Combining SJ733, an oral ATP4 inhibitor of *Plasmodium falciparum*, with the pharmacokinetic enhancer cobicistat: An innovative approach in antimalarial drug development

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Summary

Background SJ733, a newly developed inhibitor of *P. falciparum* ATP4, has a favorable safety profile and rapid antiparasitic effect but insufficient duration to deliver a single-dose cure of malaria. We investigated the safety, tolerability, and pharmacokinetics of a multidose SJ733 regimen and a single-dose pharmacoboost approach using cobicistat to inhibit CYP3A4, thereby increasing exposure.

Methods Two multidose unboosted cohorts ($n = 9$) (SJ733, 300 mg and 600 mg daily for 3 days) followed by three single-dose boosted cohorts combining SJ733 ($n = 18$) (75-, 300-, or 600-mg single dose) with cobicistat (150-mg single dose) as a pharmacokinetic booster were evaluated in healthy volunteers (ClinicalTrials.gov: NCT02661373).

Findings All participants tolerated SJ733 well, with no serious adverse events (AEs), dose-limiting toxicity, or clinically significant electrocardiogram or laboratory test findings. All reported AEs were Grade 1, clinically insignificant, and considered unlikely or unrelated to SJ733. Compared to unboosted cohorts, the SJ733/cobicistat-boosted cohorts showed a median increase in area under the curve and maximum concentration of 3·9 × and 2·6 ×, respectively, and a median decrease in the ratio of the major CYP3A-produced metabolite SJ506 to parent drug of 4·6 × . Incorporating these data in a model of parasite dynamics indicated that a 3-day regimen of SJ733/cobicistat (600 mg/150 mg daily) relative to a single 600-mg dose ± cobicistat would increase parasite clearance from 10^6 to 10^{12} parasites/μL.

Interpretation The multidose and pharmacoboosted approaches to delivering SJ733 were well-tolerated and significantly increased drug exposure and prediction of cure. This study supports the further development of SJ733 and demonstrates an innovative pharmacoboost approach for an antimalarial.

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Keywords: SJ733; Antimalarial; Pharmacoboost; Pharmacokinetics; Pharmacodynamics

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Introduction

Antimalarial drug resistance remains a threat to global control of malaria. With the spread of artemisinin resistance, new antimalarials are urgently needed.[1-3] SJ733 is

Research in context

Evidence before this study

We searched PubMed and ClinicalTrials.gov to identify *Plasmodium falciparum* ATP4 inhibitors in clinical development and the purposeful use of a pharmacoboost (pharmacoenhancement) approach with an antimalarial before December 5, 2021, by using the search terms "*Plasmodium falciparum* inhibitor", "PfATP4 inhibitor", "pharmacoenhancement", "pharmacoboost", "cobicistat", and "ritonavir". This search did not retrieve any antimalarial studies that used a pharmacoboosting approach. Prior work with antimalarial drugs and pharmacoboosters, such as ritonavir, which is part of antiretroviral therapy, has addressed drug interactions and/or adverse events/antimalarial tolerability in the setting of treatment of malaria in individuals living with HIV.

Antimalarial drug resistance remains the greatest threat to the global control of malaria. The spread of artemisinin resistance in the past decade has put the first-line, artemisinin-based combination therapies at risk, and new classes of antimalarials are urgently needed. (+)- SJ000557733 (SJ733) is an inhibitor of *P. falciparum* ATP4, a crucial sodium-proton antiporter in the malaria parasite. SJ733 is the second drug in this class to enter clinical development, following cipargamin (KAE609; a spiroindolone analogue). The first-in-human study in a malaria-naïve population of healthy volunteers demonstrated that SJ733 is safe, well-tolerated, well-absorbed, and with no relevant food effect. However, a sub-proportional increase in drug exposure was observed beyond the 600-mg dose. A single-dose, volunteer-infection study using the induced blood-stage malaria model with *P. falciparum* showed that the maximal achievable exposure was associated with rapid parasite clearance, but the decline in parasitemia may be insufficient to achieve a cure in uncomplicated malaria with a single dose. A pharmacokinetic/pharmacodynamic model based on these studies predicted that parasite decline would continue if a longer duration of exposure, with or without increased SJ733 concentrations, was achieved. This finding prompted further evaluation of the described multidose regimen and a pharmacoboost approach, which is innovative for antimalarial development.

Added value of this study

Study results demonstrate a significant increase in drug exposure and time above minimum inhibitory concentration, no drug toxicity signals, and a strong predicted parasite clearance with both the multidose and the innovative pharmacoboosted approaches of the promising new antimalarial SJ733. The described work in a malaria-naïve population of healthy volunteers supports further drug development of SJ733 as an antimalarial and serves as an innovative example of a pharmacoboost approach for an antimalarial.

Implications of all the available evidence

The safety and tolerability of unboosted and cobicistat-boosted SJ733, the significant increase in SJ733 exposure in the multidose and pharmacoboosted setting, and the PK/PD model predicted strong parasite clearance in the setting of treatment of acute uncomplicated malaria. Together, these results support testing these dosing approaches in Phase 2 clinical trials.

a newly developed orally available inhibitor of *Plasmodium falciparum* ATP4 (PfATP4), a critical sodium-proton antiporter in the parasite and the second in class, after cipargamin, to enter clinical development.[4-8] Its major metabolite SJ506 has no known antimalarial activity.

In a Phase 1 program combining a first-in-human study and a healthy volunteers induced blood-stage malaria model (IBSM), single-dose administration of SJ733 demonstrated favorable safety and tolerability profiles combined with rapid antiparasitic activity.[7] However, drug exposure was not maintained over the estimated minimum inhibitory concentration (MIC; 150 μg/L) for a sufficient duration (>96 h) to result in a single-dose cure. This was due to the combination of moderate drug clearance and a sub-proportional increase in drug exposure following a single dose above 600 mg. This prompted the evaluation of multiple dosing of SJ733 (3 daily doses). Additionally, although SJ733 is metabolized by CYP2C8, 2D6, and 3A4,[9] because the dominant metabolizing enzyme for SJ733 is CYP3A4 (unpublished data), we proposed combining a single dose of SJ733 with cobicistat, a strong inhibitor of CYP3A4.[10,11] Combining antiretrovirals that are CYP3A4 substrates, such as lopinavir, with strong CYP3A4 inhibitors that are pharmacoenhancers/pharmacoboosters, such as ritonavir or cobicistat, has been successfully used to treat HIV infection. Although studies have interrogated the impact of these anti-HIV treatments on antimalarial drug exposure and/or adverse events/tolerability in HIV-infected patients treated for malaria,[12,13] pharmacoboosting in any other infectious disease setting, including malaria, has not been reported.

We considered both ritonavir and cobicistat as pharmacoboosters and selected cobicistat because it has no antiviral activity, thereby negating any concerns when used in populations with HIV coinfection, no significant 2D6 component or off-target interactions, a more favorable safety/tolerability profile, and no restrictions with food requirements.[10] The safety/tolerability and pharmacokinetics (PK) of SJ733 after multidose administration and the innovative pharmacoboost strategy in a malaria-naÿve population of healthy volunteers are described here.

Methods

Study design and participants

This Phase 1a study was a single-center, dose-escalation, first-in-human study of single doses of SJ733 that allowed modifications to dose increments and dose-cohort size based on safety and PK results in a malaria-naÿve population of healthy volunteers. It also included an adaptation to a Phase 1b IBSM study if safety warranted. The Phase 1a component was a leapfrog design, allowing enrollment in more than one nonconsecutive dose cohort, with at least 14 days required between administrations. The single-dose escalation showed a less than proportional increase in SJ733 exposure for doses over 600 mg,[7] but promising pharmacodynamics (PD) were seen after a single 600-mg dose in the Phase 1b IBSM study. Therefore, the study protocol was amended to include both multidose (3 daily doses) and cobicistat-boosted (a single dose of SJ733 given simultaneously with cobicistat) cohorts. Study eligibility criteria for the multidose and cobicistat-boosted cohorts were similar to those previously described for the single-dose unboosted cohorts, with some additions specific to the multidose cohort (Appendix) .[7]

The primary objectives of this study amendment included assessing the safety, tolerability, and PK parameters of escalating doses of SJ733 given daily, with or without cobicistat. In addition, PK data from this study were integrated into simulations of PD effects of both multidose and cobicistat-boosted doses of SJ733 on the parasite burden by using the previously described PK/PD model established after completion of the IBSM study.[7]

Procedures

As part of the multiple ascending dose (MAD) cohort, participants received 300 and 600 mg SJ733 daily for 3 consecutive days. For the cobicistat-boosted cohorts (henceforth referred to as boosted cohorts), single ascending doses (SAD) of 75, 300, and 600 mg SJ733 in combination with cobicistat (150 mg) were planned. The starting dose of SJ733 proposed for the boosted cohorts was determined based on the PK and safety/tolerability data from the unboosted SAD cohorts,[7] unboosted MAD cohorts (described in the Results), and animal studies with cobicistat-boosted SJ733. Specifically, an increase in plasma SJ733 area under the concentration curve ($AUC_{0-\infty}$) of up to 5-fold was observed in mice when SJ733 was dosed in combination with the single dose of cobicistat (unpublished data). With an initial cohort receiving 75 mg SJ733 combined with 150 mg cobicistat based on rodent data, a maximum increase of 5-fold in SJ733 $AUC_{0-\infty}$ was hypothesized. This was well below maximum safe exposures observed in the unboosted SAD[7] and MAD cohorts (maximal $AUC_{0-\infty}$ and maximum concentration [C_{max}] values of 103,883 µg-h/L and 2182 ng/mL, respectively). Additionally, given that the unboosted SAD data showed saturable exposure between 600 and 900 mg, SJ733 doses above 600 mg in the boosted SAD and MAD studies were not planned. Furthermore, considering that food had no clinically relevant effect on the PK of either SJ733 or cobicistat, the MAD (unboosted) and the boosted SAD (MAD if needed) cohorts were planned in fasted state. Study assessments, including the PK time points for the participants in the MAD cohort and boosted single-dose cohort, are presented in Tables S1-S2.

SJ733/SJ506 pharmacokinetic analysis

Blood samples were taken predose and at 0·5, 1, 2, 3, 4, 6, 8, 10, 24, 48, 72, and 168 h post-dose to measure the concentrations of SJ733 and its major metabolite, SJ506, in plasma. Samples were analyzed using a liquid chromatography/tandem mass spectrometry method validated by the U.S. Food and Drug Administration (FDA) guidance for industry by Pyxant Labs (Colorado Springs, CO).

The population PK and individual post-hoc estimates (Empirical Bayesian Estimates) of SJ733 and SJ506 were determined by nonlinear mixed-effects modeling with Monolix (version 5.0.0, Lixoft) using the stochastic approximation expectation-maximization approach. We modeled SJ733 and SJ506 sequentially. This process reduced the complexity of the parameter estimation without affecting the ability to quantify the PK of each component. For SJ733, a linear two-compartment model with first-order absorption, an absorption lag time, and first-order elimination was applied. Enterohepatic recirculation was accounted for by using an additional gallbladder compartment that deposits drug back into the gut compartment at discrete time intervals (Figure S1). The times of the deposit were fixed at 4 and 20 h after the dose, which corresponds to the scheduled meals administered at the study site. The model for SJ506, which is produced through CYP3A metabolism of SJ733, was a modified version of the SJ733 model that accounted for the effect of cobicistat concentration on the formation of SJ506. Specifically, the cobicistat PK parameters were modeled independently (described below), and their individual post-hoc estimates were fixed and used in the PK analysis of SJ506. Additionally, the formation of SJ506 was modeled with a formation term from SJ733 that was inhibited by the cobicistat concentration (Figure S2). For all models, the interindividual and interoccasion variability of the parameters were assumed to be log-normally distributed. A proportional residual error model was used with assumed normal distribution of residuals. Concentrations below the lower limit of quantification (LLOQ=10 ng/mL) were treated as censored using the M3 method as implemented in Monolix.[14] All the secondary PK parameters,

including apparent clearance (CL/F), $AUC_{0-\infty}$, C_{max}, time of the maximum concentration (T_{max}), and terminal half-life ($t_{1/2}$) were estimated for each individual by using the post-hoc estimated PK parameters (Empirical Bayes Estimates: the mode of the conditional parameter distribution).

Cobicistat pharmacokinetic analysis

Blood samples were taken predose and at 0·5, 1, 2, 3, 4, 6, 8, 10, 24, and 48 h after the cobicistat dose to measure the concentration of the drug in plasma. Samples were analyzed using a liquid chromatography/tandem mass spectrometry method validated by the U.S. FDA guidance for industry by Pyxant Labs (Colorado Springs, CO, USA).

The population PK and individual post-hoc estimates of cobicistat PK were determined using a similar modeling approach as described above. A linear one-compartment model with first-order absorption, an absorption lag time, and first-order elimination was applied. Concentrations below the LLOQ (10 ng/mL) were treated as censored using the M3 method as implemented in Monolix.[14] The interindividual and interoccasion variabilities of the parameters were assumed to be log-normally distributed. A proportional residual error model was used with assumed normal distribution of residuals.

Pharmacodynamic simulation methods

The antimalarial efficacy of SJ733 against *P. falciparum* at different dosing regimens was simulated using the previously developed PK/PD model.[7] The simulations in the current study used the PD parameters estimated using the data from *P. falciparum*-infected participants in the Phase 1b IBSM study prior to and after 600-mg SJ733 therapy[7] and the observed PK parameters determined in the current study, with the multidose and cobicistat-boosted doses. A representative fit of the model to the parasite *vs.* time data for an individual from the Phase 1b IBSM study who was treated with a single unboosted dose of 600 mg and the simulated profiles of an individual given 3 daily doses of 600 mg ± cobicistat boost are shown in Figure S3.

Outcomes

Study outcome measures were similar to those described for the unboosted SAD/first-in-human study.[7] Electrocardiograms (ECGs) were performed in triplicate at predose and at 1·5, 4, and 8 h (MAD) or 10 h (boosted SAD) post-dose.

Statistics

The study was a single-arm, unblinded Phase 1 study that had an adaptive component in which PK and safety results from each dose cohort were reviewed by a Safety Monitoring Committee and a PK Monitoring Committee before the next dose cohort was initiated. Sample size per dose cohort was typically six and determined in consultation with the U.S. FDA, during the discussion before the Pre-Investigational New Drug application was submitted. Study eligibility criteria are noted in the appendix. Descriptive statistics were summarized for participants and adverse events.

The covariates single *vs* multidose SJ733 and concomitant administration of cobicistat were evaluated to determine their significance in explaining PK variability. These covariates were considered significant in a univariate analysis if their addition to the model reduced the objective function value by at least 3·84 units ($p < 0·05$, based on the χ^2 test for the difference in the −2 log-likelihood between two hierarchical models that differ by 1 degree of freedom).

Ethics

This study (ClinicalTrials.gov: NCT02661373) was approved by institutional review boards (Reference #: BUZZOFF (Pro00006080) and conducted at St. Jude Children's Research Hospital and at the University of Tennessee Clinical Research Center (Memphis, TN, USA) under an Investigational New Drug approval (126652) from the U.S. FDA.[7] All participants provided written informed consent.

Role of the funders

The Global Health Innovative Technology (GHIT) Fund and the American Lebanese Syrian Associated Charities (ALSAC) had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. Medicines for Malaria Venture (MMV) provided supplemental funds and subject experts, who provided input for the study design, data collection, data analysis, data interpretation, and writing of the report. This research was supported, in part, by the National Institutes of Health (NIH) Cancer Center grant (P30 CA021765); however, the content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Results

Participants

Overall, the Phase 1a study enrolled 34 healthy adult volunteers. All were male, except for one female volunteer who participated in the previously described single-dose cohort.[7] The demographic distribution of the subjects in each cohort is described in Table 1. All participants in the MAD cohort and the majority (83%) in the boosted single-dose cohort were Caucasian; there were two Black participants and one Asian participant in the boosted

	Cohort 7 (300 mg × 3 QD)[a] N = 3	Cohort 8 (600 mg × 3 QD)[b] N = 6	Total	Cohort 11 (75 mg SJ733+150 mg cobicistat SAD)[c] N = 6	Cohort 12 (300 mg SJ733+150 mg cobicistat SAD)[d] N = 6	Cohort 13 (600 mg SJ733+150 mg cobicistat SAD) N = 6	Total
Mean	31·9	27·0	29·5	25·6	30·3	23·2	26·4
Range	27·5 – 47·2	19·1 – 37·6	19·1 – 47·2	19·5 – 38·1	(21·3 – 47·95)	(18·8 – 30·8)	(18·8 – 47·95)

***Table 1*: Age of participants at screening in the Phase 1a unboosted multiple ascending dose cohorts (Cohorts 7 & 8) and the cobicistat-boosted single ascending dose cohorts (Cohorts 11, 12 and 13).**

[a] Includes 3 subjects who participated in previous single-dose cohorts. One subject participated in cohort 4 and the fed cohort; one participated in cohort 5; and one participated in cohort 6.

[b] Includes 5 subjects who participated in previous single-dose cohorts.

[c] Includes 6 subjects who participated in previous dose cohorts.

[d] Includes 2 subjects who participated in previous dose cohorts.

single-dose cohort. Between March 2017 and March 2018, nine volunteers were enrolled in the MAD cohorts (eight also participated in prior single-dose cohorts, and seven also participated in subsequent cobicistat-boosted cohorts), and 18 (eight also participated in prior cohorts) were enrolled in the cobicistat-boosted cohorts. Three participants were given 300 mg SJ733 daily for 3 days, and six were given 600 mg daily for 3 days. The 75, 300, and 600 mg SJ733 boosted with 150 mg cobicistat single-dose cohorts included six participants each (Figure 1).

Adverse events

Adverse events (AEs) in the unboosted SAD cohorts have been previously reported.[7] Those in the unboosted MAD and cobicistat-boosted SAD cohorts are summarized in aggregate in Table S3 and are individually presented in Table S4. Overall, we found no serious AEs, dose-limiting toxicities, Grade 3 or 4 AEs, clinically significant ECG/safety laboratory test findings, or study discontinuations. We adjudicated all AEs reported in the MAD and cobicistat-boosted SAD cohorts as unlikely related or unrelated to SJ733 or cobicistat (where applicable).

We did not note any baseline *versus* post-dose (1·5 h, 4 h, and 10 h) corrected QT (QTc) interval change above 60 ms, except in one instance (Day 3, 1·5-h post 300 mg, unboosted, SJ733 ECG). This AE was assessed as not clinically significant; we observed no post-dose QTc interval change above 500 ms.

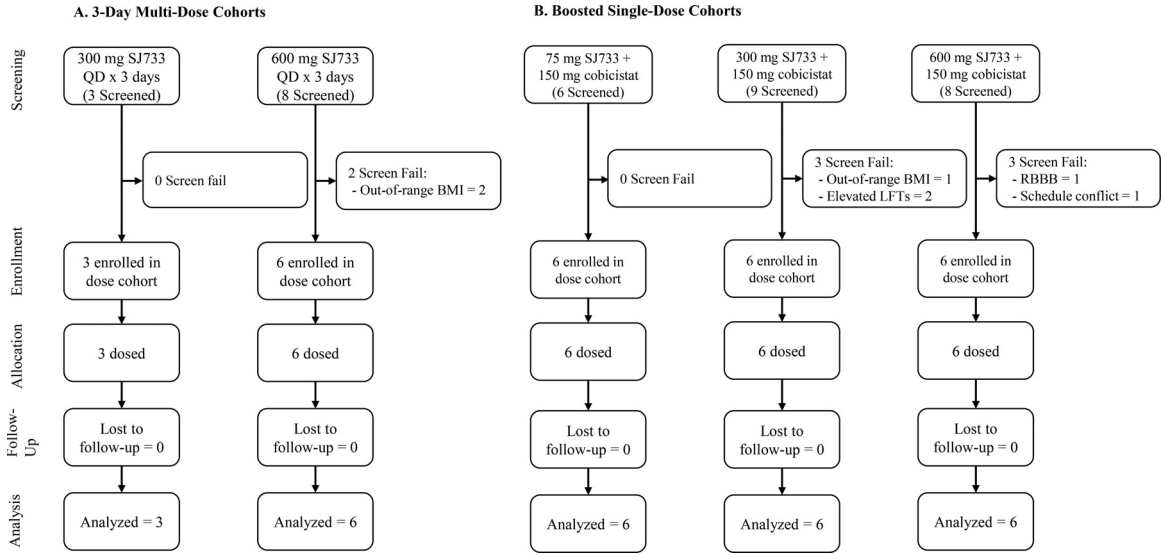

Figure 1. Consort Flow Diagram of the Phase 1a study: Multidose and cobicistat boosted dose cohorts. Flow diagram of participant progress through the study. In Phase 1a, multiple ascending doses and cobicistat-boosted doses of SJ733 were tested in two (300 mg and 600 mg SJ733 daily for 3 days, respectively) and three (75, 300, and 600 mg SJ733 combined with 150 mg cobicistat, single-dose) fasting dose cohorts, respectively. Enrolment in more than one non-consecutive dose cohort was allowed with at least 14 days required between doses.

Pharmacokinetics

The SJ733 population PK parameters estimated on data from all cohorts are described in Table S5, and the post-hoc estimated secondary parameters are summarized in Table S6. Similarly, the SJ506 population PK parameters estimated on data from all cohorts are described in Table S7, and the post-hoc estimated secondary parameters are summarized in Table S8. Plots showing the goodness-of-fit for both the models describing SJ733 and SJ506 are shown in Figures S4 and S5.

Multidose cohort pharmacokinetics

SJ733. The PK profiles of the 300 and 600 mg SJ733 (daily for 3 days) regimens are shown alongside their equivalent unboosted single-dose profiles in Figure 2 and Figure S6. None of the primary parameters were significantly different in the MAD cohorts compared to the unboosted SAD cohorts. In addition, the post-hoc estimated CL/F of SJ733 in the two MAD cohorts was similar to that of the three unboosted SAD cohorts between 75 and 600 mg (median [range]: 35·6 [19·2, 84·2] *vs.* 30·5 [15·5, 67·4] L/h, $p = 0{\cdot}4$ [Kruskal-Wallis test], respectively; Figure 3a). This translated to a proportional increase in the estimated AUC for the two MAD cohorts, compared to their respective unboosted single-dose AUC (2·3 × higher, $p = 0{\cdot}007$, [Kruskal-Wallis test]; Figure 3b). Furthermore, compared to their respective unboosted single dose, the exposure was above the MIC of 150 ng/mL longer (4·0 × and 2·0 × longer, $p = 0{\cdot}02$ and $p = 0{\cdot}004$ [Kruskal-Wallis test], for 300 mg × 3 and 600 mg × 3, respectively). The percentage of individuals for whom this time was >96 h was higher (50% *vs.* 0%, $p = 0{\cdot}046$ [Kruskal-Wallis test]), for 600 vs 300 mg SJ733 daily for 3 days (Figure 3d).

SJ506. The SJ506 concentration *versus* time profiles of the 300 mg and 600 mg SJ733 daily for 3 days are shown alongside their equivalent single-dose profiles in Figures S7 and S8. Like that of the parent drug, the PK of the metabolite showed that none of the primary parameters were significantly different in the MAD cohorts compared to the unboosted SAD cohorts. The estimated CL/F of SJ506 in the two MAD cohorts was similar to that of the three unboosted SAD cohorts between 75 mg and 600 mg (median [range]: 18·4 [10·8, 35·2] *vs.* 19·3 [6·8, 39·7] L/h, $p = 0{\cdot}9$ [Kruskal-Wallis test], respectively; Figure S9). This translates to a proportional increase in the estimated AUC for the two MAD cohorts compared to their respective unboosted single-dose AUC (3·0 × higher, $p = 3{\cdot}8 \times 10^{-4}$ [Kruskal-Wallis test]).

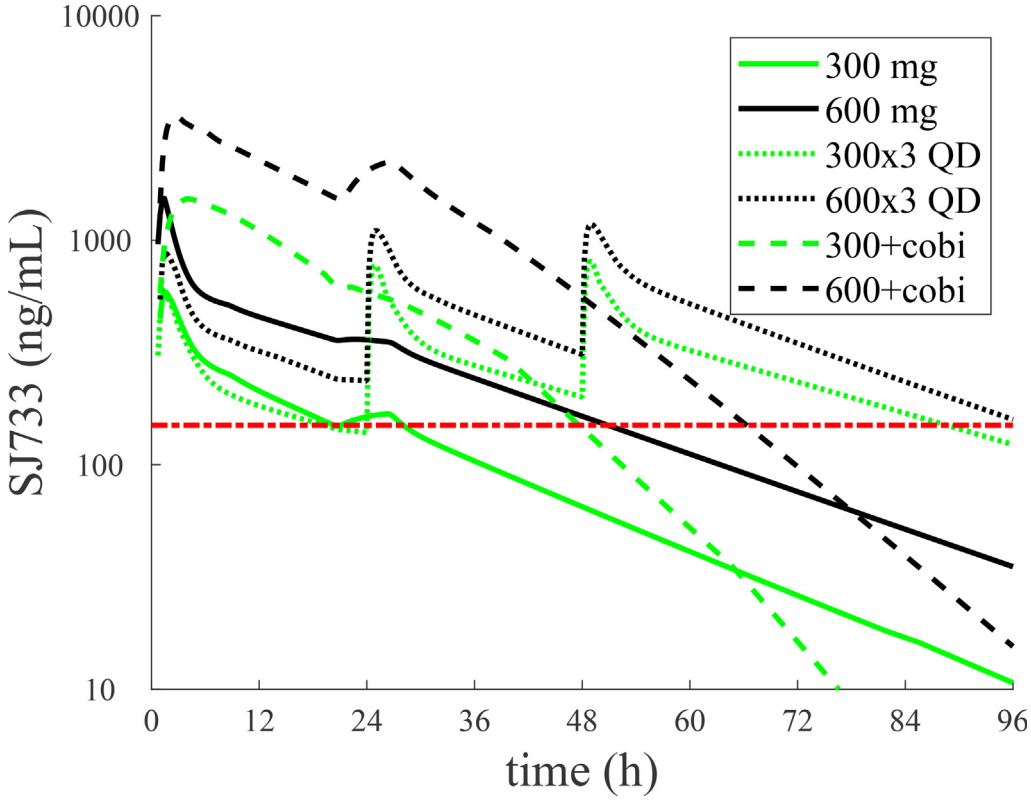

Figure 2. SJ733 median concentration *versus* time plot for unboosted and cobicistat-boosted single-dose cohorts. Estimated minimum inhibitory concentration (150 ng/mL) is shown as a horizontal red line. Abbreviations: Cobi, cobicistat; × 3 QD, daily for 3 days.

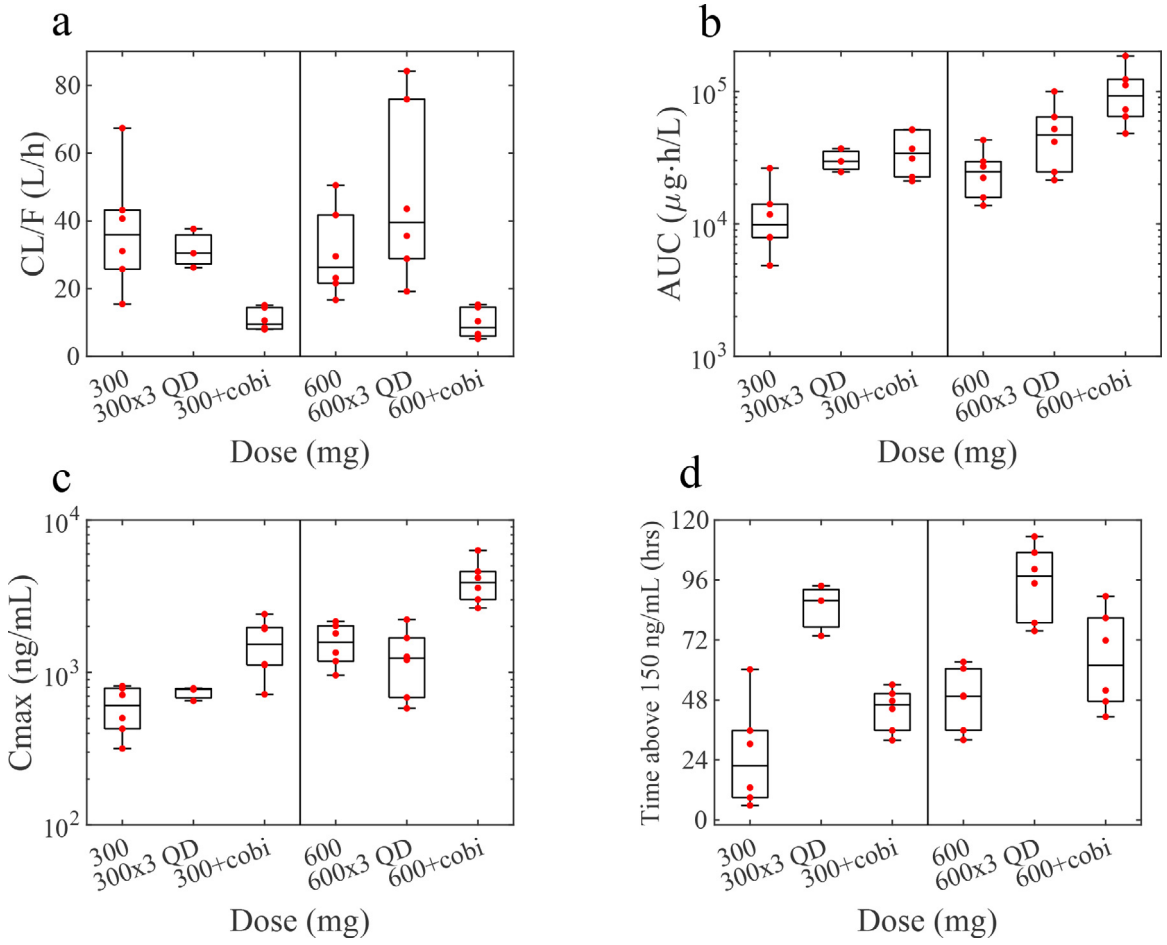

Figure 3. Secondary PK parameters after single-dose or multidose treatment with SJ733 alone or single-dose SJ733 phamacoboosted with cobicistat. (a) Apparent clearance (CL/F), (b) areas under the curve (AUC), (c) maximum concentration (C_{max}), and (d) time above 150 ng/mL, the minimum inhibitory concentration (MIC), are stratified by cohort. Horizontal Bar: median; Box: quartile range; Whiskers: range. Red dots: individual measures.

Cobicistat-boosted cohort pharmacokinetics

Cobicistat. The PK profiles of the CYP3A4 inhibitor cobicistat were described by a one-compartment model with first-order absorption (Figure S10). The estimated population PK parameters are described in Table S9. The post-hoc estimated median [range] CL/F and $t_{1/2}$ were 19·5 L/h [7·5, 111·4 L/h] and 4·1 h [1·3, 8·0 h], respectively. Given the dose of 150 mg, all individuals' cobicistat levels were below LLOQ by 48 h.

SJ733. The SJ733 PK profiles of the 300- and 600-mg doses of SJ733 with cobicistat are shown alongside their equivalent unboosted single-dose profiles in Figure 2 and Figure S6. Co-administration of cobicistat significantly increased the elimination (k_e: 0·34 vs 0·11 (L/h) with *vs* without cobicistat; $p < 2{\cdot}2 \times 10^{-16}$ [Wald test]) and decreased apparent volume (V/f: 22·9 L *vs* 336·3 L with *vs* without cobicistat; $p < 2{\cdot}2 \times 10^{-16}$ [Wald test]) and absorption (k_a: 0·16 L/h *vs* 2·26 L/h with *vs* without cobicistat; $p < 2{\cdot}2 \times 10^{-16}$ [Wald test]) of SJ733 (Table S5). This corresponded to a significant 72% decrease in the median post-hoc estimated apparent clearance of SJ733 in the two SAD cohorts without cobicistat *versus* the two cohorts with cobicistat (median [range]: 30·5 [15·5, 67·4] *vs* 8·4 [3·7, 15·2] L/h, respectively, $p = 3{\cdot}0 \times 10^{-7}$ [Kruskal-Wallis test]; Figure 3a). This decrease translated to a significant increase in SJ733 $AUC_{0\text{-}\infty}$ (3·9 ×, $p = 9{\cdot}4 \times 10^{-7}$ [Kruskal-Wallis test]) and C_{max} (2·6 ×, $p = 1{\cdot}8 \times 10^{-6}$ [Kruskal-Wallis test]) relative to the equivalent SAD cohorts without cobicistat and a slightly higher $AUC_{0\text{-}\infty}$ relative to the MAD cohorts (1·4 ×, $p = 0{\cdot}055$ [Kruskal-Wallis test]) (Figure 3c). In addition, the time above the MIC of 150 ng/mL trended longer when cobicistat was present (2·1 ×, 1·2 ×; $p = 0{\cdot}08$, $p = 0{\cdot}2$ [Kruskal-Wallis test]; given 300 or 600 mg SJ733, respectively; Figure 3d).

SJ506. The SJ506 PK profiles of the 300- and 600-mg doses of SJ733 with cobicistat are shown alongside their equivalent unboosted single-dose profiles in

Figures S7-S8. Cobicistat had a time-dependent effect on the formation of SJ506, which was directly correlated with the inhibitor's plasma concentration. Specifically, the formation of SJ506 was significantly decreased 78% in the presence of cobicistat (0·99 *vs* 0·22, $p = 2{\cdot}7 \times 10^{-15}$ [Wald test]; Table S7). Similarly, the CL/F of SJ506 was significantly higher in the presence *versus* the absence of cobicistat (median [range]: 22·3 [9·1, 42·6] *vs.* 17·7 [6·8, 39·7] L/h, respectively; $p = 0{\cdot}027$, [Kruskal-Wallis test]; Figure S9). The effects of cobicistat, therefore, translated to a lower metabolite exposure ($AUC_{0\text{-}\infty}$ and C_{max}) compared to cohorts without cobicistat (23% and 65%, $p = 0{\cdot}027$ and $2{\cdot}4 \times 10^{-6}$ [Kruskal-Wallis test], respectively). Cobicistat also significantly decreased the $AUC_{0\text{-}\infty}$ metabolic ratio (SJ506/SJ733) by 4·6 × from 1·7 (0·9, 4·1) in the cohorts without cobicistat to 0·37 (0·1, 0·7) in the cohorts with cobicistat ($p = 7{\cdot}2 \times 10^{-10}$ [Kruskal-Wallis test]).

Pharmacodynamic model simulations

Following a single unboosted 600-mg dose of SJ733, parasitemia was reduced by an average of $10^{2{\cdot}6}$ parasites/μL, and the average time to the maximum kill was ~36 h (Table 2, Figure 4a). The model simulations predict that unboosted 300 or 600 mg SJ733 daily for 3 days would enhance parasitemia reduction to $10^{6{\cdot}9}$ and $10^{8{\cdot}7}$ parasites/μL, respectively (Table 2, Figure 4b-c). Relative to a single unboosted 600-mg dose, this is an increase of 2·7 × and 3·3 ×. Additionally, these model simulations predicted an enhanced reduction of parasitemia with cobicistat-boosted SJ733 between $10^{6{\cdot}9}$ and $10^{13{\cdot}2}$ parasites/μL given a 600-mg single dose or 600 mg daily for 3 days (Table 2, Figure 4a-c). This is an increase, relative to a single unboosted 600-mg dose of 2·7 × and 5·1 ×, respectively. The simulations also showed that a reduction in parasite burden of 10^9 parasites/μL would require a minimum of 300 mg SJ733 combined with cobicistat daily for 2 days. Without a cobicistat pharmacoboost, the simulations predicted that a dose of 600 mg SJ733 daily for 3 days or 200 mg daily for 4 days would be needed to achieve the desired 10^9 or more reduction in parasites in most individuals.

Discussion

SJ733 is the second PfATP4 inhibitor to enter clinical development. Although SJ733 demonstrated a favorable safety profile and rapid antiparasitic effect, the duration of the antimalarial effect with a single dose was insufficient.[7] We demonstrated that both a standard 3-day once-daily regimen and an innovative pharmacoboosted dosing approach to treat acute, uncomplicated malaria with oral SJ733 were safe and well tolerated in healthy adult volunteers. Both regimens were also associated with drug exposures predicted to cure patients with *P. falciparum* malaria. The use of the pharmacoboost for CYP3A4 substrates is well established for antiretrovirals used to treat HIV; however, using it with an antimalarial is innovative and prompts a broader consideration of its application to other disease paradigms.

For treatment of acute uncomplicated malaria, SJ733 is considered a fast-acting drug candidate. The drug will not be used as monotherapy but combined with a second antimalarial drug that ideally would exhibit a longer t1/2. We previously demonstrated *in vitro* and *in vivo* that maximal parasite killing is achieved after a 96-h exposure to the appropriate drug levels.[4] As is shown in our recent publication, SJ733 demonstrates a biphasic killing cure. First, a very rapid, phagocyte-mediated phase clears trophozoites and schizonts, which is followed by a slow arrest and clearance of residual rings. Killing of rings is fully committed by 96 h[15] The majority of blood-stage parasites are cleared within the first 12 h after initiation of therapy, but full cure requires maintaining exposure until the residual rings have either been killed by the intrinsic mechanism or cycled and cleared by the phagocyte-mediated mechanism. Time above MIC has traditionally been the parameter targeted for antimalarial drugs and has proven a reasonable description of driving parameter for efficacy for many classes of drugs. With respect to SJ733, data from *in vitro* cellular assays and *in vivo* murine models of both *P. berghei* and *P. falciparum* suggest strongly that time above threshold near the MIC is the key driving parameter.[4,15] However, we cannot discriminate between two possible models in clinical response: efficacy requires SJ733 exposure to remain above 150 ng/

Treatment	Measure	Cohort Geometric mean (90% confidence interval)[b]		
		SJ733 600 mg × 1	SJ733 300 mg × 3	SJ733 600 mg × 3
SJ733	Max Log_{10} Kill	2·6 (2·3, 2·8)	6·9 (6·3, 7·4)	8·7 (8·2, 9·2)
	Time to Max Kill (h)	36·2 (34·7, 37·7)	90·0 (79·4, 100·5)	105·4 (91·2, 119·6)
SJ733 + Cobicistat	Max Log_{10} Kill	6·9 (6·4, 7·3)	11·9 (11·2, 12·6)	13·2 (12·5, 14·0)
	Time to Max Kill (h)	75·9 h (70·1, 81·7)	121·9 (111·8, 132·0)	133·6 (122·3, 144·9)

***Table 2*: Maximum and timing of log_{10} parasite kill[a].**

[a] These results are predictions based on the pharmacokinetic and pharmacodynamic model.

[b] Variability considered interindividual heterogeneity but excluded intraindividual heterogeneity and the breadth of potential SJ733+cobicistat pharmacokinetic profiles (*i.e.*, only maximum decline was used).

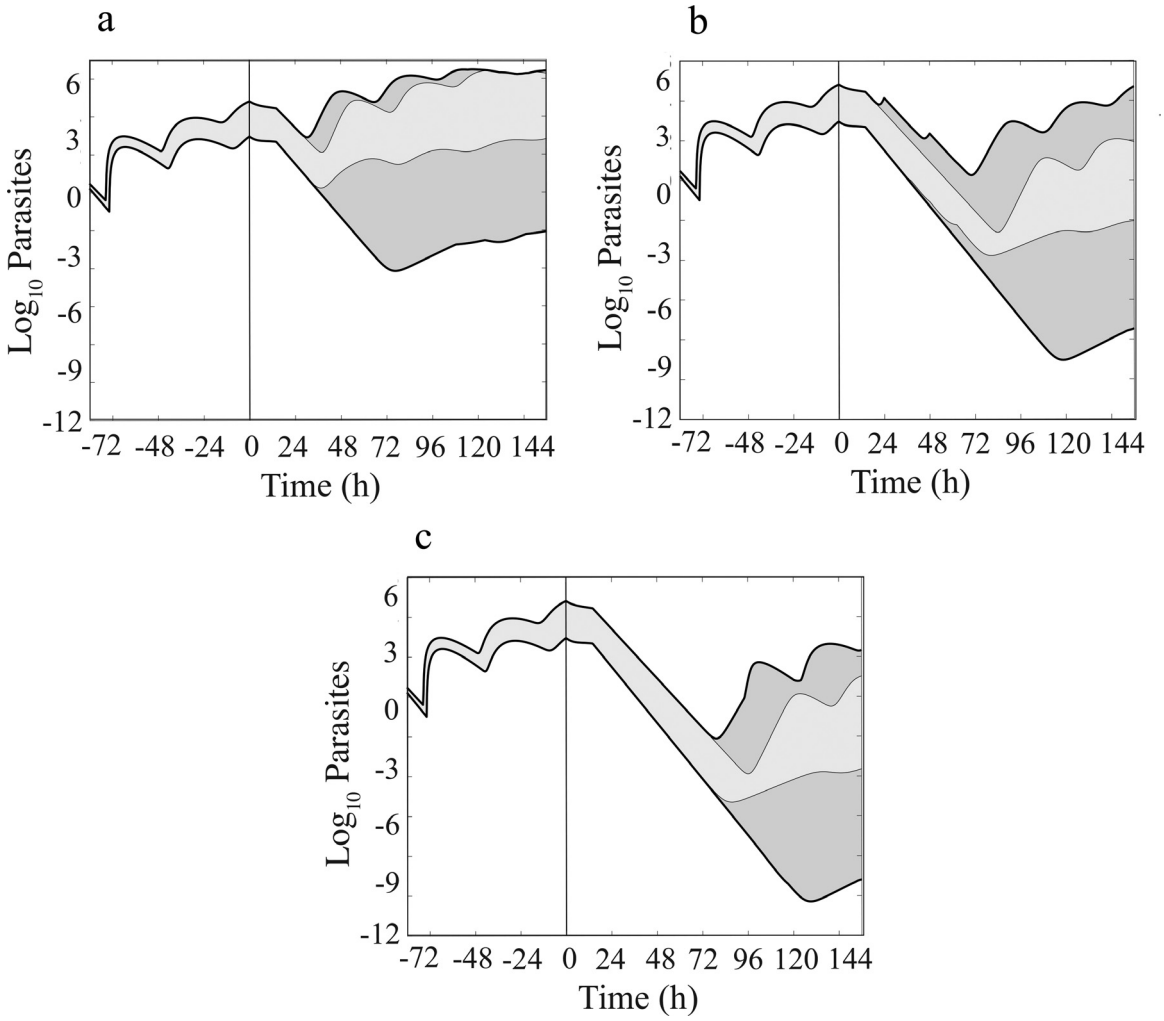

Figure 4. PK/PD simulations of the efficacy of SJ733 alone and SJ733 pharmacoboosted with cobicistat to cure malaria. (**a**) PK/PD model simulation of the effect of single-dose SJ733 (600 mg) alone (light gray) and that predicted for the same dose of SJ733 pharmacoboosted with cobicistat (dark gray). (**b-c**) The same simulations were used to assess multidose SJ733 (b: 300 mg daily for 3 days ± cobicistat and c: 600 mg daily for 3 days ± cobicistat).

mL levels for at least 4 days, or efficacy is driven by achieving maximal total exposure of SJ733 for at least 3 days. A Phase 2 trial testing these hypotheses is currently underway in Peru (ClinicalTrials.gov Identifier: NCT04709692).

In the described multidose and cobicistat-boosted cohorts, there were no AEs possibly, probably, or definitely related to SJ733, and no study discontinuations confirming the favorable safety profile of SJ733. Dose-dependent, reversible methemoglobinemia has been noted in dogs given high doses of SJ733; however, careful monitoring in our Phase 1 program, including these new investigations with the multidose and pharmacoboost administrations, did not show abnormal values. Additionally, no clinically relevant study drug-SJ733-related liver toxicity or cardiac safety (QTc prolongation) signals were noted in healthy volunteers, including in the IBSM model in Phase 1b. Concentration/QTc modeling with SJ733 and SJ506 will be needed to confirm the absence of a QTc-prolongation signal.

The reason for the observed sub-proportional increase in exposure with doses exceeding 600 mg SJ733 during the Phase 1a study remains unclear. The increased apparent clearance for doses higher than 600 mg could result from solubility-limited absorption; however, there are no data yet to confirm this hypothesis. Using our previously developed PK/PD model of *P. falciparum*[7] and the PK data from this study, we predicted a clinically meaningful parasite clearance of at least 9 $\log_{10}$ parasites/μL with the unboosted multidose approach using 600 mg SJ733 daily for 3 days or the pharmacoboosted approach using a minimum of

300 mg SJ733 plus cobicistat daily for 2 days. In addition, the co-administration of 600 mg SJ733 with cobicistat daily for 3 days, relative to a single 600-mg dose ± cobicistat, would increase parasite clearance between 10^6 parasites/μL and 10^{12} parasites/μL. For reference, clearing 10^6 parasites/μL is viewed as minimally efficacious (typically seen in uncomplicated symptomatic malaria); 10^9 parasites/μL is considered significantly efficacious; and 10^{12} parasites/μL (seen in acutely ill patients with malaria) is ideal.[16] Currently, all the approved treatments for acute, uncomplicated malaria require treatment for 3 days or longer and often require twice-daily administration. Moreover, no single-dose treatment has entered Phase 3 so far. Consequently, a 2-day, once-daily treatment would constitute an improvement over available treatments for uncomplicated malaria. Our data also suggest that SJ733 could be combined with another antimalarial that exhibits CYP3A4 inhibition and be applied as an alternative to boost with cobicistat.

The importance of PK/PD simulations and the role of pharmacometrics in optimizing dose selection for vulnerable populations who are typically excluded from clinical trials, such as children and pregnant women, has been noted.[13] The PK/PD modeling and simulation process we developed enabled us to leverage the relatively small number of PK and PD studies to evaluate new treatment options (multiple doses and dosing schedules), with or without pharmacoboosting, and determine candidate dosing approaches to move forward into Phase 2 trials against acute, uncomplicated malaria. The main advantages of this modeling and simulation process are the reduced time and cost leading up to Phase 2 trials by reducing the number of additional Phase 1 PK- or PD-dosing cohorts.

The innovative application of the pharmacoboost approach with CYP3A4 inhibition and subsequent increase in drug exposure for an antimalarial raises a number of considerations. For SJ733, it offers the opportunity to take a potentially more efficacious dosing approach into a Phase 2 setting. An adaptive open-label Phase 2a study to examine the antimalarial efficacy, safety, and tolerability of SJ733 with or without cobicistat in adult patients with uncomplicated *P. vivax* or *P. falciparum* blood-stage malaria monoinfection is currently underway in Peru. For future clinical development and Phase 2B/3 trials, SJ733 will most likely be combined with a second antimalarial, and specific drug-drug interactions will need to be considered if cobicistat is used as a pharmacobooster. Given that cobicistat has no antiretroviral activity, its use negates any concern about the development of HIV resistance when used in populations with a high HIV incidence. Additionally, the short course of antimalarial therapy that would include a strong CYP3A4 inhibitor makes concerns about clinically relevant drug-drug interactions unlikely. The added cost of the pharmacobooster and available formulation would certainly influence the generalizability of considerations for the proposed pharmacoboost strategy. Although no pediatric formulation of cobicistat is registered, clinical trials for its use with antiretrovirals in children are ongoing (ClinicalTrials.gov Identifier: NCT02016924). Ritonavir is available as a liquid formulation, has dosing guidelines available for all ages, and is generic. However, its, compared to cobicistat, inherent antiretroviral activity with theoretical concerns of perpetuating antiretroviral resistance, unpleasant taste, and being a less selective CYP inhibitor and one with enzyme-inducing effects, a higher likelihood of having drug-drug interactions with concomitant medications, reduces its appeal as a candidate pharmacobooster for SJ733 or any future applicable antimalarials.

The significant decrease in plasma exposure of SJ506, the major metabolite of SJ733, is not expected to have any clinical significance, given that it lacks antimalarial activity. This may not be the case for other antimalarials; depending on the safety or efficacy signals attributed to a metabolite, the impact of the pharmacoboost approach on the metabolite levels may be clinically meaningful.

The race/ethnicity of the volunteers in this trial is not representative of populations most affected by malaria. Under-recruiting of female participants also remains a common limitation of early-stage drug development studies, including this one.[7] These limitations are not uncommon for Phase 1 trials and are being addressed as SJ733 drug development has proceeded to the current Phase 2 trial in Peru. Of note from a cobicistat boost standpoint, there is no evidence that gene polymorphisms of *CYP3A4* have a major role in establishing ethnic differences in CYP3A4 expression.

The favorable safety and tolerability of unboosted or cobicistat-boosted SJ733 and the significant increase in SJ733 exposure in the multidose and pharmacoboosted setting with associated clinically meaningful predicted parasite clearance in the setting of uncomplicated malaria, support testing these dosing approaches in Phase 2 clinical trials. Additionally, SJ733's projected reduced susceptibility to resistance supports its further development in patients with malaria and suggests that it may afford a viable replacement for artemisinin in current treatment regimens.

Contributors

A.H.G. and R.K.G. conceptualized the overall research plan, acquired funding, administered the project, supervised and provided resources. A.H.G. and J.C.P wrote the original draft of this manuscript and the rest of the co-authors contributed to the writing of the manuscript (Review and editing). J.C.P. contributed to the project's conceptualization, data curation, formal analysis, and visualization. A.M.S. contributed to the conceptualization, formal analysis, and visualization. S.C. contributed

to the conceptualization, investigation, methodology and visualization. R.D. contributed to the conceptualization, data curation, investigation, and methodology. B.B.F. III contributed to the conceptualization and data curation. T.B.S., N.D.P., and R.N.H. contributed to the conceptualization, investigation, and methodology. L.T. contributed to the conceptualization, data curation, and formal analysis. E.J. contributed to the conceptualization and project administration. K.C.B., S.O., and J.M. contributed to the conceptualization and methodology. J.L.R. and P. M.F. contributed to the investigation and methodology. J.T.H. contributed to the formal analysis and methodology. L.B. contributed to the methodology. F.G. contributed to the conceptualization and provided resources. N.M. and T.Y. provided resources for the project. R.D., J.C.P., L.T. and A.H.G. verified the data. All authors reviewed, edited, and approved the final version of the manuscript.

Data sharing statement

Data collected for this study will be made available on request after article publication. De-identified data sets containing the variables analyzed for the primary objectives will be made available, as well as other supporting documents (*e.g.*, protocol and informed consent). Investigators who seek access to de-identified datasets and individual-level data should contact the lead authors, Aditya Gaur (aditya.gaur@stjude.org) and John C. Panetta (carl.panetta@stjude.org), with a formal request that includes: full name of requestor, affiliation, data set requested, and timing of when data is needed. The proposal will be reviewed for merit and feasibility by A. Gaur, J.C. Panetta and investigators will be notified of the decision within 30 days of receipt. If the request is accepted, a data use agreement covering relevant conditions may be required.

Declaration of Interests

A.H.G. and BBF III received grant funding from the GHIT and MMV. J.C.P. received grant funding from the GHIT and the NIH Cancer Center Support Grant P30 CA021765. R.D., T.B.S., L.T., E.J., K.C.B., S.O., N. D.P., R.N.H., J.T.H., F.G., N.M., and T.Y. received grant funding from the GHIT. P.M.F. is a member of the Merck Safety Monitoring Committee. J.S.M. received funding from the Australian National Health and Medical Research Council (NHMRC) Practitioner Fellowship (GNT1135955) and NHMRC grant Programme (GNT1132975). R.K.G. receives grant funding from and is a reviewer for the GHIT and MMV. He is also an inventor on a patent disclosing SJ733, which may generate revenue if licensed. A.M.S., J.L.R, L.B., and S.C. have no conflicts to declare.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2022.104065.

References

1 Balikagala B, Fukuda N, Ikeda M, et al. Evidence of Artemisinin-Resistant Malaria in Africa. *N Engl J Med*. 2021;385(13):1163–1171.
2 Mathenge PG, Low SK, Vuong NL, et al. Efficacy and resistance of different artemisinin-based combination therapies: a systematic review and network meta-analysis. *Parasitol Int*. 2020;74: 101919.
3 Phillips MA, Burrows JN, Manyando C, van Huijsduijnen RH, Van Voorhis WC, Wells TNC. Malaria. *Nat Rev Dis Prim*. 2017;3:17050.
4 Jimenez-Diaz MB, Ebert D, Salinas Y, et al. +)-SJ733, a clinical candidate for malaria that acts through ATP4 to induce rapid host-mediated clearance of Plasmodium. *Proc Natl Acad Sci USA*. 2014;111(50):E5455–E5462.
5 White NJ, Pukrittayakamee S, Phyo AP, et al. Spiroindolone KAE609 for falciparum and vivax malaria. *N Engl J Med*. 2014;371 (5):403–410.
6 Schmitt EK, Ndayisaba G, Yeka A, et al. Efficacy of cipargamin (KAE609) in a randomized, Phase II dose-escalation study in adults in sub-Saharan Africa with uncomplicated Plasmodium falciparum malaria. *Clin Infect Dis*. 2021. https://doi.org/10.1093/cid/ciab716. Epub ahead of print.
7 Gaur AH, McCarthy JS, Panetta JC, et al. Safety, tolerability, pharmacokinetics, and antimalarial efficacy of a novel Plasmodium falciparum ATP4 inhibitor SJ733: a first-in-human and induced blood-stage malaria phase 1a/b trial. *Lancet Infect Dis*. 2020;20(8):964–975.
8 Spillman NJ, Kirk K. The malaria parasite cation ATPase PfATP4 and its role in the mechanism of action of a new arsenal of antimalarial drugs. *Int J Parasitol Drugs Drug Resist*. 2015;5(3):149–162.
9 Chen Y, Zhu F, Hammill J, et al. Selecting an anti-malarial clinical candidate from two potent dihydroisoquinolones. *Malar. J*. 2021;20(1):107.
10 Gong Y, Haque S, Chowdhury P, et al. Pharmacokinetics and pharmacodynamics of cytochrome P450 inhibitors for HIV treatment. *Expert Opin Drug Metab Toxicol*. 2019;15(5):417–427.
11 Sherman EM, Worley MV, Unger NR, Gauthier TP, Schafer JJ. Cobicistat: review of a pharmacokinetic enhancer for HIV infection. *Clin Ther*. 2015;37(9):1876–1893.
12 Hoglund RM, Byakika-Kibwika P, Lamorde M, et al. Artemether-lumefantrine co-administration with antiretrovirals: population pharmacokinetics and dosing implications. *Br J Clin Pharmacol*. 2015;79(4):636–649.
13 Hughes E, Wallender E, Mohamed Ali A, Jagannathan P, Savic RM. Malaria PK/PD and the role pharmacometrics can play in the global health arena: malaria treatment regimens for vulnerable populations. *Clin Pharmacol Ther*. 2021;110(4):926–940.
14 Beal SL. Ways to fit a PK model with some data below the quantification limit. *J Pharmacokinet Pharmacodyn*. 2001;28(5):481–504.
15 SheelaNair A, Romanczuk AS, Aogo RA, et al. Similarly efficacious anti-malarial drugs SJ733 and pyronaridine differ in their ability to remove circulating parasites in mice. *Malar J*. 2022;21(1):49.
16 Burrows JN, Duparc S, Gutteridge WE, et al. New developments in anti-malarial target candidate and product profiles. *Malar J*. 2017;16(1):26.