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Title
Population Pharmacokinetics and Exposure-Response Relationships of Baloxavir Marboxil in Patients Infected with Influenza at High Risk of Influenza Complications

Running title (50/54 characters including space)
PK/PD of Baloxavir in High-Risk Influenza Patients

Authors
Hiroki Koshimichi,a,# Sylvie Retout,b Valerie Cosson,b Vincent Duval,c Stefan De Buck,b
Yoshiyuki Tsuda,a Toru Ishibashi,a Toshihiro Wajimaa

Affiliation
a Clinical Pharmacology & Pharmacokinetics, Project Management Department, Shionogi & Co., Ltd., Osaka, Japan
Roche Pharma Research and Early Development, Pharmaceutical Sciences, Roche Innovation Center Basel, Switzerland

Certara, Data Science Services, Basel, Switzerland

# Address correspondence to Hiroki Koshimichi, hiroki.koshimichi@shionogi.co.jp
ABSTRACT (250/250 words)

Baloxavir marboxil, a prodrug of cap-dependent endonuclease inhibitor, baloxavir acid, reduces the time to improvement of influenza symptoms in patients infected with type A or B influenza virus. To characterize its pharmacokinetics, a population pharmacokinetic model for baloxavir acid was developed using 11846 plasma concentration data items from 1827 subjects including 2341 plasma concentration data items from 664 patients at high risk of influenza complications. A three-compartment model with first-order elimination and first-order absorption with lag time well described the plasma concentration data. Body weight and race were found to be the most important factors influencing clearance and volume of distribution. The exposures in high-risk patients were similar to those in otherwise healthy patients, and no pharmacokinetic difference was identified regarding any risk factors for influenza complications.

Exposure-response analyses were performed regarding the time to improvement of symptoms and the reduction in the influenza virus titer in high-risk patients. The analyses suggested that body weight-based dosage, 40 mg for patients weighing < 80 kg and 80 mg for patients weighing ≥ 80 kg, can shorten the time to improvement of influenza symptoms and reduce virus titer for both type A and B influenza virus regardless of the exposure.
levels of the high-risk patients as well as for the otherwise healthy influenza patients.

The results of our population pharmacokinetic and exposure-response analyses in patients with risk factors of influenza complications should provide useful information on the pharmacokinetic and pharmacodynamic characteristics of baloxavir marboxil and also for the optimization of dose regimens.
KEYWORDS

Baloxavir marboxil, cap-dependent endonuclease inhibitor, influenza, population pharmacokinetics, S-033188, exposure-response, high risk of influenza complications
INTRODUCTION

Baloxavir marboxil (product code S-033188, Xofluza®) is a prodrug of baloxavir acid, a potent and selective inhibitor of cap-dependent endonuclease necessary for the replication of both influenza A and B viruses. Baloxavir marboxil has been approved in Japan and the United States for the treatment of adults and adolescents (at least 12 years old) with acute uncomplicated influenza (Xofluza package insert).

After single oral administration, baloxavir marboxil is metabolized to baloxavir acid mainly by arylacetamide deacetylase (AADAC) in the intestine and liver, and baloxavir acid is eliminated from the plasma via a metabolism by uridine diphosphate-glucuronosyltransferase 1A3 (UGT1A3) in the liver with minor contribution from CYP3A4 (1). In thorough QTc study (Supplementary Table S1), in which 39 males and 24 females were assessed in the pharmacokinetic analyses in the same study, time to maximum plasma concentration was slightly longer in females (median at 80 mg: 4 hours) compared to male subjects (median at 80 mg: 3 hours), indicating a potential gender effect on absorption.

A randomized, double-blind, controlled phase 2 study in otherwise healthy adults (hereafter, phase 2 OwH study) as well as phase 3 study in otherwise healthy adults and

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adolescents (CAPSTONE-1, hereafter, phase 3 OwH study) were conducted with patients suffering from acute uncomplicated influenza (2). Baloxavir marboxil significantly reduced the time to alleviation of influenza (the primary endpoint) compared to placebo. Interestingly, on the secondary endpoint (viral load reduction), baloxavir marboxil showed a significantly faster reduction in viral load compared to not only the placebo treatment group but also the oseltamivir treatment groups. Using two clinical studies with otherwise healthy influenza patients and ten phase 1 studies, population pharmacokinetic analysis and exposure-response analysis of baloxavir acid were conducted (1). Body weight and race (Asian or non-Asian) were identified as the most significant covariates on the apparent total clearance (CL/F) and the apparent central volume of distribution (Vc/F) of baloxavir acid. In addition, a hepatic function marker (alanine aminotransferase; ALT) on CL/F, gender on absorption rate constant (Ka), and food intake on bioavailability (F), were identified as significant covariates. Exposure-response analyses suggested that the treatment of baloxavir marboxil for otherwise healthy influenza patients significantly shortened the time to alleviation of symptoms and reduced viral load compared to the placebo treatment group, with an apparent near maximum response over a wide dose and concentration range in both Asian and non-Asian patients, despite the race effect on the pharmacokinetics of baloxavir
In addition, no apparent safety concerns were observed, suggesting that baloxavir acid has a wide therapeutic window. In addition to the two clinical trials with otherwise healthy influenza patients, a clinical study for treatment of influenza in patients at high risk of influenza complications (CAPSTONE-2, hereafter, phase 3 HR study) was conducted as a double-blind, placebo- and oseltamivir-controlled study with adults and adolescents in 2016-2018 (3). The definition of high risk of influenza complications was adapted from Centers for Disease Control and Prevention (CDC) criteria (http://www.cdc.gov/flu/about/disease/high_risk.htm), which included influenza patients with asthma or chronic lung disease, aged ≥ 65 years, morbid obesity (see reference [3] for more details). The median time to improvement of influenza symptoms (the primary endpoint) in the baloxavir treatment group was 29.1 hours shorter than that in the placebo treatment group (73.2 hours versus 102.3 hours) and similar to that in the oseltamivir treatment group (81.0 hours). The treatment with baloxavir marboxil demonstrated a significantly larger viral load decline compared to the placebo and oseltamivir treatment groups with the median reductions from baseline in virus titer on Day 2 of 3.45 log10[TCID50/mL] in the baloxavir treatment group versus 1.80 log10[TCID50/mL] in the...
oseltamivir treatment group, and $1.20 \log_{10}[\text{TCID}_{50}/\text{mL}]$ in the placebo treatment group.

Patient populations infected with influenza at high risk of influenza complications are different from otherwise healthy influenza patients, primarily in terms of risk factors which are heterogenous in nature. It cannot be ruled out beforehand whether any such factors may present a relevant covariate on the pharmacokinetics and/or exposure-effect relationship of baloxavir acid. In the phase 3 HR study, a total of 2341 additional plasma concentrations in 664 patients were obtained from different patient populations along with the efficacy data.

The aim of this study was to construct a population pharmacokinetic model based on the pooled data from all clinical trials including the phase 3 HR study, and to assess the effects of background characteristics including risk factors of influenza complications on the pharmacokinetics of baloxavir acid. In addition, exposure-response relationships were investigated in the patients at high risk of influenza complications. This paper is the first report which includes the population pharmacokinetic analysis and exposure-response analysis in influenza patients at high-risk of developing influenza-related complications, which may result in hospitalization or death, for not only baloxavir marboxil but also other drugs against influenza.
RESULTS

Population pharmacokinetic analysis. Background characteristics, i.e., demographic, clinical laboratory test results, and disease-related parameters, from 13 clinical studies used for the population pharmacokinetic analyses are summarized in Table 1. The model-building process is shown in Supplementary Table S2. A three-compartment model with first-order elimination and first-order absorption with lag time was selected as the structural model (4). Inferential assessments were conducted based on the full model (Model No. 407) to quantify the importance of the detected covariate effects on the pharmacokinetics. The ratios of parameters of interest calculated at the lower and upper range of covariate distributions in the database relative to the reference value (median for continuous covariates and mode for categorical covariates) and their 95% confidence intervals (CIs) were estimated and shown in Supplementary Figure S1. The covariates with a ratio considered to be small (point estimates of the ratios close to 1 and their 95% CIs within or close to the range of 0.80 to 1.25) were removed from the model. The final model (Model No. 523) includes the effect of body weight on CL/F, Q1/F, and Q2/F (the same exponent), the effect of body weight on Vc/F, Vp1/F, and Vp2/F (the same exponent), the effects of race (Asian or non-Asian) on CL/F and Vc/F, and the effect of gender on Ka.
Relationships of ηs with covariates are shown in Supplementary Figure S2. The tendencies of ηs for CL/F and Vc/F against body weight and race (Asian or non-Asian) visible in the base model (Supplementary Figure S3, Supplementary Table S3) disappeared in the final model (Supplementary Figure S2), indicating that the final model could appropriately explain the effects of background characteristics on the pharmacokinetics of baloxavir marboxil.

Diagnostic plots for the final model are shown in Supplementary Figure S4. The final model adequately described the plasma concentrations, showing a good fit to the observed data and the absence of obvious bias. As shown in Figure 1 and Supplementary Figure S5, the prediction corrected-visual predictive check (pc-VPC) (5) indicated that the model well captured the central trend of the observed data with a lack of bias as the observed plasma concentration data and the median profiles and 95% predicted intervals (PIs) of the model were consistent. The stability and robustness of the final model were evaluated using the nonparametric bootstrap procedure (6) (Table 2). The parameter estimates in the final model had little bias and that the final model was fairly robust as the population parameter estimates obtained from 5000 bootstrap sample sets were comparable to the estimates in the final model and the 80.1% of runs successfully completed.
Pharmacokinetics in Patients with Risk Factors of Influenza Complications. The individual exposure indices of baloxavir acid (maximum plasma concentration $[C_{\text{max}}]$), area under the plasma concentration-time curve [AUC], and observed plasma concentration at 24 hours after administration [$C_{24}$]) were summarized by dose (body weight group) and race for the phase 3 OwH and HR studies (Table 3). All exposure parameters were lower in non-Asians than in Asians. The exposures in the phase 3 HR study were similar to those in the phase 3 OwH study regardless of dose or race. Relationships of $\eta$s with risk factors in the final model are shown in Supplementary Figure S6. None of the risk factors evaluated in this study showed clear tendencies regarding $\eta_{\text{CL/F}}$, suggesting that the pharmacokinetics of baloxavir acid would not be affected by the risk factors of influenza complications.

Exposure-Response Analysis. The summary statistics of time to improvement of influenza symptoms (the primary endpoint, hereafter, TTIIS) and the change from baseline in virus titer on Day 2 (the secondary endpoint) by groups of exposure indices ($C_{\text{max}}$, AUC and $C_{24}$) and by virus type are presented in Table 4 and Table 5, respectively. The box plots
of virus titer for C_{24} are shown in Figure 2. For the three highest exposure categories of C_{24} ≥ 20 ng/mL, baloxavir shortens the TTIIS as compared to the placebo and, overall, is similar to oseltamivir for virus type A. For virus type B, for the two highest exposure categories, baloxavir shortens the TTIIS compared to both placebo and oseltamivir, although TTIIS was highly variable in all groups and did not show clear exposure-dependency. Other exposure indices show similar tendencies.

For type A virus, the change from baseline in virus titer on Day 2 was more obvious in C_{24} groups of ≥ 20 ng/mL with the median change of -3.8 to -4.5 \log_{10}[TCID_{50}/mL] compared with the placebo treatment group (-1.4 \log_{10}[TCID_{50}/mL]) and the oseltamivir treatment group (-2.3 \log_{10}[TCID_{50}/mL]). For type B virus, the change from baseline in virus titer on Day 2 showed the exposure-dependent declines with the median change of -2.3 to -4.0 \log_{10}[TCID_{50}/mL] for C_{24} groups of ≥ 20 ng/mL, which were larger reductions compared with the placebo treatment group (-0.8 \log_{10}[TCID_{50}/mL]) and the oseltamivir treatment group (-1.0 \log_{10}[TCID_{50}/mL]). In the lowest exposure group, C_{24} < 20 ng/mL, less obvious reductions in TTIIS or virus titer were found for both type A and type B viruses. However, since the numbers of subjects in this group were limited, it is difficult to discuss the magnitude of the responses related to exposure levels.
DISCUSSION

A population pharmacokinetic model of baloxavir acid was developed using data from 13 clinical studies including healthy subjects as well as patients who were either otherwise healthy or at high risk of influenza complications. Over the entire dose range explored, plasma drug exposure of baloxavir acid was well described by a three-compartment model with first-order elimination and absorption, indicating linear pharmacokinetics. All fixed and random effect parameters were precisely estimated with relative standard error (%RSE) below 20%. The η shrinkages for both CL/F and Vc/F were low (4.4% and 10.9%, respectively), indicating that the sampling scheme was informative enough to obtain reliable empirical Bayesian-estimates while the η shrinkage for Vp1/F and Vp2/F was large, above 60%, indicating that the pharmacokinetic sampling scheme was much less informative for estimating the between-subject variability of disposition phase, which is not unexpected with a sparse sampling scheme.

Race and body weight were found to be relevant covariates of CL/F of baloxavir acid, which was consistent with findings from the previous population pharmacokinetic analysis (1). In both Asian and non-Asian patients, CL/F and Vc/F of baloxavir acid increased with increasing body weight in a less than proportional manner, as shown by their allometric
exponents estimated at 0.362 for CL/F and 0.833 for Vc/F, which were both lower than the classical/theoretical ones of 0.75 and 1 (7, 8), respectively. Interestingly, an attempt to optimize body weight effect on both Vc/F and CL/F for each race separately did not relevantly improve model fit (Model No. 213 and 214, Supplementary Table S2-c), indicating that body weight effect on drug disposition may by itself not be ethnic sensitive. In vitro data have shown that metabolic clearance of baloxavir involves primarily UGT1A3 and CYP3A mediated metabolism which is not known to be ethnic sensitive. In addition, as excretion of unchanged baloxavir marboxil in urine or feces is primarily passive, neither is likely to be ethnic sensitive. Moreover, the race effect was fairly similar on both CL/F and Vc/F, suggesting the ethnic difference in drug disposition may represent a shift in oral bioavailability via F rather than a difference in systemic clearance (CL), although some minor ethnic differences in CL cannot be fully ruled out. The intrinsic and/or extrinsic factor driving ethnic difference in bioavailability is currently not known, but might involve an interplay between prodrug conversion and the rate of downstream intestinal metabolism of baloxavir acid. A gender effect was detected on the rate of absorption with a 31.8% slower rate constant in females. Gender differences in gut transit times, lipid solubility of an agent, activities of
certain CYP enzymes, diets, etc., have been reported to influence drug absorption and bioavailability (9). However, in case of baloxavir, the gender difference in absorption may be multifactorial and difficult to elucidate, since the absorption process is likely to be a complex interplay that may involve biopharmaceutical factors (disintegration and dissolution in the gut), physiological factors (transit to absorption site), and bio- or physicochemical factors (food interaction, conversion from baloxavir marboxil to baloxavir acid by AADAC, and downstream intestinal metabolism or efflux). Since baloxavir acid has a wide therapeutic window with an apparent near-plateau in response over the dose range explored, this finding is not deemed clinically relevant. No relevant difference in pharmacokinetics were found between otherwise healthy influenza patients and patients who are at high risk of influenza complications.

In the population pharmacokinetic analysis, a tendency of lower baloxavir acid exposure was seen when baloxavir marboxil was taken with food, in line with an observation made from a dedicated phase 1 food-effect study (10). However, the magnitude of the overall food effect on F was small, and the covariate was eventually removed during refinement (backward deletion procedure) of the final model, since the estimates of the ratio on F approached 1 and the 95% CI was within the range of 0.80 to 1.25 (Supplementary Figure 238).
This finding is in accordance with the previous population pharmacokinetic model in otherwise healthy influenza patients where the effect of food intake on baloxavir acid exposure was found to be small (0.869-fold) and not clinically relevant (1). Indeed, considering its wide therapeutic window, the minor decrease in drug exposure when taken with food supports the label claim that baloxavir marboxil can be taken regardless of food intake (Xofluza package insert).

Body weight-based dosing used in both phase 3 OwH and HR studies (40 mg for 40 kg to < 80 kg and 80 mg for ≥ 80 kg) successfully avoided underexposure in higher body weight individuals, regardless of race (Table 3). The lowest drug exposure was generally seen in non-Asian patients with body weight < 80 kg, in both OwH and HR patients. However, the drug exposure remained well above the mean exposure (C_{max}, 27.8 ng/mL; AUC, 2105 ng.h/mL, C_{24}, 15.1 ng/mL) observed at the lowest dose (10 mg) explored in the phase 2 OwH study (1, 11), which was demonstrated to be effective for both type A (statistically) and type B (numerically) influenza virus compared with the placebo treatment group (2).

Thus, since baloxavir marboxil is safe and well tolerated up to 80 mg regardless of race, and is efficacious over a wide exposure range, its therapeutic window appears to be wide and allows a globally aligned body weight-based dosing regimen that provides efficacious
and safe exposure of baloxavir acid regardless of race or gender.

In the exposure-response analysis for influenza patients at high risk of influenza complications, the median changes from the baseline in virus titer on Day 2 were -3.8 to -4.5 log_{10}[TCID_{50}/mL] for type A virus and -2.3 to -4.0 log_{10}[TCID_{50}/mL] for type B virus for C_{24} exposure groups above or equal to 20 ng/mL, which were similar to those seen in the otherwise healthy influenza patients (-4.5 to -5.0 log_{10}[TCID_{50}/mL] for type A virus and -2.5 to -4.1 log_{10}[TCID_{50}/mL] for type B virus) reported in the previous paper (1). In the C_{24} exposure group < 20 ng/mL, the median changes from the baseline in virus titer on Day 2 were -2.0 log_{10}[TCID_{50}/mL] for type A virus and 0.1 log_{10}[TCID_{50}/mL] for type B virus, which were smaller than those in OwH patients (-4.5 log_{10}[TCID_{50}/mL] for type A virus and -2.55 log_{10}[TCID_{50}/mL] for type B virus). However, the numbers of subjects are much smaller (9 for type A virus and 6 for type B virus) than those reported in the analysis with otherwise healthy influenza patients (66 for type A virus and 16 for type B virus) (1). This difference in the lowest C_{24} exposure group between OwH and HR patients is thought to be due to the small sample size in the phase 3 HR study, in which the effect of lower dose levels of 10 mg and 20 mg was not investigated. These results suggested that risk factors of influenza complications did not affect the virus reductions.
In conclusion, the population pharmacokinetics of baloxavir acid was successfully described by a three-compartment model with first-order elimination and first-order absorption with lag time based on the integrated data collected from healthy subjects, otherwise healthy patients and patients at high risk of influenza complications. The model well described the plasma concentration data, and the body weight and race were found to be the most important factors influencing clearance and volume of distribution. The exposures in high-risk patients were similar to those in otherwise healthy patients, and no pharmacokinetic difference was identified regarding any risk factors for influenza complications. The exposure-response analyses in high-risk patients showed that the body weight-based dose regimen (40 mg for the patients weighing less than 80 kg and 80 mg for the patients weighing at least 80 kg) shortened TTIIS and reduced virus titer for both type A and B influenza virus regardless of exposure levels to baloxavir acid of $\geq 20$ ng/mL $C_{24}$.

The population pharmacokinetic model and exposure-response analysis results in the patients with risk factors of influenza complications are useful for understanding the pharmacokinetic and pharmacodynamic characteristics of baloxavir marboxil and also for optimization of dose regimens in clinical situations.
MATERIALS AND METHODS

Data for Analysis. Table S1 provides information of clinical studies used for the population pharmacokinetic analysis. All clinical studies were approved by the ethics committees for each site and conducted in compliance with the Declaration of Helsinki and good clinical practice (GCP). Baloxavir marboxil was administered orally in all these clinical studies. The details of study designs and dosages in the phase 2 OwH, phase 3 OwH, and phase 3 HR studies have been described in previous papers (2, 3). The patients in the phase 3 HR study were considered to be at high risk of influenza complications due to the presence of at least one of the inclusion criteria defined by CDC.

Determination of plasma baloxavir acid concentrations were conducted using a validated liquid chromatography-tandem mass spectrometry method (more details in reference [1]) at Sumika Chemical Analysis Service, Ltd. (Osaka, Japan) for phase 1 studies and a phase 2 study, or at LGC Limited. (Teddington, UK) for two phase 3 studies.

Pharmacokinetic and pharmacodynamic data from the phase 3 HR study were used for the exposure-response analysis. The TTIIS and the change from baseline in the influenza virus titer on Day 2 were used as efficacy parameters. The TTIIS was defined as the time from the start of study treatment to the improvement of influenza symptoms. The virus titers on May 5, 2020 by guest
were determined, and cytopathic effects were evaluated by the methods described in reference (1).

**Population Pharmacokinetic Analysis.** Nonlinear mixed effect modeling software, NONMEM (version 7.3; ICON Development Solutions, US), was used for the population pharmacokinetic analysis with a PREDPP library and NM-TRAN preprocessor. A first-order conditional estimation with interaction was used for the analysis.

First, using 11848 concentration data points from 1827 subjects, a basic structural population pharmacokinetic model was constructed without any covariate. One-, two-, and three-compartment models with first-order elimination and first-order absorption with lag time were tested for the structural model. The inter-individual variability (IIV) on pharmacokinetic parameters was assumed to follow a log-normal distribution. A model for residual error variability was selected from a proportional error model, an additive error model, and a combined error model.

Next, a covariate model was constructed by combining the preliminary covariates screening through univariate regression analysis with a forward selection and a stepwise backward deletion. The tested covariates are summarized in Table 1 with pharmacokinetic
parameters. The body-surface-area-adjusted estimated glomerular filtration rate (eGFR) and creatinine clearance (CLcr) were calculated by the same methods described in reference (1). A power model for a continuous variable and a multiplicative model for a categorical variable were used for the analysis as described in reference (1). The significance level of 0.05 based on the $\chi^2$ test ($p < 0.05$, difference in objective function value $[\Delta OBJ]$ is less than $-3.84$ for one degree of freedom) was used for the screening and forward selection. The significant covariates at screening were tested in the forward selection to construct a full model. The significance level of 0.01 based on the $\chi^2$ test ($p < 0.01$, $\Delta OBJ$ is more than $6.63$ for one degree of freedom) was used for the stepwise backward deletion to construct the final model.

The points with absolute conditional weighted residuals with interaction (CWRESI) value $\geq 6$ in the base model and the final model were excluded from the analysis as outlier concentrations.

**Model Evaluation.** The population pharmacokinetic model was evaluated using diagnostic plots of the observed plasma concentration (DV) versus the mean predicted plasma concentration (PRED), DV versus individual predicted plasma concentration.
concentrations (IPRED), absolute individual weighted residuals (IWRES) versus IPRED, CWRESI versus PRED, and CWRESI versus time after the reference dose. A nonparametric bootstrap resampling procedure (6) was performed using Perl-speaks-NONMEM (version 4.2) to assess the stability of the final parameter estimates and the robustness of the final model. The 5000 bootstrap sample sets were resampled from the original data set, and the parameter estimates for each of the 5000 sample sets were estimated using the final model. The medians and 95% CIs of the 5000 parameters estimated from bootstrap sample sets were compared with the means and its standard error of the final parameter for each parameter estimate.

A pc-VPC (5) was performed and the final model was evaluated by comparing the observed plasma concentrations with the 95% PIs simulated from the final population pharmacokinetic parameters. Five hundred data sets were simulated from the final population pharmacokinetic parameters using the original data set as a simulation template. For each simulation run, the median, the 2.5th and 97.5th percentiles of pharmacokinetic time course were first calculated. Next, for each of those statistics, the 95% CIs were computed over the 500 simulated runs and compared with the observations.
Exposure-Response Analysis. The individual systemic exposures of baloxavir acid, $C_{\text{max}}$ and AUC, were calculated using the individual post-hoc pharmacokinetic parameter estimates derived from the final model using an empirical Bayesian estimation.

Exposure-response analyses were performed for TTIIS on a total of 658 measurements and for the change from the baseline in the virus titer on Day 2 on a total of 568 measurements from the phase 3 HR study. These estimated individual $C_{\text{max}}$, individual AUC as well as $C_{24}$ (sampling time window: 20-28 hours post-dose) were used as exposure indices. The TTIIS and change from baseline in virus titer on Day 2 were plotted against the exposure indices (Supplementary Figures S7 and S8). In addition, four groups of patients were created based on different exposure levels: either based on $C_{\text{max}}$ levels (< 40 ng/mL, 40 to < 80 ng/mL, 80 to < 120 ng/mL, and ≥ 120 ng/mL), AUC levels (< 2500 ng·h/mL, 2500 to < 5000 ng·h/mL, 5000 to < 7500 ng·h/mL, and ≥ 7500 ng·h/mL), or $C_{24}$ levels (< 20 ng/mL, 20 to < 40 ng/mL, 40 to < 60 ng/mL, and ≥ 60 ng/mL); the TTIIS and change from baseline in virus titer on Day 2 were then summarized and graphically presented for comparison across the baloxavir acid exposure groups and with the oseltamivir and placebo treatment groups (Figure 2, Supplementary Figure S9, S10). To facilitate the comparison with the previous exposure-response analyses for otherwise
healthy influenza patients (1), the ranges of the exposure categories were kept the same.
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Table 1  Subject characteristics and pharmacokinetic parameters on which the covariates were tested

| Characteristics | Healthy subjects | Patients | Pharmacokinetic Parameters |
|-----------------|------------------|----------|----------------------------|
|                 | Asian (n = 231)  | Non-Asian (n = 46) | Asian (n = 844) | Non-Asian (n = 706) |
| Age (yr)        |                  |                      |                  |                      |
| Mean (SD)       | 29.1 (7.6)       | 49.2 (11.8)          | 40.0 (15.9)      | 44.3 (17.6)          |
| Median (range)  | 27 (20 - 49)     | 48 (26 - 70)         | 38 (12 - 85)     | 45 (12 - 84)         |
| No. of male / female subjects | 207 / 24 | 37 / 9 | 487 / 357 | 289 / 419 |
| Ht (cm)         |                  |                      |                  |                      |
| Mean (SD)       | 170.3 (6.7)      | 172.3 (9.8)          | 165.4 (9.1)      | 166.8 (10.1)         |
| Median (range)  | 171.0 (148.3 - 187.5) | 173.0 (153.0 - 205.0) | 166.0 (137.5 - 192.0) | 166.1 (121.9 - 195.6) |
| Body wt (kg)    |                  |                      |                  |                      |
| Mean (SD)       | 61.7 (6.9)       | 83.1 (13.9)          | 63.4 (13.5)      | 83.1 (21.7)          |
| Median (range)  | 61.1 (46.0 - 77.2) | 82.7 (56.7 - 118.9) | 61.9 (36.0 - 118.4) | 80.0 (40.8 - 217.3) |
| BMI (kg/m²)     |                  |                      |                  |                      |
| Mean (SD)       | 21.2 (1.5)       | 27.9 (3.8)           | 23.0 (3.9)       | 29.8 (7.0)           |
| Median (range)  | 21.1 (18.5 - 24.9) | 28.3 (21.0 - 37.8) | 22.3 (15.3 - 37.8) | 28.6 (16.5 - 69.4) |
| AST (U/L)       |                  |                      |                  |                      |
| Mean (SD)       | 17.1 (4.0)       | 23.9 (7.8)           | 25.1 (19.8)      | 25.6 (22.3)          |
| Median (range)  | 17 (9 - 32)      | 23 (14 - 62)         | 21 (10 - 428)    | 21 (11 - 355)        |
| ALT (U/L)       |                  |                      |                  |                      |
| Mean (SD)       | 15.9 (7.1)       | 23.4 (8.8)           | 23.4 (20.7)      | 26.9 (31.0)          |
| Median (range)  | 14 (6 - 39)      | 22 (10 - 50)         | 17 (6 - 320)     | 20 (6 - 552)         |
| eGFRadj (mL/min/1.73m²) | CL/F             |                      |                  |                      |
| Mean (SD)       | 87.8 (11.4)      | 114.0 (26.3)         | 78.9 (20.8)      | 94.6 (26.6)          |
| Parameter                      | Median (range) | Mean (SD) | Median (range) | Mean (SD) |
|-------------------------------|---------------|-----------|---------------|-----------|
| eGFRabs (mL/min)              | 87.4 (63.3 - 114.9) | 65.1 (11.7) | 86.0 (60.0 - 121.6) | 111.1 (15.3) |
| CL/F                          | 114.9 (60.4 - 176.8) | 100.2 (28.7) | 130.3 (76.5 - 209.2) | 101.4 (28.4) |
| CLCAH (mL/min)                | 76.3 (27.4 - 173.9) | 99.6 (26.3 - 225.6) | 158.8 (84.4 - 431.7) | 125.2 (48.3) |

No. of patients in OwH study / HR study:
- No. of influenza virus infected patients:
  - 0 (0) / 0 / 658 / 186 / 228 / 478
  - 0 (0) / 807 (95.6) / 319 (45.2)

No. (%) of subjects by food condition:
- Fasted:
  - 231 (100.0) / 46 (100.0) / 236 (28.0) / 299 (42.4)
- Intermediate:
  - 0 (0.0) / 0 (0.0) / 275 (32.6) / 165 (23.4)
- Fed:
  - 0 (0.0) / 0 (0.0) / 333 (39.5) / 242 (34.3)

No. (%) of subjects with asthma or chronic lung disease:
- 0 (0.0) / 0 (0.0) / 62 (7.3) / 219 (31.0) / CL/F

No. (%) of subjects with endocrine disorders:
- 0 (0.0) / 0 (0.0) / 59 (7.0) / 148 (21.0) / CL/F

No. (%) of subjects with neurological and neurodevelopmental disorders:
- 0 (0.0) / 0 (0.0) / 9 (1.1) / 29 (4.1) / CL/F

No. (%) of subjects with heart disease:
- 0 (0.0) / 0 (0.0) / 16 (2.0) / 59 (8.4) / CL/F

No. (%) of subjects ≥ 65 years of age:
- 0 (0.0) / 5 (10.9) / 75 (8.9) / 106 (15.0) / CL/F

No. (%) of subjects with ≥ 25 years of age:
- 0 (0.0) / 36 (4.3) / 26 (3.7) / CL/F
metabolic disorders

| No. (%) of subjects with morbid obesity (BMI ≥ 40) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 73 (10.3) | CL/F |

Ht, height; Wt, weight; BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; eGFRadj, body-surface-area adjusted estimated glomerular filtration rate; eGFRabs, absolute estimated glomerular filtration rate; CL\textsubscript{CR}, creatinine clearance

\(^4\) Race (Asian or non-Asian) was also tested as a covariate on CL/F and Vc/F.

\(^5\) Fasted, dosing ≥ 4 hours before and after food intake; Intermediate, dosing within 2 to 4 hours before or after food intake; Fed, dosing < 2 hours before or after food intake
Table 2  Population pharmacokinetic parameter estimates in the final model

| Parameter                | Units | Final model | Bootstrap estimates |
|--------------------------|-------|-------------|---------------------|
|                          |       | Estimate    | %RSE    | Median | 95% CI      |
| **Pharmacokinetic parameters** |       |             |         |        |             |
| CL/F                     | L/h   | 10.8        | 1.8     | 10.8   | 10.4 - 11.3 |
| Vc/F                     | L     | 565         | 3.0     | 565    | 528 - 604   |
| Q1/F                     | L/h   | 12.4        | 6.9     | 12.4   | 10.3 - 14.2 |
| Vp1/F                    | L     | 141         | 3.1     | 141    | 130 - 152   |
| Q2/F                     | L/h   | 1.43        | 4.3     | 1.42   | 1.25 - 1.59 |
| Vp2/F                    | L     | 139         | 2.4     | 139    | 131 - 147   |
| Ka                       | l/h   | 1.03        | 6.3     | 1.03   | 0.905 - 1.20|
| Lag time                 | h     | 0.345       | 3.3     | 0.344  | 0.323 - 0.371|
| Effect of body wt on CL/F, Q1/F, and Q2/F |       | 0.362       | 10.4    | 0.363  | 0.278 - 0.445|
| Effect of body wt on Vc/F, Vp1/F, and Vp2/F |       | 0.833       | 5.0     | 0.833  | 0.743 - 0.926|
| Effect of race (Asian) on CL/F |       | 0.519       | 2.2     | 0.519  | 0.495 - 0.544|
| Effect of race (Asian) on Vc/F |       | 0.564       | 3.6     | 0.564  | 0.523 - 0.609|
| Effect of gender on Ka   |       | 0.682       | 9.1     | 0.679  | 0.566 - 0.819|
| % CV for IIIV for CL/F (sh_ηp) | %     | 41.1 (4.4)  | 4.4     | 41.1   | 39.3 - 42.9 |
| % CV for IIIV for Vc/F (sh_ηp) | %     | 62.7 (10.9) | 4.6     | 62.6   | 59.7 - 65.4 |
| % CV for IIIV for Vp1/F (sh_ηp) | %     | 29.3 (61.5) | 16.7    | 29.3   | 23.9 - 34.3 |
| % CV for IIIV for Vp2/F (sh_ηp) | %     | 35.4 (60.4) | 18.2    | 35.1   | 26.2 - 41.1 |
| % CV for IIIV for Ka (sh_ηp) | %     | 123.7 (33.9)| 6.9     | 123.4  | 115.4 - 132.5|
| Covariance between CL/F and Vc/F | %     | 0.209       | 4.7     | 0.209  | 0.190 - 0.228|
| % CV for proportional residual error (sh_ε) | %     | 20.2 (17.8) | 2.1     | 20.2   | 19.4 - 21.0 |

CI, confidence interval; CV, coefficient of variation; IIIV, inter-individual variability; RSE, relative standard error; sh, shrinkage.

\[
CL/F = 10.8 \times (\text{Body wt/67.7})^{0.362} \times 0.519^{\text{Asian}}; \quad Q1/F = 12.4 \times (\text{Body wt/67.7})^{0.362}; \quad Q2/F = 1.43 \times (\text{Body wt/67.7})^{0.362},
\]

\[
Vc/F = 565 \times (\text{Body wt/67.7})^{0.833} \times 0.564^{\text{Asian}}; \quad Vp1/F = 141 \times (\text{Body wt/67.7})^{0.833}; \quad Vp2/F = 139 \times (\text{Body wt/67.7})^{0.833};
\]
$Ka (\text{h}) = 1.03 \times 0.682^{\text{Gender}}$

Asian = 0 for non-Asian subject and 1 for Asian subject. Gender = 0 for male and 1 for female.
| Study                                      | Dose | Race            | No. of subjects (n for $C_{24}$) | $C_{\text{max}}$ (ng/mL) | AUC (ng.h/mL) | $C_{24}$ (ng/mL) |
|-------------------------------------------|------|-----------------|----------------------------------|--------------------------|---------------|-----------------|
| Phase 3 otherwise healthy patients       | 40 mg| Asian           | 309 (194)                        | 97.6 (20.1 - 221)        | 6210 (1399 - 13200) | 59.8 (5.81 - 158) |
|                                           |      | Non-Asian       | 59 (39)                          | 63.9 (11.1 - 133)        | 3648 (809.5 - 7609) | 37.2 (7.35 - 81.4) |
|                                           | 80 mg| Asian           | 34 (26)                          | 136 (30.9 - 253)         | 9741 (4527 - 16340) | 74.9 (17.5 - 209)  |
|                                           |      | Non-Asian       | 44 (30)                          | 94.6 (27.1 - 196)        | 6345 (2247 - 15040) | 88.7 (39.3 - 142)  |
|                                           | Overall patients |                | 446 (289)                       | 95.8 (11.1 - 253)        | 6154 (909.5 - 16340) | 59.7 (5.81 - 209)  |
| Phase 3 patients at high risk of influenza complications | 40 mg| Asian           | 138 (79)                         | 104 (24.0 - 382)         | 6380 (2294 - 14690) | 64.0 (25.4 - 231)  |
|                                           |      | Non-Asian       | 96 (58)                          | 60.6 (12.2 - 158)        | 3661 (720.3 - 8571) | 35.9 (5.77 - 90.2)  |
|                                           | 80 mg| Asian           | 26 (14)                          | 137 (40.9 - 241)         | 9733 (4893 - 16640) | 87.6 (33.8 - 126)  |
|                                           |      | Non-Asian       | 118 (81)                         | 84.9 (9.21 - 240)        | 5737 (890.8 - 14810) | 58.7 (5.86 - 198)  |
|                                           | Overall patients |                | 378 (232)                        | 89.1 (9.21 - 382)        | 5719 (720.3 - 16640) | 56.5 (5.77 - 231)  |

Mean (range)

$C_{\text{max}}$, AUC: post-hoc Bayesian estimation based on the population pharmacokinetic model

$C_{24}$: the observed plasma concentration at 20 to 28 hours post-dose
| Parameter | Range of pharmacokinetic parameter | Virus type A | Difference from placebo treatment (h) | Difference from oseltamivir phosphate treatment (h) | Virus type B | Difference from placebo treatment (h) | Difference from oseltamivir phosphate treatment (h) |
|-----------|-----------------------------------|--------------|--------------------------------------|---------------------------------------------------|--------------|---------------------------------------|-------------------------------------------------|
| C<sub>max</sub> (ng/mL) | < 40 | 38 | 101.5 | 0.4 | 35.0 | 21 | 93.5 | 0.3 | -4.5 |
|           | 40 to < 80 | 70 | 73.6 | -25.5 | 9.1 | 52 | 75.1 | -18.1 | -22.9 |
|           | ≥ 120 | 44 | 50.3 | -50.8 | -16.2 | 45 | 67.3 | -25.9 | -30.7 |
| AUC (ng·h/mL) | < 2500 | 24 | 101.1 | 0.0 | 34.6 | 11 | 85.3 | -7.9 | -12.7 |
|           | 2500 to < 5000 | 79 | 88.7 | -12.4 | 22.2 | 58 | 84.6 | -8.6 | -13.4 |
|           | ≥ 7500 | 64 | 54.5 | -46.6 | -12.0 | 54 | 69.8 | -23.4 | -28.2 |
| C<sub>24</sub> (ng/mL) | < 20 | 11 | 165.2 | 84.1 | 98.7 | 6 | 89.0 | -4.2 | -9.0 |
|           | 20 to < 40 | 33 | 77.0 | -24.1 | 10.5 | 30 | 90.7 | -2.5 | -7.3 |
|           | 40 to < 60 | 32 | 92.7 | -8.4 | 26.2 | 25 | 68.6 | -24.6 | -29.4 |
|           | ≥ 60 | 44 | 60.3 | -40.8 | -6.2 | 48 | 67.9 | -25.3 | -30.1 |

Placebo 214 101.1 - 34.6 167 93.2 - -4.8
Oseltamivir phosphate 236 66.5 -34.6 - 148 98.0 4.8 -
Table 5  Change from baseline in virus titer on Day 2 by predicted C<sub>max</sub>, predicted AUC, and observed C<sub>24</sub> groups

| Parameter | Range of pharmacokinetic parameter | Virus type A | Virus type B |
|-----------|-------------------------------------|--------------|--------------|
|           | N Median (log<sub>10</sub> TCID<sub>50</sub>/mL) | Difference from placebo treatment (log<sub>10</sub> TCID<sub>50</sub>/mL) | Difference from oseltamivir phosphate treatment (log<sub>10</sub> TCID<sub>50</sub>/mL) | N Median (log<sub>10</sub> TCID<sub>50</sub>/mL) | Difference from placebo treatment (log<sub>10</sub> TCID<sub>50</sub>/mL) | Difference from oseltamivir phosphate treatment (log<sub>10</sub> TCID<sub>50</sub>/mL) |
| C<sub>max</sub> (ng/mL) | | | | | | |
| < 40 | 31 | -3.0 | -1.6 | -0.7 | 20 | -1.1 | -0.3 |
| 40 to < 80 | 58 | -5.8 | -2.4 | -1.5 | 47 | -2.3 | -1.5 |
| 80 to < 120 | 48 | -4.4 | -3.0 | -2.1 | 44 | -2.7 | -1.9 |
| ≥ 120 | 39 | -4.2 | -2.8 | -1.9 | 38 | -4.7 | -3.9 |
| AUC (ng.h/mL) | | | | | | |
| < 2500 | 20 | -3.0 | -1.6 | -0.7 | 9 | -0.8 | 0.0 |
| 2500 to < 5000 | 64 | -3.8 | -2.4 | -1.5 | 52 | -2.3 | -1.5 |
| 5000 to < 7500 | 54 | -3.9 | -2.5 | -1.6 | 52 | -2.65 | -1.85 |
| ≥ 7500 | 38 | -4.9 | -3.5 | -2.6 | 36 | -3.9 | -3.1 |
| C<sub>24</sub> (ng/mL) | | | | | | |
| < 20 | 9 | -2.0 | -0.6 | -0.3 | 6 | 0.1 | 0.9 |
| 20 to < 40 | 28 | -4.0 | -2.6 | -1.7 | 27 | -2.3 | -1.5 |
| 40 to < 60 | 29 | -3.8 | -2.4 | -1.5 | 24 | -3.3 | -2.5 |
| ≥ 60 | 35 | -4.5 | -3.1 | -2.2 | 45 | -4.0 | -3.2 |
| Placebo | 185 | -1.4 | - | 0.9 | 154 | -0.8 | - |
| Oseltamivir phosphate | 207 | -2.3 | -0.9 | - | 133 | -1.0 | -0.2 |
**Figure legends**

**Figure 1** Prediction-corrected visual predictive check

Observed median (solid line), 2.5th/97.5th percentiles (dotted lines) of plasma concentrations were compared with their 95% prediction intervals (grey areas) simulated based on the final model. The figures are for Phase 2/3 Asian patients in (a) linear and (b) semi-logarithmic axis, and for Phase 2/3 non-Asian patients in (c) linear and (d) semi-logarithmic axis.
Figure 2  Relationships of change from baseline in virus titer on day 2 with $C_{24}$ by virus type in the patients at high risk of influenza complications.

(a) virus type A, and (b) virus type B. Thick center line represents median, top and bottom of the box represent the 1st and 3rd quartiles, and whiskers represent the 10th/90th percentile. The dashed line represents median value for placebo treatment. The number in the brackets is the number of subjects in each category.
