} and b\textsubscript{U} are derived as follows:
\[ b_L = 1 + \ln(\theta_L)/\ln(r) \quad \text{and} \quad b_U = 1 + \frac{\ln(\theta_U)}{\ln(r)} \quad (Eq. 3) \]

with \( r \) as the ratio of doses (highest dose/lowest dose) and \( \theta_L (=0.8) \) and \( \theta_U (=1.25) \) being the standard bioequivalence limits. Summary statistics of skin tissue (only in MAD) and blister fluid LYS006 concentrations were calculated by timepoint and cohort. Concentration ratios relative to plasma and blood were summarized in the same way. The analysis was conducted on all participant data at the end of the study.

**In Vitro Investigations of Clinically Relevant Uptake and Efflux transporters**

A detailed description of all in vitro transport assays is provided in [Supp. Methods S2](#) (including [Tables S5-7](#)). In brief, in vitro phenotyping studies of human solute carrier (uptake) transporters were conducted to investigate whether LYS006 was a substrate of the organic anion drug transporters OAT1, OAT3, and OAT4 and the organic cation transporter OCT2 using transfected human embryonic kidney (HEK293) cell lines. In addition, LYS006 was investigated as a substrate of efflux transporters of the human ATP-binding cassette family using bidirectional permeability measurements across Madin-Darby canine kidney strain II cell (MDCKII) monolayers overexpressing P-glycoprotein (P-gp) or breast cancer resistance protein (BCRP) and using inside-out membrane vesicles prepared from cells overexpressing multidrug resistance-associated proteins (MRPs), namely MRP2 and MRP4. Transport of LYS006 was measured across a wide concentration range to determine kinetic parameters. To verify transport by a specific transporter, reference inhibitors were included in all assays. In addition, untransfected HEK293 and MDCKII control cells were used in all uptake and transcellular monolayer studies as additional control, and the active net transport was calculated by subtracting the clearance in the control cells from the one in the transfected cells. For vesicle
assays, LYS006 accumulation was measured in the presence of either ATP or AMP to calculate energy-dependent active net transport. Where feasible, measured data were fitted to a Michaelis-Menten model as follows:

\[
PS_{app} = PS_m + PS_c = PS_m + \frac{V_{c,\text{max}}}{K_m + S} = PS_m + \frac{PS_{c,\text{max}} \times K_m}{K_m + S} \quad (Eq. 4)
\]

where \(PS_{app}\), \(PS_m\), \(PS_c\), and \(PS_{c,\text{max}}\) are the apparent, passive, carrier-mediated (saturable), and maximum carrier-mediated uptake permeability (\(\mu\text{L/min/mg protein}\)), respectively; \(V_{c,\text{max}}\) is the maximum transporter uptake velocity rate (pmol/min/mg protein); and \(K_m\) is the Michaelis-Menten constant (\(\mu\text{M}\)).
Results

Participants

In total, 121 participants (SAD, N=69; FE, N=12; MAD, N=40) were enrolled and completed the study. There were no reported dosing administration errors. All demographic and other baseline characteristics were similar across treatment groups. The majority of participants were white (SAD, 92.8%; FE, 91.7%; MAD, 100%) and male (SAD, 94.2%; FE, 91.7%; MAD, 95%) with a mean age (± standard deviation [SD]) of 33.0±7.02 years (SAD), 35.1±7.87 years (FE), and 35.6±6.92 years (MAD), and mean (±SD) BMI of 24.4±2.48 kg/m² (SAD), 25.2±2.19 kg/m² (FE), and 24.6±2.60 kg/m² (MAD). Additional demographic characteristics are provided in Supp. Table S8.

Plasma Pharmacokinetics

The plasma PK parameters for SAD and MAD are presented in Table 1A, B.

Single-Dose PK. The mean plasma concentration-time profiles for LYS006 in the SAD arm are presented in Figs. 2A, B. The concentration-time profiles of LYS006 were characterized by an initial sharp peak ~1-hour post-dose, indicating rapid absorption (Fig. 2A and Table 1A). Concentrations subsequently dropped in a multiphasic way, initially quickly and within 12–36 hours across dose levels up to ~1 ng/mL; the later decline was slow, and concentrations were consistently measurable up to the last sampling point after 2 weeks (Fig. 2B). Terminal concentrations were all close to 1 ng/mL despite the large dose range. In contrast, shortly after dosing, exposure was largely proportional to the dose (Table 1A). With an increase in the dose from 5 to 100 mg (i.e., 20-fold), C_{max} increased by 21.7-fold. AUC_{0–24h} increased by 75-fold with a 40-fold increase in daily dose (5 mg to two times 100 mg
administered 12 hours apart to limit $C_{\text{max}}$ while increasing SAD dose) (Table 1A). For the dose range of 5 mg q.d. to 100 mg b.i.d. (40-fold) for AUC and 5–100 mg for $C_{\text{max}}$ following the first dose (20-fold), the estimated slope of the power model for $C_{\text{max}}$ was 1.08 (90% confidence interval [CI]: 1.01, 1.15); for AUC$_{\text{last}}$, the slope was 1.00 (90% CI: 0.96; 1.04), and for AUC$_{0-24}$, the slope was 1.20 (90% CI: 1.15; 1.24). Dose proportionality over the whole dose range was demonstrated for AUC$_{\text{last}}$ but not for $C_{\text{max}}$ and AUC$_{0-24}$. For $C_{\text{max}}$ and AUC$_{0-24}$, PK can be considered dose proportional only over a smaller dose range: 4.5-fold and 2.6-fold increase in dose, respectively (Smith et al., 2000). Dose proportionality was not observed for late timepoints, because the plasma concentrations of all dose groups converged with increasing time from dosing. Mean concentrations across cohorts 1 week after dosing were in the range of 0.458–0.828 ng/mL, i.e., less than 2-fold different despite a 40-fold dose range.

**Food Effect.** When LYS006 10 mg was administered after completion of a moderate-fat breakfast, the median $T_{\text{max}}$ was delayed from 1 to 2 hours. The mean terminal half-life was not affected. There was no or only a marginal decrease in AUC$_{0-12}$, AUC$_{0-24}$, and AUC$_{0-240}$ under fed conditions (AUC$_{0-12}$ fed/fasted, AUC$_{0-24}$ fed/fasted, and AUC$_{0-240}$ fed/fasted were ~0.9). In contrast, there was a more pronounced decrease in $C_{\text{max}}$ when administered with food compared with administration under fasted condition ($C_{\text{max}}$ fed/fasted: 0.6), with the %CV in $C_{\text{max}}$ also lower under fed conditions. Overall, these data indicate that food has a clinically relevant effect on LYS006 peak exposure and increases time to peak.

**Multiple-Dose PK.** The mean plasma concentration-time profiles of LYS006 in MAD are presented in Fig. 2C. Mean accumulation (derived for q.d. as AUC$_{0-24}$ Day 12/AUC$_{0-24}$ Day 1 and for b.i.d. as AUC$_{0-12}$ Day 12/AUC$_{0-12}$ Day 1) to steady state
was 2.1-fold for the lowest dose (5 mg q.d.), but for all higher q.d. or b.i.d. doses, accumulation was ≤1.4-fold (Table 1B). For the three cohorts with b.i.d. regimen, pre-dose concentrations on the morning of Day 12 were ~2- to 3-fold higher than the 12 hours post-dose concentrations, even though in both cases time to previous dose was 12 hours. From Day 3 to 4, no consistent or major increase in morning pre-dose concentrations was observed, suggesting that by Day 3, steady state was reached, in line with the limited observed accumulation. The CL/F derived from steady exposure was ~21.0 L/h on average for doses ranging from 5 mg q.d. to 40 mg b.i.d. but was lower at the highest tested dose (80 mg b.i.d.). Correspondingly, dose proportionality criteria were met for AUC_{τ,ss} from 5 mg q.d. to 40 mg b.i.d. For the dose range of 5 to 80 mg (16-fold), the estimated slope for C_{max,ss} was 1.19 (90% CI: 1.08, 1.30), and for AUC_{τ,ss}, the slope was 1.07 (90% CI: 1.01; 1.12). Dose proportionality over the whole dose range was not demonstrated for both C_{max,ss} and AUC_{τ,ss}. However, C_{max,ss} and AUC_{τ,ss} PK can be considered dose proportional for up to 2.12- and 6.29-fold increase in doses, respectively. The change in feeding state from 15 mg q.d. to 20 mg b.i.d. led to a decrease in mean C_{max,ss} despite an increase in dose, in line with results from the FE part.

**Blood Pharmacokinetics**

The relation of blood-to-plasma concentrations is provided in Fig. 3, which displays all blood-to-plasma concentration ratios across the three study parts (SAD, FE, and MAD) over the range of observed plasma concentrations. Samples collected up to 1-hour post-dose were excluded because no distribution equilibrium was reached. At LYS006 concentrations around and above 100 ng/mL, blood-to-plasma ratios were close to 1.0, indicating similar concentrations in blood and plasma. At low LYS006 concentrations of ~1 ng/mL during the terminal phase, blood-to-plasma ratios
increased to >30, indicating a predominant distribution into blood cells. In SAD, the mean blood exposure of LYS006 increased with dose. After a peak at ~1.5 hours after dosing, the blood concentration consistently decreased but remained higher than plasma concentrations resulting from high blood cell concentrations.

**Skin Blister Fluid and Skin Tissue Trough Exposure**

In SAD, skin blister fluid was assessed in the 30-mg treatment group, with the samples collected at 24 hours post-dose, and the mean concentration of LYS006 was 4.67 ng/mL. The blister fluid concentrations were on average ~2-fold higher than the plasma concentrations at the same time and 14% of the corresponding blood concentrations. In MAD, the mean pre-dose concentrations of LYS006 in blister fluid increased with dose from 4.6 ng/mL at 15 mg q.d. to 233 ng/mL at 80 mg b.i.d. In skin tissue from skin biopsies collected pre-dose in MAD, the mean concentrations changed slightly (33.7–50.9 ng/g) with dose. Skin tissue concentrations were comparable with blood concentrations, with skin/blood ratios ranging from 0.4 to 0.9. Skin and blister fluid concentration data are presented in Supp. Table S9.

**Urine Pharmacokinetics**

**Single-Dose PK.** The urine PK parameters for LYS006 SAD are summarized in Table 2, and the full dataset of urine PK parameters is presented in Supp. Table S10. The mean percentage of LYS006 recovered in urine ranged from 54.4% to 95.0% of the dose in the dose range of 5–2*100 mg b.i.d. Mean values below 60% were only observed for the 5- and 10-mg doses, whereas at higher doses, the values were >70%. The mean CLr values across the different dose groups and urine collection intervals (0–72 hours) covered a wide range, 2.5–26.9 L/h. However, for high plasma concentrations during the early collection intervals up to 4 hours, post-
dosing values of ~20 L/h were observed: for the 0- to 2-hour urine pools, the median of the mean CLr across the different dose groups was 20.9 L/h (range, 18.8–26.9 L/h) (Table 2). At later timepoints with lower plasma concentrations, CLr dropped to ~10.0 L/h, suggesting a concentration dependency. For the 48- to 72-hour collection interval in the SAD part, the median of the mean CLr across the different dose groups was 9.4 L/h (range, 4.9–14.2 L/h) (Table 2).

**Urinary Excretion in Crossover FE Part**: The mean fraction of the LYS006 dose recovered in urine was similar in both fasted and fed groups (69.1% and 64.4%, respectively), in line with the similar AUC. Independent of the feeding state/cohort, the mean fraction of the LYS006 dose recovered in urine was <60% after the first dose and >70% after the second dose administered 2 weeks later (Table 2).

**Multiple-Dose PK**. The urine PK parameters for LYS006 in MAD are summarized in Table 2, and the full dataset of urine PK parameters is presented in Supp. Table S10. The average excretion over one dosing interval at steady state was ≥90% of the dose at all dose levels, except for 20 mg b.i.d.; incomplete urine collection may explain the lower recovery of LYS006 in urine at this dose level because this deviation is otherwise difficult to explain for a dose in the middle of the tested range. As observed in SAD, CLr was ~20.0 L/h at the initial high plasma concentrations and dropped to ~10.0 L/h at later time points with lower plasma concentrations.

**Metabolite Analysis and Drug Disposition**

Seven metabolites were identified in human plasma and urine and characterized by LC-MS/MS analysis. Four of these metabolites were semi-quantified with calibration curves of either synthetic standard compounds or radioactive standard samples from...
a rat ADME study where rats were dosed with [³H]-LYS006. The calibration curves are provided in Supp. Fig. S1.

The exposure (AUC₀⁻₂₄h) of LYS006 and metabolites in plasma and amount excreted into urine at steady state after daily doses of 15 mg of LYS006 is detailed in Table 3, and a schematic summary of the metabolites identified in human plasma and urine is illustrated in Fig. 4. LYS006 and its metabolites excreted into urine over one dosing interval at steady state accounted for 95% of the dose. Metabolite pathways involve N-dealkylation (M4), carnitine conjugation (M1, M9), beta-oxidation (M3), deamination (M5, M9), and alkyl chain elongation (M6, M7). Although the exact structures of metabolites M6 and M7 could not be determined by mass spectrometry, the addition of two carbon atoms to the alkyl chain is most likely to correspond to fatty acid chain elongation via fatty acid synthesis pathway, especially given that LYS006 is also metabolized by the related beta-oxidation pathway. LYS006 was the major component in urine and plasma. All metabolites were minor components in urine. M4 was the only metabolite with a plasma exposure above 10% of total AUC₀⁻⁻₂₄h. Minor metabolites M5, M7, and M9 could not be successfully quantified in plasma and urine. The structures and the MS/MS spectra of LYS006 and the metabolites are presented in Supp. Fig. S2.

**In Vitro Study: Transporter Analysis**

Based on the observed predominant renal elimination, LYS006 was characterized in vitro as a substrate of transporters from solute carrier (OAT1, OAT3, OAT4, and OCT2) and ATP-binding cassette families (P-gp, BCRP, MRP2, and MRP4). Uptake experiments in recombinant HEK293 cells overexpressing the individual transporters indicated that LYS006 was a substrate of OAT3 and OAT4 with kinetic parameters shown in Table 4. Transport activity was significantly inhibited in the presence of the
reference inhibitors probenecid and MK571 for OAT3 and benz bromarone and
diclofenac for OAT4. LYS006 was not identified as a substrate of OAT1 and OCT2.
Studies of efflux transporters indicated that LYS006 is a substrate of BCRP and
MRP4 (Table 4). In the bidirectional permeability measurement in MDCKII-BCRP
cells, LYS006 showed 3.8-fold higher permeability in the B-A direction than in the A-
B direction, which was reduced close to unity (1.57-fold) in the presence of a
selective BCRP inhibitor (Ko143), indicating that LYS006 is a BCRP substrate.
The experiments with MRP4-vesicles showed significantly higher uptake of LYS006
in the presence of ATP compared to MRP4 vesicles incubated with AMP. LYS006
uptake was reduced by the MRP4 inhibitor MK571, indicating that LYS006 was a
substrate of MRP4. LYS006 was not identified as substrate of P-gp and MRP2.

Discussion and Conclusions
The human PK of LYS006 is characterized by a $T_{\text{max}}$ at 1–2 hours post-dosing,
followed by a multiphasic decline in concentration including an initial fast decline that
slowed considerably after 1–3 days. In line with the preclinical findings (Markert et
al., 2021), nonlinearity in the PK of LYS006 was evident: most prominently in a
highly concentration-dependent in vivo blood distribution as well as in plasma
concentrations, while close to dose proportional over the dosing interval becoming
largely dose independent with increasing time from dosing. Saturable binding of
LYS006 in blood cells, skin, and likely other tissues combined with slow redistribution
might explain most of the nonlinearity. Moreover, renal clearance, the major
mechanism of LYS006 elimination, decreased 2-fold at lower plasma concentrations.
Seven metabolites of LYS006 were identified in plasma and urine. In urine collected
over a dosing interval at 15 mg q.d. steady state, 95% of the dose was recovered.
Profiling of renal transporters identified OAT3 as basolateral uptake transporter for
LYS006 and BCRP, MRP4, and OAT4 as efflux transporters. A new FDA draft guideline (CDER, 2022) suggests that a radiolabeled mass balance study may not be required for drugs such as LYS006 with a very high urinary excretion (≥90% of dose).

**Systemic PK and Effects of Target Binding.** The steady-state exposure (AUC) to LYS006 was dose proportional from 5 mg q.d. to 40 mg b.i.d. Despite a rapid concentration decline following C\(_{\text{max}}\), a long apparent terminal half-life was observed with mean values across dose groups and study parts of 245–513 hours, i.e., 10–21 days. The accuracy of this estimation is limited as values are high relative to the sampling period (14 days), and also, the decline may not be adequately described with a half-life owing to the underlying non-linearity. Steady-state exposure was reached on ~Day 3 with little accumulation despite the slow terminal concentration decline. This indicates little relevance of the terminal phase for steady-state exposure and elimination, which is rapid. The fast initial concentration decline (~100-fold over 24 hours) provided the rationale to explore b.i.d. dosing in the MAD part because q.d. dosing requires higher daily doses to keep blood concentrations above IC\(_{90}\) over the 24-hour dosing interval.

LYS006 is an inhibitor of LTA4H, which is expressed in blood neutrophils, monocytes, and erythrocytes (McGee and Fitzpatrick, 1985). In the preclinical investigations, a concentration-dependent blood-to-plasma distribution of LYS006 was observed because of saturable target binding (Markert et al., 2021). The extent of saturable binding observed in blood and skin in this study as well as in earlier work suggests that erythrocytes and other nonimmune cells also contribute to the saturable target binding observed for LYS006. In this study, the sigmoid-shaped blood-to-plasma distribution curve was well in line with the in vitro potency of
LYS006 measured in human whole blood (IC₅₀ 53 nM; 20.8 ng/mL and IC₉₀ 148 nM; 58 ng/mL) (Markert et al., 2021). A slow redistribution of target-bound LYS006 into plasma is a plausible reason for the long terminal half-life during a largely dose-independent terminal phase. The specific binding capacity for LYS006 but not the dose is important for its exposure during this washout phase, which can therefore be described as TMDD. Interestingly, in both treatment sequences of the FE part, 1–2 mg more LYS006 was recovered in urine following the second dose in comparison to the first dose despite a 2-week washout period. This provides an initial estimate of the amount of LYS006 bound to LTH4H in the human body 2 weeks after dosing.

**Skin Blister Fluid and Skin Tissue.** As LYS006 is considered for the treatment of inflammatory skin diseases, its concentration was measured in skin and blister fluid samples collected at pre-dose steady state. Skin concentrations were largely independent of the dose, whereas blister fluid concentrations increased with the dose. A possible explanation is that pre-dose skin concentrations mostly reflect LTA4H-bound LYS006, and because of high degree of saturation already at 15 mg q.d., minimal increases were observed at higher doses. In contrast, LYS006 in blister fluid was mostly free or bound to plasma proteins such as albumin, for which no saturation was expected in the covered concentration range. Therefore, the concentrations increased with increasing dose and were about twice as high as pre-dose plasma concentrations. As LYS006 is detected in skin tissue and blister fluid in concentrations comparable to systemic exposure, inflammatory skin disorders may be effectively treated with LYS006.

**LYS006 Elimination, Active Transport, and Metabolism.** Urinary excretion of unchanged LYS006 was identified as the major route of elimination. At steady state, ~90% of the dose was excreted as LYS006 into urine over a dosing interval.
Considering the additional excretion of metabolites, this indicates that absorption and oral bioavailability of LYS006 are high (at least 90%). Higher plasma protein binding at lower LYS006 concentrations (~<3 ng/mL) was observed in vitro. This may partly explain the decrease in CLr at lower concentrations. Moreover, the increasing blood-to-plasma ratio can reduce active renal secretion (Kumar et al., 2020). The CLr during 0–4 hours (~20 L/h) was compared with the renal filtration clearance (CLr,f), which was calculated as the product of the unbound fraction of LYS006 in plasma (7.9%) (Markert et al., 2021) and the human glomerular filtration rate (125 mL/min) (Davies and Morris, 1993). The resulting CLr,f (0.59 L/h) represents only ~3% of the CLr, indicating that renal elimination of LYS006 is predominantly determined by transporter-mediated secretion. Therefore, transport of LYS006 by kidney transporters was assessed. OAT3 likely mediates the uptake of LYS006 from blood into proximal kidney tubule cells. Several transporters, including BCRP, MRP4, and OAT4, secrete LYS006 into the proximal tubule lumen. Inhibition of OAT3 is expected to affect LYS006 exposure; therefore, OAT3 inhibitors are excluded in ongoing clinical trials. Inhibition of any of the apical transporters (BCRP, MRP4, OAT4) is unlikely to change LYS006 exposure relevantly, because inhibition of a single transporter is expected to be compensated by the others. In general, parallel elimination pathways lower the risk of drug-drug interaction (DDI) (Weiss et al., 2021). Also, there is limited or no clinical evidence of DDI due to inhibition of these kidney transporters.

Metabolism was investigated at steady state with 15 mg q.d. dose. These data are expected to be relevant for any dose in the range investigated in this study considering the largely linear steady-state PK and the intention of chronic use. Although metabolism makes a minor contribution to LYS006 elimination, various
metabolites, formed via uncommon pathways including beta-oxidation, carnitine conjugation, and fatty acid chain elongation, were identified. Beta-oxidation is a pathway that metabolizes fatty acids and has been reported to metabolize xenobiotics with structural moieties related to fatty acids (Swizdor et al., 2012). Carnitine conjugation is a metabolic step that often occurs in conjunction with beta-oxidation and facilitates uptake of substrates to mitochondria, where beta-oxidation takes place (Testa and Krämer, 2008; Houten and Wanders, 2010). Fatty acid chain elongation is important for the synthesis of fatty acids and is not commonly reported as a xenobiotic metabolism pathway but was reported for the metabolism of small carboxylic acids (Dodds, 1991). Metabolite M4 was the only metabolite of relevant abundance in plasma and was not considered a safety concern as it was also present at high concentrations in the animals used for toxicological investigations.

Considering the predominant excretion of unchanged LYS006 into urine, no relevant DDI upon inhibition of metabolism is expected. Moreover, the identified metabolic pathways are generally not associated with DDI.

In the three b.i.d. cohorts of the MAD part, the mean concentrations observed on Day 12 in the morning pre-dose were 2- to 3-fold higher than concentrations measured in the evening, although both samples were collected 12 hours after the previous dose. A circadian rhythm of renal function with higher glomerular filtration, renal blood flow, and urine production during the day could explain this difference considering the predominant renal elimination of LYS006.

In conclusion, the findings of the FIH study of LYS006 indicate a high absorption and bioavailability, a predominant renal elimination, and nonlinearity in PK mostly apparent at low concentration. The nonlinearity is due to a concentration-dependent distribution of LYS006, at least partly caused by target binding with some...
contribution from concentration dependency of CLr. The predominant renal excretion (90% of dose) in combination with characterization of metabolites in plasma and urine allowed mass balance to be achieved based on the steady-state data. As a result, the metabolism contributing little to elimination and the renal excretion pathway, which drives most of the elimination of LYS006 could be well characterized without the need for a radiolabeled human ADME study. Despite the underlying complexity in PK, LYS006 is an attractive drug candidate based on its high absorption and bioavailability, its emerging DDI, quantitative disposition profile, and a largely linear steady-state PK. Ongoing patient trials will provide insights into the efficacy and safety of LYS006 in diseases involving neutrophil-driven inflammation.

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Authorship Contributions

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*Conducted experiments*: Cyrille Marvalin, Ester Tor Carreras, Patrick Schweigler

*Contributed new reagents or analytical tools*: NA

*Performed data analysis*: Martin Fink, Ester Tor Carreras, Birk Poller, David Pearson, Patrick Schweigler, H Markus Weiss

*Wrote or contributed to the writing of the manuscript*: Birk Poller, David Pearson, Luc Alexis Leuthold, Martin Fink, Astrid Jullion, Patrick Schweigler, Ester Tor Carreras, Cyrille Marvalin, Christian Loesche, H Markus Weiss
Data Sharing Statement

Novartis will not provide access to patient-level data if there is a reasonable likelihood that individual patients could be reidentified. Phase 1 studies by their nature present a high risk of patient reidentification; therefore, patient individual results for phase 1 studies cannot be shared. In addition, clinical data in some cases have been collected subject to contractual or consent provisions that prohibit transfer to third parties. Such restrictions may preclude granting access under these provisions. If co-development agreements or other legal restrictions prevent companies from sharing particular data, companies will work with qualified requestors to provide summary information when possible.
References


Footnotes

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Legends for Figures

Fig. 1. Overview of the study design. FE, food effect; MAD, multiple ascending dose; SAD, single ascending dose.

Fig. 2. Arithmetic mean (±SD) linear and semi-logarithmic plasma concentration-time profiles for SAD and MAD: (A) Linear view of plasma concentration-time profiles on Day 1 (SAD); (B) Semi-logarithmic view of plasma concentration-time profiles from Day 1 to 336 hours post-LYS006 administration (SAD); (C) Linear view of plasma concentration-time profiles on Day 12 (MAD). Note that the b.i.d. doses in the MAD part were administered under fed conditions resulting in a later peak. MAD, multiple ascending dose; SAD, single ascending dose; SD, standard deviation.

Fig. 3. Relationship of the blood-to-plasma concentration ratios to the LYS006 plasma concentration across the three study parts from 1 to 336 hours post-dosing. Samples collected less than 1 hour after dosing had often lower ratios due to incomplete equilibration and were not included.

Fig. 4. A schematic summary of the metabolites identified in human plasma and urine.
Table 1A. Summary statistics of pharmacokinetic parameter of LYS006 in plasma: Single ascending dose. Dosing was under fasted condition except for the last three (20 mg b.i.d., 40 mg b.i.d., and 80 mg b.i.d.) cohorts in multiple ascending dose

<table>
<thead>
<tr>
<th></th>
<th>Tmax (h)</th>
<th>T1/2 (h)</th>
<th>Cmax (ng/mL)</th>
<th>AUClast (h*ng/mL)</th>
<th>AUC0-12h (h*ng/mL)</th>
<th>AUC0-24h (h*ng/mL)</th>
<th>AUC12-24h (h*ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LYS006 5 mg</td>
<td>1.00</td>
<td>513±228</td>
<td>65.5±12.1</td>
<td>327±56.5</td>
<td>148±24.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>n=6</td>
<td>(0.87, 1.50)</td>
<td>(44.5)</td>
<td>(18.5)</td>
<td>(17.3)</td>
<td>(16.2)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>10 mg</td>
<td>1.00</td>
<td>458±127</td>
<td>118±33.1</td>
<td>508±81.2</td>
<td>283±56.7</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>n=6</td>
<td>(1.00, 2.00)</td>
<td>(27.8)</td>
<td>(27.9)</td>
<td>(16.0)</td>
<td>(20.0)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>20 mg</td>
<td>1.50</td>
<td>364±111</td>
<td>301±58.2</td>
<td>1040±55.9</td>
<td>790±50.6</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>n=6</td>
<td>(0.75, 2.00)</td>
<td>(30.3)</td>
<td>(19.3)</td>
<td>(5.4)</td>
<td>(6.4)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>30 mg</td>
<td>1.50</td>
<td>339±91.1</td>
<td>358±92.6</td>
<td>1460±177</td>
<td>1160±159</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>n=6</td>
<td>(0.75, 3.02)</td>
<td>(26.8)</td>
<td>(25.9)</td>
<td>(12.1)</td>
<td>(13.7)</td>
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<td>—</td>
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<tr>
<td>45 mg</td>
<td>1.00</td>
<td>260±49.8</td>
<td>767±318</td>
<td>2140±529</td>
<td>1840±483</td>
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<td>—</td>
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<tr>
<td>n=6</td>
<td>(1.00, 1.50)</td>
<td>(19.2)</td>
<td>(41.4)</td>
<td>(24.7)</td>
<td>(26.3)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>70 mg</td>
<td>1.50</td>
<td>328±157</td>
<td>906±177</td>
<td>2830±388</td>
<td>—</td>
<td>2530±410</td>
<td>—</td>
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<tr>
<td>n=5</td>
<td>(0.75, 1.50)</td>
<td>(47.8)</td>
<td>(19.5)</td>
<td>(13.7)</td>
<td>(16.2)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Dose</td>
<td>n</td>
<td>Tmax (h)</td>
<td>Cmax (ng/mL)</td>
<td>AUC0–12h (ng*h/mL)</td>
<td>AUC0–24h (ng*h/mL)</td>
<td>AUC12–24h (ng*h/mL)</td>
<td>AUClast (ng*h/mL)</td>
</tr>
<tr>
<td>------------</td>
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<td>--------------</td>
<td>--------------------</td>
<td>--------------------</td>
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<td>--------------------</td>
</tr>
<tr>
<td>70 mg b.i.d.</td>
<td>6</td>
<td>1.00</td>
<td>381±162</td>
<td>1280±286</td>
<td>8320±906</td>
<td>3680±465</td>
<td>7680±922</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(42.5)</td>
<td>(22.2)</td>
<td>(10.9)</td>
<td>(12.6)</td>
<td>(12.0)</td>
</tr>
<tr>
<td>100 mg b.i.d.</td>
<td>6</td>
<td>1.50</td>
<td>245±123</td>
<td>1420±341</td>
<td>11800±1740</td>
<td>4720±1090</td>
<td>11100±1740</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(50.3)</td>
<td>(24.0)</td>
<td>(14.7)</td>
<td>(23.1)</td>
<td>(15.8)</td>
</tr>
<tr>
<td>40 mg b.i.d.</td>
<td>4</td>
<td>1.25</td>
<td>250±130</td>
<td>747±182</td>
<td>5780±627</td>
<td>2380±361</td>
<td>5290±624</td>
</tr>
<tr>
<td>(limited hydration)</td>
<td></td>
<td></td>
<td>(52.0)</td>
<td>(24.4)</td>
<td>(10.9)</td>
<td>(15.2)</td>
<td>(11.8)</td>
</tr>
</tbody>
</table>

Statistics are mean±SD (CV%) and CV%=SD/mean*100.
*For 5 and 45 mg LYS006 dose groups n=5 for T1/2.
For Tmax, statistics are median (min, max).
AUC0–12h, area under the plasma concentration-time curve from time zero to 12 hours after drug administration; AUC0–24h, area under the plasma concentration-time curve from time zero to 24 hours after drug administration; AUC12–24h, area under the plasma concentration-time curve from 12 to 24 hours after drug administration; AUClast, area under the plasma concentration-time curve from time zero to the time of the last quantifiable concentration; b.i.d., twice daily; Cmax, observed maximum plasma concentration following drug administration; CV, coefficient of variation; h, hour; n, number of participants in individual cohort; ng, nanogram; q.d., once daily; SD, standard deviation; T1/2, terminal half-life; Tmax, time to reach maximum plasma concentration after drug administration.
Table 1B. Summary statistics of pharmacokinetic parameter of LYS006 in plasma: Multiple ascending dose

<table>
<thead>
<tr>
<th>Dose</th>
<th><strong>Profile Day 1</strong></th>
<th><strong>Profile Day 12</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (h)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</td>
<td>C&lt;sub&gt;max/dose&lt;/sub&gt;</td>
</tr>
<tr>
<td>5 mg q.d.</td>
<td>1.00 (0.75, 2.00)</td>
<td>52.8±15.9 (30.2)</td>
</tr>
<tr>
<td>15 mg q.d.</td>
<td>1.00 (0.75, 2.00)</td>
<td>213±36.5 (17.1)</td>
</tr>
<tr>
<td>20 mg b.i.d.</td>
<td>2.50 (1.50, 3.00)</td>
<td>194±54.4 (28.1)</td>
</tr>
<tr>
<td>40 mg b.i.d.</td>
<td>2.00 (2.00, 3.00)</td>
<td>352±38.7 (11.0)</td>
</tr>
<tr>
<td>80 mg b.i.d.</td>
<td>2.00 (1.50, 3.00)</td>
<td>1010±179 (17.7)</td>
</tr>
</tbody>
</table>
Statistics are mean±SD (CV%) [n] and CV%=SD/mean*100.

*For 5 mg q.d. and 80 mg b.i.d. LYS006 dose groups, T1/2 was not calculated and hence n=5 for T1/2.

*For T_max, statistics are median (min, max) [n].

Racc calculated as (AUC_{ss}, Day 12)/(AUC_{ss}, Day 1).

AUC_{0-12h}, area under the plasma concentration-time curve from time zero to 12 hours after drug administration; AUC_{0-24h}, area under the plasma concentration-time curve from time zero to 24 hours after drug administration; AUC_{0-12h,ss}, area under the plasma concentration-time curve from time zero to 12 hours after drug administration at steady state; AUC_{0-24h,ss}, area under the plasma concentration-time curve from time zero to 24 hours after drug administration at steady state; AUC_{0-24h}, area under the plasma concentration-time curve from 12 to 24 hours after drug administration; AUC_{0-24h}, area under the plasma concentration-time curve from 12 to 24 hours after drug administration; AUC_{last}, area under the plasma concentration-time curve from time zero to the time of the last quantifiable concentration; AUC_{max}, area under the plasma concentration-time curve from time zero to the end of the dosing interval τ; b.i.d., twice daily; CL/F_{ss}, apparent systemic clearance from plasma following extravascular administration at steady state; C_{max}, observed maximum plasma concentration following drug administration; C_{max,ss}, observed maximum plasma concentration following drug administration at steady state; CV, coefficient of variation; n, number of participants in individual cohort; ng, nanogram; PK, pharmacokinetics; q.d., once daily; Racc, accumulation ratio calculated using AUC values obtained from a dosing interval; SD, standard deviation; T_{1/2}, terminal half-life; T_{max}, time to reach maximum plasma concentration after drug administration; T_{max,ss}, time to reach maximum plasma concentration after drug administration at steady state; V_{ss}/F, apparent volume of distribution at steady state.
Table 2. Pharmacokinetic parameters of LYS006 in urine: Single ascending dose and multiple ascending dose

<table>
<thead>
<tr>
<th>Single Ascending Dose - Profile Day 1</th>
<th>Amount recovered (mg), [n]</th>
<th>Fraction of dose excreted (%), [n]</th>
<th>$\text{CL}_\text{R0-2h}$ (L/h), [n]</th>
<th>$\text{CL}_\text{R48-72h}$ (L/h), [n]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 mg</td>
<td>2.7±0.4 (13.0) [6]</td>
<td>54.4±7.1 (13.0) [6]</td>
<td>18.8±5.2 (27.9) [6]</td>
<td>4.9±0.9 (17.9) [6]</td>
</tr>
<tr>
<td>10 mg</td>
<td>5.8±0.7 (12.1) [6]</td>
<td>57.5±6.9 (12.1) [6]</td>
<td>20.9±5.0 (24.1) [6]</td>
<td>8.1±1.2 (15.0) [6]</td>
</tr>
<tr>
<td>30 mg</td>
<td>23.3±2.6 (11.3) [6]</td>
<td>77.5±8.8 (11.3) [6]</td>
<td>22.7±5.0 (22.2) [6]</td>
<td>9.4±1.3 (13.2) [6]</td>
</tr>
<tr>
<td>45 mg</td>
<td>38.2±4.8 (12.5) [6]</td>
<td>84.9±10.6 (12.5) [6]</td>
<td>24.5±8.1 (33.2) [6]</td>
<td>9.8±2.3 (23.1) [6]</td>
</tr>
<tr>
<td>70 mg</td>
<td>51.3±11.9 (23.2) [5]</td>
<td>73.3±17.0 (23.2) [5]</td>
<td>20.0±4.4 (21.9) [5]</td>
<td>10.4±2.3 (21.5) [5]</td>
</tr>
<tr>
<td>70 mg b.i.d.</td>
<td>102.0±6.0 (5.9) [6]</td>
<td>73.0±4.3 (5.9) [6]</td>
<td>19.6±3.3 (16.6) [6]</td>
<td>9.3±4.3 (46.9) [4]</td>
</tr>
<tr>
<td>100 mg b.i.d.</td>
<td>190.0±9.0 (4.7) [6]</td>
<td>95.0±4.5 (4.7) [6]</td>
<td>26.9±6.5 (24.3) [6]</td>
<td>14.2±3.1 (22.0) [6]</td>
</tr>
<tr>
<td>40 mg b.i.d. (limited hydration)</td>
<td>72.8±0.4 (0.6) [2]</td>
<td>91.0±0.5 (0.6) [2]</td>
<td>19.2±1.7 (9.1) [4]</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Food Effect</th>
<th>Amount recovered (mg), [n]</th>
<th>Fraction of dose excreted (%), [n]</th>
<th>$\text{CL}_\text{R0-2h}$ (L/h)</th>
<th>$\text{CL}_\text{R48-72h}$ (L/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mg fasted period 1</td>
<td>6.0±0.3 (4.8) [6]</td>
<td>59.6±2.9 (4.8) [6]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10 mg fasted period 2</td>
<td>7.9±0.7 (8.7) [6]</td>
<td>78.5±6.8 (8.7) [6]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10 mg fasted overall</td>
<td>6.9±1.1 (16.0) [12]</td>
<td>69.1±11.0 (16.0) [12]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10 mg fed period 1</td>
<td>5.7±1.0 (17.0) [6]</td>
<td>57.4±9.8 (17.0) [6]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10 mg fed period 2</td>
<td>7.1±0.5 (7.4) [6]</td>
<td>71.4±5.3 (7.4) [6]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10 mg fed overall</td>
<td>6.4±1.1 (16.3) [12]</td>
<td>64.4±10.5 (16.3) [12]</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Multiple Ascending Dose - Profile Day 12</th>
<th>Amount recovered (mg), [n]</th>
<th>Fraction of dose excreted (%), [n]</th>
<th>$\text{CL}_\text{R0-2h,ss}$ (L/h), [n]</th>
<th>$\text{CL}_\text{R48-72h,ss}$ (L/h), [n]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td>q.d.</td>
<td>b.i.d.</td>
<td>(91.0 \pm 16.4) [6]</td>
<td>(22.5 \pm 3.7) [36%] [6]</td>
</tr>
<tr>
<td>-------</td>
<td>------</td>
<td>--------</td>
<td>-----------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>5 mg</td>
<td>4.6±0.8 (18.0) [6]</td>
<td>13.6±1.3 (9.6) [6]</td>
<td>15.4±2.2 (14.3) [6]</td>
<td>40.0±3.1 (7.8) [6]</td>
</tr>
<tr>
<td>15 mg</td>
<td>9.0±8.7 (9.6) [6]</td>
<td>90.4±8.7 (9.6) [6]</td>
<td>76.8±11.0 (14.3) [6]</td>
<td>99.9±7.8 (14.3) [6]</td>
</tr>
<tr>
<td>30 mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Statistics are mean±SD (CV%) [n] and CV%=SD/mean*100.

q.d., once daily; b.i.d., twice daily; CLr, renal clearance from plasma; CLr, ss, renal clearance from plasma at steady state; CV, coefficient of variation; n, number of participants in each individual cohort; q.d., once daily; SD, standard deviation.
Table 3. LYS006 and metabolite exposure in plasma and urine excretion at steady state

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>AUC_{0-24h} (% total AUC)</th>
<th>Urine excretion (% dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>0.9</td>
<td>1.2</td>
</tr>
<tr>
<td>M3</td>
<td>4.3</td>
<td>2.9</td>
</tr>
<tr>
<td>M4</td>
<td>24.3</td>
<td>0.1</td>
</tr>
<tr>
<td>M5</td>
<td>Not quantified</td>
<td>Not quantified</td>
</tr>
<tr>
<td>M6</td>
<td>4.3</td>
<td>0.2</td>
</tr>
<tr>
<td>M7</td>
<td>Not quantified</td>
<td>Not quantified</td>
</tr>
<tr>
<td>M9</td>
<td>Not detected</td>
<td>Not quantified</td>
</tr>
<tr>
<td>LYS006</td>
<td>66.1</td>
<td>90.4</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>94.8</td>
</tr>
</tbody>
</table>

\(AUC_{0-24h}\), area under the plasma concentration-time curve for all quantified drug related material from time zero to 24 hours after drug administration at 15 mg q.d. at steady state; M, metabolite. Urine was collected over one 24-hour dosing interval at steady state.

Table 4. In vitro assessment of transport kinetics

<table>
<thead>
<tr>
<th>Transporter</th>
<th>In vitro system</th>
<th>(K_m) (μM)</th>
<th>(V_{max}) (pmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-gp</td>
<td>MDCKII-MDR1 cells</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>BCRP</td>
<td>MDCKII-BCRP cells</td>
<td>&gt;300</td>
<td>ND</td>
</tr>
<tr>
<td>MRP2</td>
<td>HEK293-MRP2 vesicles</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>MRP4</td>
<td>HEK293-MRP4 vesicles</td>
<td>&gt;150</td>
<td>~&gt;180</td>
</tr>
<tr>
<td>OAT1</td>
<td>HEK293-OAT1 cells</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>OAT3</td>
<td>HEK293-OAT3 cells</td>
<td>31.1</td>
<td>1013</td>
</tr>
<tr>
<td>OCT2</td>
<td>HEK293-OCT2 cells</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>OAT4</td>
<td>HEK293-OAT4 cells</td>
<td>0.085</td>
<td>1.25</td>
</tr>
</tbody>
</table>

BCRP, breast cancer resistance protein; HEK293, human embryonic kidney cells; \(K_m\), intrinsic transporter affinity or Michaelis-Menten constant; MDCKII, Madin-Darby canine kidney cells; MDR1, multidrug-resistant protein 1; MRP, multidrug resistance-associated protein; NA, no active transporter measured; ND, parameter could not be determined due to the absence of saturation up to the highest tested LYS006 concentration (300 μM); OAT, organic anion transporter; OCT, organic cation transporter; P-gp, P-glycoprotein; \(V_{max}\), maximal transporter velocity.
Figure 1.
Figure 2.
Figure 3.
Figure 4.