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Randomized clinical study of safety, pharmacokinetics, and pharmacodynamics of RIPK1 inhibitor GSK2982772 in healthy volunteers

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Abstract

GSK2982772 is a highly selective inhibitor of receptor-interacting protein kinase 1 (RIPK1) being developed to treat chronic inflammatory diseases. This first-in-human study evaluated safety, tolerability, pharmacokinetics (PK), and exploratory pharmacodynamics (PD) of GSK2982772 administered orally to healthy male volunteers. This was a Phase I, randomized, placebo-controlled, double-blind study. In Part A, subjects received single ascending doses of GSK2982772 (0.1-120 mg) or placebo in a crossover design during each of 4 treatment periods. In Part B, subjects received repeat doses of GSK2982772 (20 mg once daily [QD] to up to 120 mg twice daily [BID]) or placebo for 14 days. Part C was an open-label relative bioavailability study comparing 20-mg tablets vs capsules. Safety, tolerability, pharmacokinetics (PK), RIPK1 target engagement (TE), and pharmacodynamics (PD) were assessed. The most common adverse events (AEs) were contact dermatitis and headache. Most AEs were mild in intensity, and there were no deaths or serious AEs. The PK of GSK2982772 was approximately linear over the dose range studied (up to 120 mg BID). There was no evidence of drug accumulation upon repeat dosing. Greater than 90% RIPK1 TE was achieved over a 24-hour period for the 60-mg and 120-mg BID dosing regimens. Single and repeat doses of GSK2982772 were safe and well tolerated. PK profiles showed dose linearity. The high levels of RIPK1 TE support progression into Phase II clinical trials for further clinical development.

KEYWORDS

anti-inflammatory agents, GSK2982772, pharmacodynamics, pharmacokinetics, RIPK1, safety

Abbreviations: AEs, adverse events; ANA, antinuclear antibody; ECG, electrocardiography; EDTA, ethylenediaminetetraacetic acid; SAEs, serious adverse events; TE, target engagement; TNF, tumor necrosis factor.

1 | INTRODUCTION

Necroptosis, or programmed necrosis, is a form of cell death that causes inflammation by damaging cell function and/or releasing damage-associated molecular patterns.[1] Emerging evidence indicates that necroptosis may play a critical role in mediating a variety of human disease pathologies, including ischemia-reperfusion injury, pancreatitis, myocardial infarction, atherosclerosis, liver disease, neurodegenerative disorders, and immune-mediated inflammatory diseases such as inflammatory bowel diseases, rheumatoid arthritis, and psoriasis.[2,3] Small-molecule inhibitors of necroptosis are currently being developed as a new class of anti-inflammatory agents with broad therapeutic potential.[3]

Receptor-interacting protein kinase 1 (RIPK1) has emerged as an important therapeutic target for small-molecule kinase inhibitors because of its central role in mediating necroptosis in response to a variety of upstream activators, including the signaling that follows engagement of the tumor necrosis factor (TNF) receptor 1.[4] In addition to regulating necroptosis, RIPK1 can also activate inflammatory apoptotic cell death and proinflammatory cytokine production, highlighting its central role in promoting cell damage and inflammation.[4] Moreover, inhibition of RIPK1 kinase activity by small-molecule inhibitors or by targeted disruption of RIPK1 enzymatic activity protects against cellular damage and prevents inflammation in numerous animal models of human disease pathologies.[2]

A number of other reported RIPK1 kinase inhibitors have been developed and used in preclinical models, including the necrostatin series.[5] However, moderate potency and poor developability properties for this series, including the leading exemplar Nec-1, appear to have precluded further clinical development.[6]

GSK2982772 is the first oral, monoselective, small-molecule inhibitor of RIPK1 and necroptosis to advance into human clinical studies. GSK2982772 binds in an allosteric pocket of the RIPK1 kinase domain and is a potent inhibitor of RIPK1-mediated cell death and cytokine production in preclinical models. The objective of this first-in-human study was to evaluate the safety, tolerability, pharmacokinetics (PK), exploratory target engagement, and pharmacodynamics (PD) of single and repeat doses of GSK2982772 in healthy male volunteers in order to support future clinical development in chronic inflammatory diseases.

2 | MATERIALS AND METHODS

2.1 | Participants

This study was approved by the National Research Ethics Service committee of East of England, Hatfield; Rolling Mill Road, Jarrow; Tyne and Wear; NE32 3DT (IRB identification number 14/EE/1209). All subjects provided informed consent. Healthy male subjects (aged 18-65 years) with a body mass index of 19-30 kg/m^2 were eligible for this study. Exclusion criteria included abnormal laboratory findings, any history or current evidence of abnormal organ function, active infections (including hepatitis B and C, human immunodeficiency virus, and tuberculosis) or a positive result from the prestudy drug, and alcohol screening test. Other exclusion criteria included previous participation in a clinical trial that required receiving an investigational agent within 30 days (or 5 half-lives of the agent) of the first dosing day, exposure to more than 4 new chemical entities in the 12 months prior to the first dosing day, and an inability or unwillingness to swallow multiple size 00 capsules.

Additional exclusion criteria that were applied only in Part B of the study included cholesterol levels ≥300 gm/dL or triglyceride levels ≥250 mg/dL, significant risk or history of suicidality and positive antinuclear antibody (ANA) results outside of the normal reference range.

The "all screened subjects" population was defined as all subjects who were screened for eligibility. The "all subjects" population was defined as all subjects who received at least 1 dose of study medication. The "PK population" was defined as subjects in the "all subjects" population for whom at least 1 PK sample was obtained and analyzed.

2.2 | Permitted/prohibited drugs

Subjects were prohibited from taking any prescription or nonprescription drugs, including vitamins and dietary or herbal supplements, prior to the first dose of study medication until completion of the follow-up visit, unless the investigator and sponsor judged that the medication would not interfere with the study drug. However, paracetamol/acetaminophen at doses of ≤2 g/day was permitted for use any time during the study. Other concomitant medications were considered on an individual basis.

2.3 | Study design

This single center, randomized, double-blind, placebo-controlled study (NCT02302404; GSK trial 200975) was conducted at the GlaxoSmithKline Clinical Unit Cambridge, Addenbrooke's Centre for Clinical Investigation, Cambridge, UK. The study was designed in accordance with International Conference on Harmonisation (ICH 1998) and European Medicines Agency regulatory guidance for first-in-human studies (EMA 2007).

The study consisted of 3 parts with a total planned enrollment of 76 subjects (Figure 1) (79 subjects were actually enrolled when replacements are considered). Part A (N = 16) evaluated single, oral, ascending doses of GSK2982772 in 2 cohorts of subjects. Subjects were randomized equally (1:1:1:1) to 1 of 4 treatment sequences within Cohort 1 (n = 8) or Cohort 2 (n = 8). Each cohort comprised 4 study periods. For Cohort 1, 6 of the 8 subjects were randomized to receive a single oral dose of GSK2982772 (0.1 mg [Period 1], 0.5 mg [Period 2], 2.5 mg [Period 3], or 10 mg [Period 4]), and 2 subjects received placebo in each period, all under fasted conditions. Each subject received 3 doses of drug and 1 dose of placebo during the course of the study. Similarly, in Cohort 2 during each period, 6 of the 8 subjects were randomized to receive a single dose of

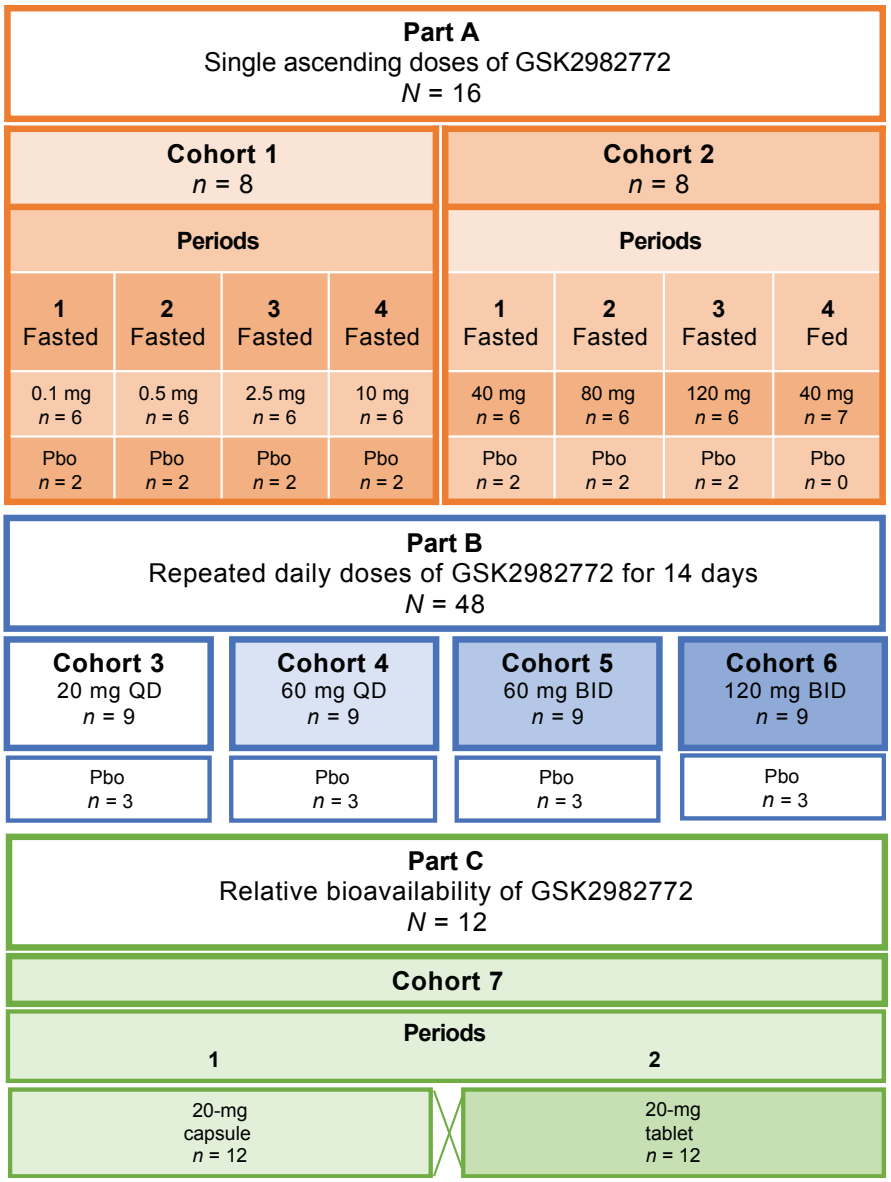

FIGURE 1 Study design. In Part A, subjects in Cohorts 1 and 2 were randomized 1:1:1:1 to 1 of 4 treatment sequences. During each period of Part A, 6 of 8 subjects in Cohorts 1 and 2 received the assigned dose level of GSK2982772 and 2 subjects received placebo, except for Cohort 2 during Period 4 (fed state) where all subjects received GSK2982772 with a high-fat meal. In Part B, subjects received the assigned dose of GSK2982772 for 14 days of continuous treatment. Cohorts at the next higher dose level began once the preceding dose was deemed safe. In Part C, subjects were randomized to receive either GSK2982772 20-mg capsules or tablets. BID, twice daily; Pbo, placebo; QD, once daily

GSK2982772 (40 mg [Period 1], 80 mg [Period 2], or 120 mg [Period 3]), and 2 subjects were randomized to receive placebo treatment, all under fasted conditions. Seven subjects in Cohort 2 participated in a final treatment period (Period 4) in which they received a single dose of GSK2982772 (40 mg after a high-fat meal). No subjects received placebo during this period. In both cohorts, there was a 6-day minimum washout period between each single-dose treatment.

Part B ($N = 48$) consisted of 14 days of repeated daily dosing regimens in a randomized, double-blind, placebo-controlled, sequential group analysis. Twelve subjects were randomized to GSK2982772 or placebo in a 3:1 ratio in each of the 4 sequential cohorts (Figure 1; Cohorts 3, 4, 5, and 6). Nine subjects were randomized to receive GSK2982772, 20 mg once daily (QD), 60 mg QD, 60 mg twice daily (BID), or 120 mg BID in Cohorts 3, 4, 5, and 6, respectively. The 3 remaining subjects in each cohort were randomized to receive placebo.

Part C ($N = 12$) consisted of a randomized, open-label, 2-period, 2-sequence crossover design to compare the bioavailability of a single 20-mg dose of GSK2982772 delivered in 20-mg tablets vs 20-mg capsules. Subjects were randomized to receive either capsules or tablets first, with a 6-day washout period between administration of the alternate formulation. The GSK2982772 dosages of less than 5 mg were administered as a solution in water with 5% ethanol (0.1 mg/mL), and dosages of 10-120 mg were administered as drug in capsules. Identical appearance, placebo-control solutions and capsules were administered. Dose escalation of GSK2982772 during Parts A and B was based on formal interim analyses of safety, tolerability, and PK data from the previous dose.

2.4 | Endpoints

The primary endpoint was the safety and tolerability of GSK2982772 as determined by the rates of adverse events (AEs)

and serious adverse events (SAEs), changes in laboratory values, electrocardiography (ECG), vital signs, and physical examinations. The secondary endpoints were blood/plasma PK parameters for GSK2982772 (area under the blood/plasma concentration-time curve from time zero to infinity [$AUC_{0-\infty}$] for single dose administration and AUC over the dosing interval [AUC_{0-tau}] for repeat dose administration, maximum blood/plasma concentration [C_{max}], time to reach C_{max} [t_{max}], and terminal elimination half-life [$t_{½}$]).

The plasma 4β-hydroxycholesterol-to-cholesterol ratio pretreatment and following repeat dosing of GSK2982772 was used as a marker to evaluate the potential of GSK2982772 to induce cytochrome P450 (CYP3A4).[7] In addition, the ex vivo GSK2982772 blood to plasma concentration ratio over a range of concentrations and percent blood cell association was assessed.

Exploratory PD endpoints were measured in Parts A and B, which included determining the level of RIPK1 target engagement (TE) and levels of macrophage inflammatory protein (MIP)-1α and MIP-1β after TNF pathway activation in whole blood samples.[8] These cytokines are produced in a RIPK1-dependent fashion following ex vivo stimulation.

2.5 | PK methods

PK parameters from Parts A and B were planned to be derived based on GSK2982772 concentrations obtained in blood to compare safety margins vs the nonclinical safety studies. In Part A, only whole blood samples were collected for PK analysis; in Part B, both blood and plasma samples were processed for PK analysis; and in Part C, only plasma samples were processed for PK analysis.

Blood samples were collected into ethylenediaminetetraacetic acid (EDTA) tubes using venipuncture technique for PK analysis and metabolite identification. Samples for blood assay were diluted with an equal volume (1:1) of deionized water. The plasma samples were processed by centrifugation at 1500 *g* for 10 minutes at 2-8°C. Both blood and plasma samples were analyzed for GSK2982772 concentrations using a validated analytical method based on protein precipitation, followed by high-performance liquid chromatography–tandem mass spectrometry analysis. The lower limit of quantitation was 0.2 ng/mL and the upper limit of quantitation was 100 ng/mL.

PK parameters were determined from the blood/plasma GSK2982772 concentration-time data using a standard noncompartmental analysis in Phoenix WinNonlin Version 6.4 (Certara, Princeton, NJ, USA) using actual blood sampling times. Accumulation ratios were determined where applicable using mixed effects models.

2.6 | Analysis of metabolism

Residual blood/water and plasma PK samples, urine samples, and bile samples were analyzed for relative levels of GSK2982772 and any metabolites as part of a separate study. Urine samples were collected at various time points. Bile samples were collected via an Entero-Test[9] from 1 cohort in Part B on Days −1 and 7 based on emerging safety and PK data during the study. The Entero-Test was administered approximately 2 hours following the subjects' morning GSK2982772 dose administration.

2.7 | PD and biomarker assessment

Plasma samples collected pretreatment and at the end of repeat dosing in Part B were analyzed for 4β-hydroxycholesterol and cholesterol levels using a validated liquid chromatography-mass spectrometry method. In Part B Cohorts 3, 4, and 5, the ratio of 4β-hydroxycholesterol to cholesterol was compared between baseline and Day 14, and for Part B Cohort 6, the comparison was made between baseline and Day 15.

The PD response in blood was assessed using an ex vivo challenge assay to assess RIPK1 activity-dependent signaling. Preclinical validation studies identified MIP-1α and MIP-1β, proinflammatory cysteine-cysteine family chemokines, as suitable analytes.[10] These cytokine levels were analyzed following ex vivo stimulation of cells with a stimulation cocktail in TruCulture (Myriad RBM, Austin, TX, USA) tubes (unpublished data on file, GlaxoSmithKline, Collegeville, PA). Whole blood (1 mL) was drawn into TruCulture tubes containing 2 mL of stimulation cocktail (medium containing TNF, zVAD, and SMAC mimetic) and incubated for 6 hours at 37°C.[8, 11] The assay was optimized in preclinical studies by pretreating whole blood with GSK2982772 for 1 hour and then transferring 1 mL of the treated blood to TruCulture tubes containing stimulation cocktail in 2 mL of media. Plasma was separated and concentrations of MIP-1α and MIP-1β were quantified using the Meso Scale Discovery platform (Meso Scale Diagnostics, Rockville, MD, USA).[8]

RIPK1 TE by GSK2982772 in the blood was assessed using a novel immunoassay, which detects a conformational change induced by inhibitor binding. The percent (%) TE was calculated based on the ratio of the signals from 2 antibodies that differentially bind "drug-bound" vs "unbound" RIPK1 protein (unpublished data on file, GlaxoSmithKline, Collegeville, PA). Two parallel sets of samples were evaluated. Initially, TE was assessed in the PD samples using the cellular fraction recovered from the TruCulture tubes. A secondary post hoc analysis was performed using a parallel set of unstimulated blood samples obtained at the same time as the PD samples. Lysates were prepared from both sets of blood samples, and TE was evaluated using identical assay conditions.

2.8 | Statistical analysis

PK parameters for Parts A, B, and C were summarized by treatment using descriptive statistics.

For Part A, dose proportionality and food effects were assessed via descriptive statistics and graphical displays. No formal statistical analyses were performed. In Part B, attainment of steady state from Day 2 to Day 14 trough concentrations, plasma accumulation ratio (AUC_{0-tau} Day 14/AUC_{0-tau} Day 1), and plasma steady-state ratios (AUC_{0-tau} Day 14/$AUC_{0-\infty}$ Day 1) were calculated using mixed effects models. In Part C, the relative bioavailability for AUCs and C_{max} between the tablet and capsule formulations were assessed

using mixed effects models and by nonparametric methods for t_{max}.[12]

The percentages of inhibition of MIP-1α and MIP-1β were assessed from the analysis of change from baseline using a $\log_e$ transformation using a mixed effects repeated measures analysis adjusted for baseline concentrations. TE using the difference in $\log_e$-transformed free TE assay for RIPK1 (TEAR) and $\log_e$-transformed total TEAR1 was assessed using a mixed effects repeated measures analysis adjusted for baseline-free and total TEAR1.

3 | RESULTS

3.1 | Demographics

A total of 79 subjects were enrolled in the study (19 in Part A, 48 in Part B, and 12 in Part C). Subject demographics are described in Table 1. In Part A, 4 of the 19 randomized subjects withdrew from the study, 3 of whom were replaced by other subjects during subsequent crossover periods. Of the 4 subjects who withdrew, 1 subject in Cohort 1 withdrew after Period 1 due to investigator discretion. Three subjects in Cohort 2 withdrew due to investigator discretion (Period 1), diagnosis of herpes zoster 27 days after placebo treatment and 42 days after the last dose of GSK2982772 (80 mg) (Period 2) and personal reasons (Period 3), respectively. There were no withdrawals among the 48 subjects in Part B, and only 1 of 12 subjects withdrew from Part C due to investigator discretion.

3.2 | Adverse events

In Part A, 14 of 19 subjects reported AEs. The most commonly reported AEs were contact dermatitis (n = 5) followed by headache (n = 3), nasopharyngitis (n = 2), and abnormal dreams (n = 2). All AEs were of mild intensity except for neck pain and fatigue in 1 subject each, which were of moderate intensity. AEs were not dose dependent (data not shown). The overall relative risk ratio for contact dermatitis and headache was approximately 2, which shows that GSK2982772 treatment increases the risk of this event when compared with placebo, although both CIs contained 1.

In Part B, the most common AEs were contact dermatitis and headache (Table 2). No dose-dependent effect was observed. Most AEs were of mild intensity; AEs of moderate intensity included headache in 2 subjects (1 treated with placebo and 1 with GSK2982772 60 mg BID), nasopharyngitis in 1 subject (GSK2982772 120 mg BID), a ligament sprain in 1 subject (GSK2982772 60 mg QD), depressed mood in 1 subject (GSK2982772 60 mg BID), and an upper respiratory tract infection in 1 subject (GSK2982772 20 mg QD). The overall relative risk ratio for the events when compared with placebo was 1 for nausea and contact dermatitis, and <1 for rash, nasopharyngitis, medical device site reaction, headache, and fatigue.

A total of 3 drug-related AEs, all of mild intensity, were reported in 2 subjects in Part A. One subject experienced a herpes zoster infection of mild intensity that led to the subject's withdrawal from the study. This occurred in a 36-year-old male, 27 days after placebo treatment and 42 days after last dose of GSK2982772 (80 mg). The rash affected a single dermatome (T11) and did not cross the midline. After treatment with acyclovir, the event resolved. Another subject experienced headache and lethargy.

In Part B, 4 subjects experienced drug-related AEs in the GSK2982772 60-mg BID group, and 3 subjects experienced drug-related AEs in both the GSK2982772 20-mg QD group and placebo group, with nausea and headache being the most frequent drug-related AEs. All drug-related AEs in Part B were of mild intensity except for an upper respiratory tract infection reported in 1 subject in the GSK2982772 20-mg QD group, which was of moderate intensity.

No serious AEs, suicidality or deaths were reported during any part of the study. Furthermore, no AEs involving clinical laboratory

TABLE 1 Demographics and baseline characteristics

	Part A	Part B				
Demographics	**Total (N = 19)**	**Placebo (n = 12)**	**20 mg QD (n = 9)**	**60 mg QD (n = 9)**	**60 mg BID (n = 9)**	**120 mg BID (n = 9)**
Age (y), mean (SD)	42.6 (8.02)	41.6 (10.51)	36.4 (13.03)	44.3 (11.61)	33.1 (12.58)	41.6 (8.09)
Males, n (%)	19 (100)	12 (100)	9 (100)	9 (100)	9 (100)	9 (100)
BMI (kg/m^2), mean (SD)	24.96 (1.97)	26.48 (2.98)	25.16 (3.50)	24.18 (2.19)	24.69 (2.84)	26.09 (2.55)
Height (cm), mean (SD)	177.5 (5.96)	178.1 (7.82)	180.8 (6.42)	177.0 (6.18)	177.0 (9.18)	176.9 (9.55)
Weight (kg), mean (SD)	78.89 (9.38)	83.95 (10.72)	81.78 (8.45)	75.92 (9.58)	78.10 (15.49)	81.97 (13.13)
Ethnicity, n (%)						
Hispanic or Latino	0	0	0	1 (11)	0	0
Non-Hispanic or Latino	19 (100)	12 (100)	9 (100)	8 (89)	9 (100)	9 (100)
Race, n (%)						
Asian	1 (5)	0	0	2 (22)	0	0
White	17 (89)	11 (92)	7 (78)	6 (67)	9 (100)	9 (100)
African American	1 (5)	1 (8)	2 (22)	1 (11)	0	0

BID, twice daily; QD, once daily.SD, standard deviation.

TABLE 2 Part B adverse events occurring in ≥2 subjects[a]

Adverse events, n (%)	Part B				
	20 mg QD (*n* = 9)	60 mg QD (*n* = 9)	60 mg BID (*n* = 9)	120 mg BID (*n* = 9)	Placebo (*n* = 12)
Subjects with any AE	7 (78)	6 (67)	9 (100)	6 (67)	10 (83)
Contact dermatitis	1 (11)	3 (33)	5 (56)	0	3 (25)
Headache	4 (44)	0	3 (33)	1 (11)	3 (25)
Nasopharyngitis	0	0	1 (11)	3 (33)	2 (17)
Rash	0	3 (33)	1 (11)	0	2 (17)
Catheter-site erythema	0	1 (11)	2 (22)	0	0
Application-site erythema	0	0	0	2 (22)	0

AE, adverse event; BID, twice daily; QD, once daily.
[a]Not all adverse events are listed.

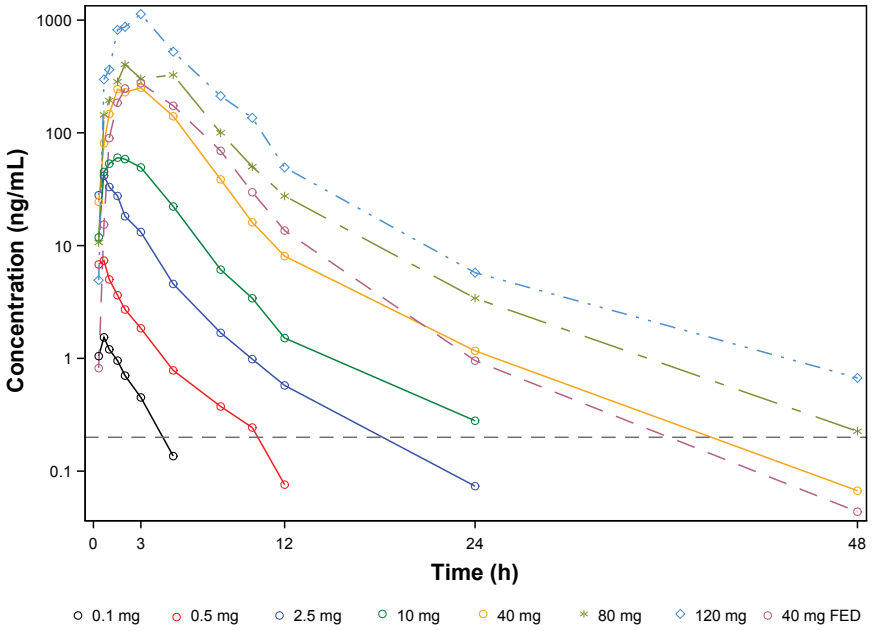

FIGURE 2 Part A. Mean blood GSK2982772 concentration-time plots by treatment, semi-logarithmic scale. The dotted line represents lower limit of quantitation of 0.2 ng/mL

TABLE 3 Summary of derived plasma GSK2982772 pharmacokinetic parameters after repeated oral administrations of GSK2982772 (geometric mean (% CV))

PK parameters (units)	GSK2982772 dose regimen							
	20 mg QD		60 mg QD		60 mg BID		120 mg BID	
Visit	Day 1	Day 14	Day 1	Day 14	Day 1	Day 14	Day 1	Day 14
C_{max}, ng/mL	281 (36.1)	300 (31.2)	–	646 (22.3)	1086 (32.9)	845 (21.5)	1715 (16.7)	1437 (21.1)
$AUC_{0-\infty}$, h·ng/mL	1138 (27.6)	1149 (25.1)	–	2605 (18.9)	4420 (21.9)	3173 (28.9)	6795 (23.2)	7224 (17.7)
AUC_{0-tau}, h·ng/mL	1164 (27.1)	1144 (25.1)	–	2591 (18.8)	4328 (21.9)	3099 (26.4)	7053 (23.5)	6994 (18.9)
t_{max}[2], h (median and range)	2.00 (1.50, 3.01)	1.50 (0.99, 5.02)	–	2.00 (1.00, 3.00)	2.00 (1.50, 3.03)	1.50 (1.00, 3.02)	2.00 (1.00, 3.02)	2.00 (1.50, 5.00)
$t_{½}$, h	3.84 (13.6)	3.79 (11.3)	–	3.97 (17.5)	1.74 (14.4)	2.19 (22.4)	2.13 (19.8)	2.40 (15.1)

$AUC_{0-\infty}$, area under the blood/plasma concentration-time curve from time zero to infinite time; AUC_{0-tau}, area under the blood/plasma concentration-time curve from time zero to the time of the last quantifiable concentration; BID, twice daily; C_{max}, maximum blood/plasma concentration; CV, coefficient of variance; PK, pharmacokinetics; QD, once daily; $t_{½}$, terminal elimination half-life; t_{max}, time to reach C_{max}.

abnormalities or electrocardiogram abnormalities were reported during the study. No clinically significant Holter abnormality was reported.

There were no clinically significant abnormal findings identified through vital signs or during physical examinations, neurological examinations, or respiratory assessments.

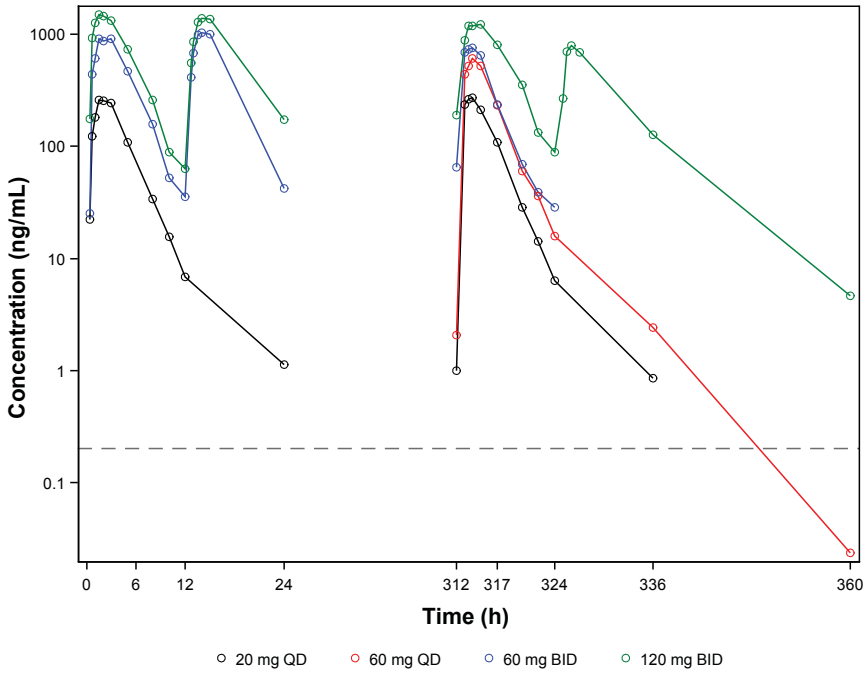

FIGURE 3 Part B. Mean plasma GSK2982772 concentration-time plots by treatment. The dotted line represents lower limit of quantitation of 0.2 ng/mL

3.3 | Pharmacokinetics

Issues were identified with the water dilution method process (pipettes were not calibrated) for the whole blood samples. Thus, the accuracy of the reported whole blood concentrations was deemed unreliable. Hence, PK parameters collected during Part A were summarized descriptively without any statistical analysis, and blood to plasma ratios in Part B were not calculated.

3.3.1 | Part A

Following single-dose administration of GSK2982772 (0.1, 0.5, and 2.5 mg) in solution, median time to C_{max} was achieved within 1 hour postdose. The time to C_{max} was higher following administration of GSK2982772 in a capsule, and increased with increasing dose (1.5-2.5 hours postdose for 10 mg and 120 mg, respectively).

Under fed conditions, there was a slight lag in time to peak concentration of GSK2982772 resulting in a median t_{max} of 3 hours compared with a median t_{max} of 2 hours in the fasted state. Based on the $AUC_{0-\infty}$ values, the overall extent of absorption appeared to be similar in the fed and fasted state.

After attainment of C_{max}, blood concentrations declined rapidly until approximately 12 hours postdose with a $t_{½}$ of approximately 2-3 hours followed by a slower terminal phase $t_{½}$ of approximately 5-6 hours (Figure 2). The majority of systemic exposure was associated with the shorter half-life of 2-3 hours.

3.3.2 | Part B

For Part B, the PK parameters were derived using GSK2982772 plasma concentrations instead of whole blood because of the issues identified with the blood sample preparation in Part A. Table 3 shows the PK parameters for the increasing doses of GSK2982772. No PK parameters were estimated for subjects receiving a 60-mg QD dose on Day 1 due to coagulation in the samples. Increases in C_{max} and AUC were observed with increasing doses of GSK2982772. No dose-dependent changes were observed for T_{max} or $t_{½}$.

The GSK2982772 plasma concentration-time profiles were similar on Day 1 and Day 14 of dosing (Figure 3). The accumulation ratio was close to unity indicating no accumulation upon repeat dosing (Table 4). Slopes estimated from log_e-transformed plasma predose concentration from Days 2 to 14 showed values near zero, reflecting trough concentration having reached a plateau by Day 2.

3.3.3 | Part C

For Part C, both the rate and extent of absorption were similar for the tablet and capsule formulations, based on the point estimates of the relative bioavailability ratios being close to 1 and the 90%

TABLE 4 Summary of statistical analysis estimating accumulation ratio

	Adjusted geometric Mean $AUC_{(0\text{-}tau)}$ (h·ng/mL)		Accumulation ratio $AUC_{(0\text{-}tau)}$ Day 14/$AUC_{(0\text{-}tau)}$ Day 1	
Visit	Day 14	Day 1	Estimate	90% CI
20 mg QD	1144	1164	0.98	(0.88, 1.09)
60 mg QD	2591	NE	NE	NE
60 mg BID	3099	4328	0.72	(0.63, 0.81)
120 mg BID	6994	7053	0.99	(0.87, 1.14)

AUC, area under the concentration-time curve from time zero to the time of the last quantifiable concentration; BID, twice daily; CI, confidence interval; NE, not evaluated; QD, once daily.

confidence intervals being within the 0.80-1.25 criterion associated with bioequivalence.

3.4 | Metabolites in blood, urine, and bile

Unchanged GSK2982772 and a metabolite formed by *N*-glucuronidation were the major circulating drug-related components observed in blood and plasma extracts after both single and repeat oral dosing. In addition, a minor desmethyl metabolite was also present in plasma. The *N*-glucuronide metabolite was also identified in urine along with trace levels of unchanged GSK2982772. Similarly, GSK2982772 (possibly originating from unabsorbed dose), the *N*-glucuronide metabolite, and a second isomeric *N*-glucuronide metabolite were identified in extracts of duodenal bile collected at 6.5 hours on Day 7 after repeat 60-mg BID dosing.

3.5 | Pharmacodynamics

3.5.1 | Target engagement

In Part A, there was an increase in percent TE across the GSK2982772 dose range of 0.1-120 mg with >90% TE being

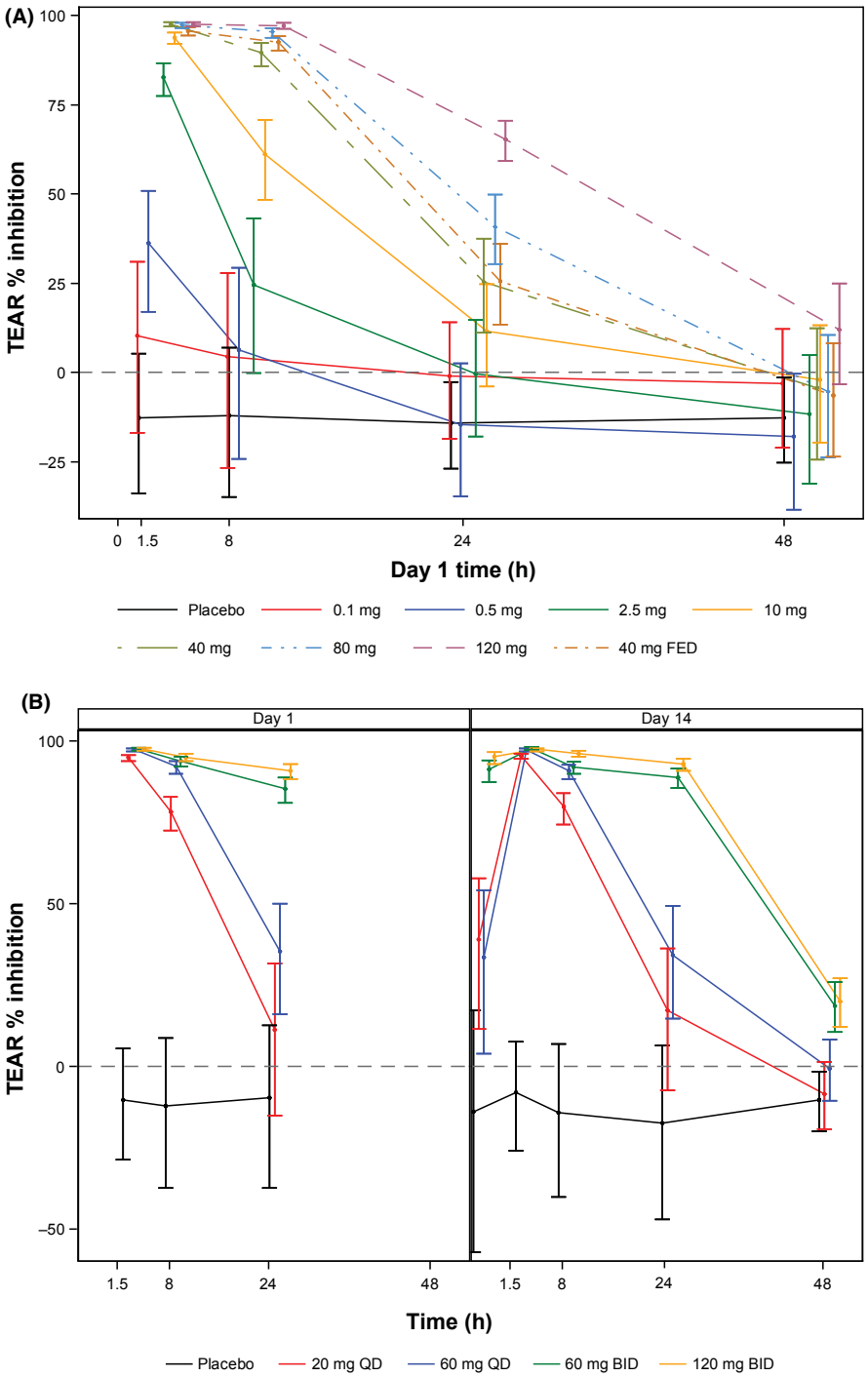

FIGURE 4 Adjusted mean (95% CI) of TEAR1 percent target engagement for Part A (A) and for Part B on Day 1 (B, left panel) and Day 14 (B, right panel)

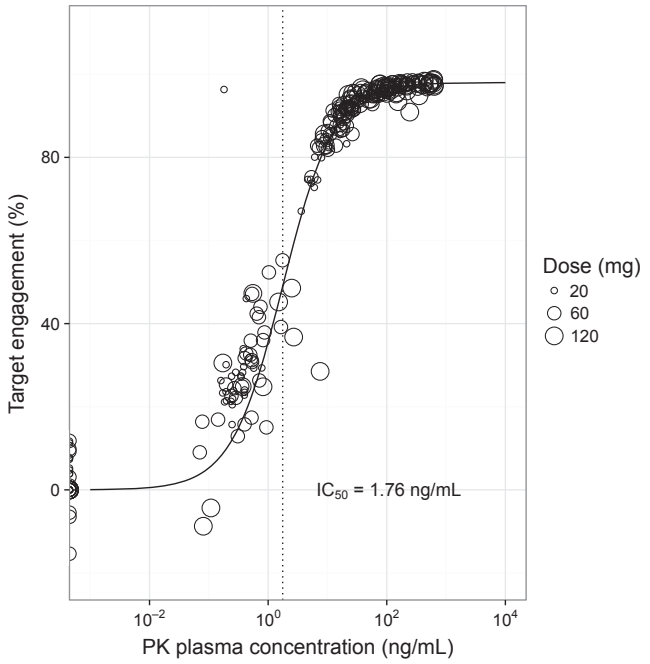

FIGURE 5 Concentration-response plot of TEAR1 target engagement

achieved at 1.5 hours postdose for doses of 10 mg and above (Figure 4A). This level of TE was sustained at 8 hours postdose for doses of 40 mg and above. At 24 hours postdose, the percent TE declined for all dose groups and was negligible by 48 hours postdose. In Part B, on Day 14 of repeat dosing, the mean TE was approximately 95% at 1.5 hours postdose for all GSK2982772 dose regimens (20 and 60 mg QD and 60 and 120 mg BID) (Figure 4B, right panel). The BID dosing regimens maintained approximately 90% TE over 24 hours. There was a concentration-dependent relationship between GSK2982772 and TE with an estimated half maximal inhibitory concentration (IC_{50}) of 1.76 ng/mL (Figure 5).

3.5.2 | Pathway engagement—*MIP-1α* and *MIP-1β*

In Part A, a dose-dependent percent inhibition of MIP-1α and MIP-1β production was observed, which paralleled the TE response. On Day 14 of Part B, maximal inhibition (mean of MIP-1α and MIP-1β) was observed at 1.5 hours and 8 hours postdose for all treatment groups. For doses of 60 mg and 120 mg BID, maximal inhibition was observed out to 24 hours posttreatment. The MIP-1α vs time profile is presented in Figure 6. There was a concentration-dependent relationship between GSK2982772 and MIP-1α with an estimated IC_{50} of 1.36 ng/mL (Figure 7). IC_{50} for MIP-1β inhibition was similar to that for MIP-1α (data not shown).

3.5.3 | 4β-Hydroxycholesterol biomarker

No increase in the mean plasma 4β-hydroxycholesterol to cholesterol ratio was detected following repeated dose administration of GSK2982772 at all dosage levels, indicating that there was no evidence of CYP3A4 induction by GSK2982772 following 14 days of dosing.

4 | DISCUSSION

The majority of the AEs reported were mild in intensity in this first-in-human study of GSK2982772 dosages ranging from a total dose of 0.1-120 mg given QD or BID per day. Neither the frequency nor the intensity of AEs demonstrates a relationship to increasing dose. The most common AEs were contact dermatitis and headache, which

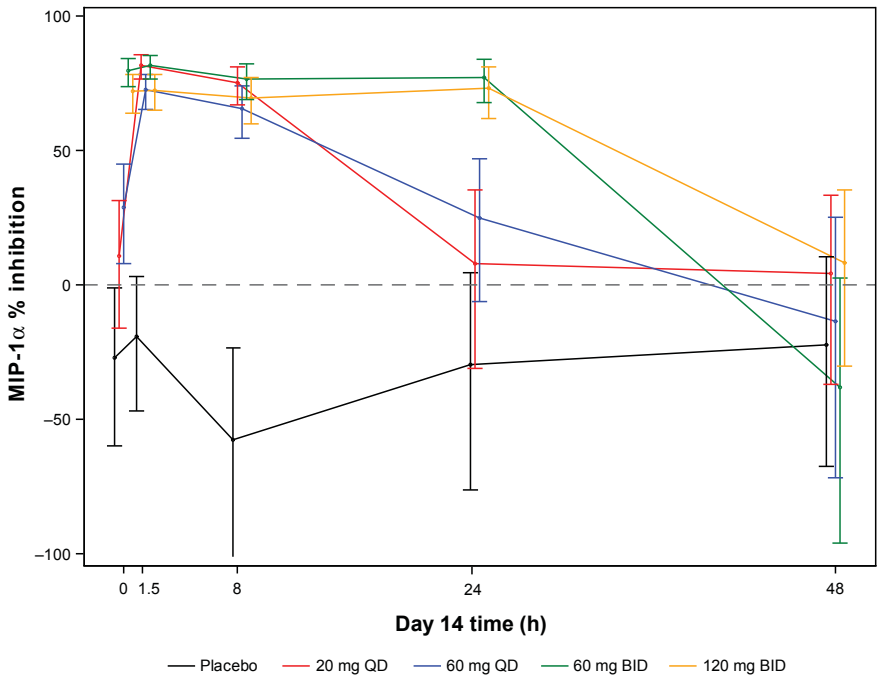

FIGURE 6 Adjusted mean (95% CI) of MIP-1α % inhibition on Day 14

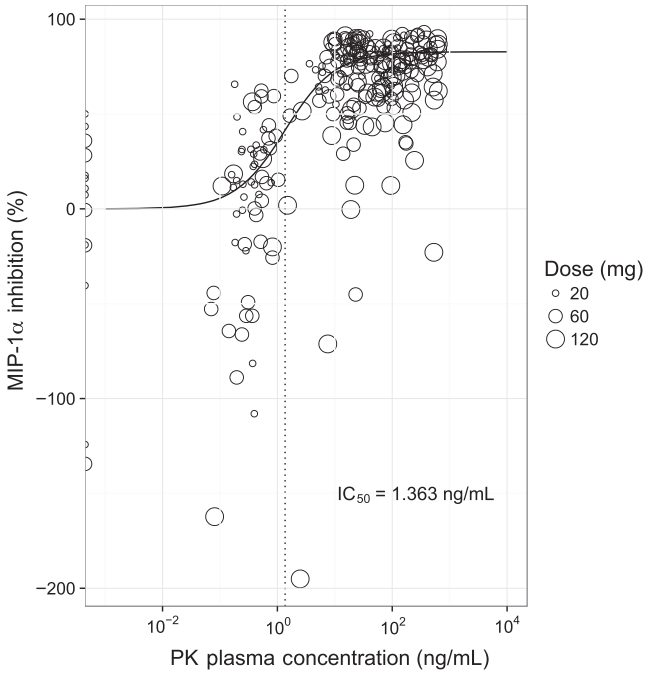

FIGURE 7 Concentration-response plot of MIP-1α target engagement

were reported both in subjects receiving placebo and those receiving active therapy. However, the overall relative risk ratio for contact dermatitis and headache showed that this event is more likely to occur on administration of GSK2982772 as compared with placebo. No SAEs or deaths occurred.

The PK assessments after single dosing showed that after attaining C_{max}, GSK2982772 concentrations in plasma/blood declined rapidly until approximately 12 hours postdose, with a $t_{½}$ of approximately 2-3 hours. This was followed by a slower terminal phase half-life of approximately 6 hours. The majority of the systemic exposure to GSK2982772 was observed within the first 12 hours after administration. GSK2982772 displayed approximately linear kinetics over the dose range of 0.1-120 mg, and food had no significant impact on the PK of GSK2982772, aside from a slight delay in the rate of absorption.

A BID dosing regimen and a QD dosing regimen were evaluated in Part B of the study. Following repeat dosing of GSK2982772, there was no evidence of drug accumulation for either the QD or BID dosing regimens.

GSK2982772 inhibited RIPK1 activity in blood, in a concentration-dependent manner, as measured by the inhibition of MIP-1α and MIP-1β production in an ex vivo challenge assay.[8] The level of GSK2982772 binding to RIPK1 in blood, measured by the TE assay, exhibited a similar concentration-dependent profile. The concentration of GSK2982772 needed to elicit a half-maximal response in all assays was similar (approximately 1.4 ng/mL). For both the QD and BID dosing regimens, the mean RIPK1 TE was ≥95% at 1.5 hours postdose. The 60-mg and 120-mg BID dosing regimens achieved approximately 90% TE over 24 hours, whereas TE fell to below 40% by 24 hours postdose for the QD dosing regimen. A limitation of the study included a blood dilution error in Part A of this study, which precluded statistical analyses of the single-dose PK parameters.

Currently, there are 3 ongoing clinical trials to assess the safety, tolerability, and clinical efficacy of GSK2982772 in patients with inflammatory disorders. These include Phase II trials enrolling patients with moderate to severe rheumatoid arthritis (NCT02858492), ulcerative colitis (NCT02903966), or plaque-type psoriasis (NCT02776033).

5 | CONCLUSIONS

Single and repeat doses of GSK2982772 were generally safe and well tolerated in healthy adult male volunteers. The favorable results of this first-in-human study support further development of the drug in a Phase II trial.

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DISCLOSURE

T. Sahota and J.G. Wang were employees of GlaxoSmithKline during study conduct and reporting. T. Sahota is currently as employee of AztraZeneca, Cambridge, UK, and J.G. Wang is currently an employee of Novartis Pharma, East Hanover, NJ, USA.

AUTHOR CONTRIBUTIONS

K.W., T.R., K.P., N.S., L.R.P., and S.M. contributed to the conception and design of the study, acquisition of the data, data analysis, and data interpretation. M.L.W. and B.V. contributed to the acquisition of the data, and data analysis and interpretation. J.B. and T.S. contributed to conception and study design, and data analysis and interpretation. A.W. contributed to the conception and study design and acquisition of the data. M.R., P.G., G.W., and P.H. contributed to the conception and design of the study. D.T., M.S., and A.V. contributed to data analysis and interpretation. J.L. and J.F contributed to the acquisition of the data.

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