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ARTICLE

A phase I randomized study to evaluate safety, pharmacokinetics, and pharmacodynamics of SIR2446M, a selective RIPK1 inhibitor, in healthy participants

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Abstract

Activation of receptor-interacting protein kinase 1 (RIPK1), a broadly expressed serine/threonine protein kinase, by pro-inflammatory cytokines and pathogens can result in apoptosis, necroptosis, or inflammation. RIPK1 inhibition has been shown to reduce inflammation and cell damage in preclinical studies and may have therapeutic potential for degenerative and inflammatory diseases. SIR2446 is a potent and selective novel small molecule RIPK1 kinase inhibitor. This phase I, randomized, double-blind, placebo-controlled study in Australia (ACTRN12621001621808) evaluated the safety (primary objective), pharmacokinetics, and pharmacodynamics of single (3–600 mg) and multiple (5–400 mg for 10 days) ascending oral doses of SIR2446M (SIR2446 magnesium salt form) in healthy adults from Nov 24, 2021, until May 01, 2023. All treatment-emergent adverse events (TEAEs) were mild/moderate. The most reported TEAEs were vascular access site pain, headache, and rash morbilliform. SIR2446M plasma half-lives ranged from 11 to 19 h and there were no major deviations from dose proportionality for maximum concentration and area under the curve across doses. Renal excretion of unchanged SIR2446 was minimal. No marked accumulation was observed (mean accumulation ratio, 1.2–1.6) after multiple daily doses. A high-fat meal mildly reduced the exposure but was not considered clinically significant. SIR2446M had a rapid and sustained inhibitory effect on the activity of RIPK1, with an overall 90% target engagement at repeated doses ranging from 30 to 400 mg in peripheral blood mononuclear cells ex vivo stimulated to undergo necroptosis. The favorable safety, pharmacokinetic, and pharmacodynamic profile of SIR2446M in healthy participants supports its further clinical development in patients with degenerative and inflammatory diseases.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Receptor-interacting protein kinase 1 (RIPK1), a broadly expressed serine/threonine protein kinase activated most notably by tumor necrosis factor α signaling, is involved in inflammation, apoptosis, and necroptosis. Inhibition of RIPK1 activity using various inhibitors in animal models has been shown to protect against inflammation and tissue damage. A few RIPK1 inhibitors have been evaluated in patients with neurodegenerative and inflammatory diseases, as well as in healthy participants. Most inhibitors exhibited good tolerability. No efficacy has been reported to date, but many studies are still ongoing.

WHAT QUESTION DID THIS STUDY ADDRESS?

SIR2446 is a potent, selective, and orally administered small molecule RIPK1 inhibitor. This first-in-human phase I study was conducted to evaluate the safety, tolerability, pharmacokinetics (PK), including the effect of food, and pharmacodynamics (PD) of single and multiple doses of SIR2446M (SIR2446 magnesium salt form) compared with placebo in healthy adult participants.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

SIR2446M was well tolerated following single (up to 600 mg) or multiple (up to 400 mg) oral ascending dose administrations, with a favorable PK profile. SIR2446M demonstrated an effective peripheral PD effect with an overall 90% target engagement achieved at repeated 30 mg to 400 mg dosing throughout the entire treatment period.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

The favorable safety and PK profile and effective target engagement of SIR2446M in healthy adult participants support its further clinical development in patients with degenerative and inflammatory diseases.

INTRODUCTION

Regulated cell death, including apoptosis and necroptosis, plays a central role in the elimination of infected or potentially neoplastic cells, contributing to homeostasis and host defense against infectious or inflammatory diseases, cancer, and other pathologies.[1] Abnormalities in the regulation of cell death pathways have been associated with pathogenesis of a wide range of human diseases.[2] Receptor-interacting protein kinase 1 (RIPK1), a broadly expressed serine/threonine protein kinase, is involved in inflammation, apoptosis, and necroptosis once activated, and has emerged as an important therapeutic target for multiple diseases.[3]

RIPK1 can be activated by pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α or interferon-γ, as well as by pathogens including bacteria and viruses, leading to either cell death or pro-inflammatory cytokine production.[1,3,4] When TNF-α binds to its receptor TNFR1, RIPK1 regulates between the "pro-survival" and kinase-independent nuclear factor kappa B (NF-κB) pathway or the "pro-death" and kinase-dependent apoptosis/necroptosis pathways,[5] as determined by the post-translational modifications of the RIPK1 protein, including ubiquitination and phosphorylation.[6] Ubiquitinated RIPK1 leads to the activation of NF-κB pathway and induction of pro-inflammatory cytokines.[2] Un-ubiquitinated RIPK1 can dissociate from TNFR1 and interact with caspase-8 in the cytoplasm to mediate apoptosis, or autophosphorylated RIPK1 can bind RIPK3, which phosphorylates mixed lineage kinase domain-like (MLKL) and results in necroptosis.[2]

The kinase activity of RIPK1 is involved in regulating cell death.[2,5] Inhibition of RIPK1 activity using various inhibitors in animal models has been shown to protect against inflammation and cell damage,[7] prevent TNF-α-induced systemic inflammatory response syndrome (SIRS)[8] and male reproductive system impairment,[9] and alleviate the progression of Alzheimer's disease,[10] amyotrophic lateral sclerosis,[11] chemotherapy-associated acute kidney injury[12] or ischemia reperfusion-induced organ injury including acute kidney injury,[13] and

multiple sclerosis.[14] These preclinical studies suggest that RIPK1 inhibitors may provide a potential new approach for the treatment of neurodegenerative and systemic inflammatory diseases. A few RIPK1 inhibitors have been evaluated in patients with Alzheimer's disease or amyotrophic lateral sclerosis,[15] psoriasis,[16] rheumatoid arthritis,[17] ulcerative colitis,[18] and COVID-19,[19] as well as in healthy participants.[20,21] Most inhibitors were well tolerated.[16–20] To date, no efficacy has been reported for these RIPK1 inhibitors,[15–19] but many studies are still ongoing.

SIR2446 is a potent, selective, and oral small molecule RIPK1 inhibitor under development for the treatment of degenerative and inflammatory diseases. Its inhibitory activity of RIPK1 kinase has been demonstrated in in vitro activity and cell necroptosis assay. In a SIRS mice model, SIR2446 efficiently reduced TNF-α–induced hypothermia and cytokine storm in a dose-dependent manner. The safety of SIR2446 has been established in both in vitro systems and animal models (Unpublished data, Sironax Aus Pty Ltd, a Subsidiary of Sironax, Ltd). There were no metabolites of SIR2446 with UV% ≥10% in human hepatocytes or liver microsomes and compared with the tox animal species tested, no unique metabolites were observed in human hepatocytes or liver microsomes, which is supporting its further development in humans. The objectives of this first-in-human study were to evaluate the safety, tolerability, pharmacokinetics (PK), including food effect, and pharmacodynamics (PD) of single and multiple doses of SIR2446M (SIR2446 magnesium salt form) compared with placebo in healthy participants.

MATERIALS AND METHODS

Study design

This phase I, randomized, double-blind, placebo-controlled, sequential cohort study was conducted at a single clinical research unit (CRU) in Australia (ACTRN12621001621808). The study included two parts, single ascending dose (SAD) with evaluation of food effect (Part 1) and multiple ascending dose (MAD) involving 10 days of administration (Part 2) (Figure 1). The Safety Review Committee made the decision of dose escalation and/or the adjustment of planned doses or PK timepoints based on the review of safety, tolerability, and PK (when possible) data from the previous dose cohort.

Part 1 included 7 SAD cohorts. Within each cohort, participants were randomized to receive a single oral dose of SIR2446M capsule (6 participants per cohort), at 3, 10, 30, 100, 200, 400, or 600 mg, or a matching placebo (two participants per cohort) after an overnight/~8-h fast (fasted condition). This range of dose regimen was selected based on the minimal anticipated biological effective level and the human equivalent dose of the no-observed-adverse-effect level in preclinical studies (Appendix S1). Part 1 also included a separate cohort (eight participants) for the

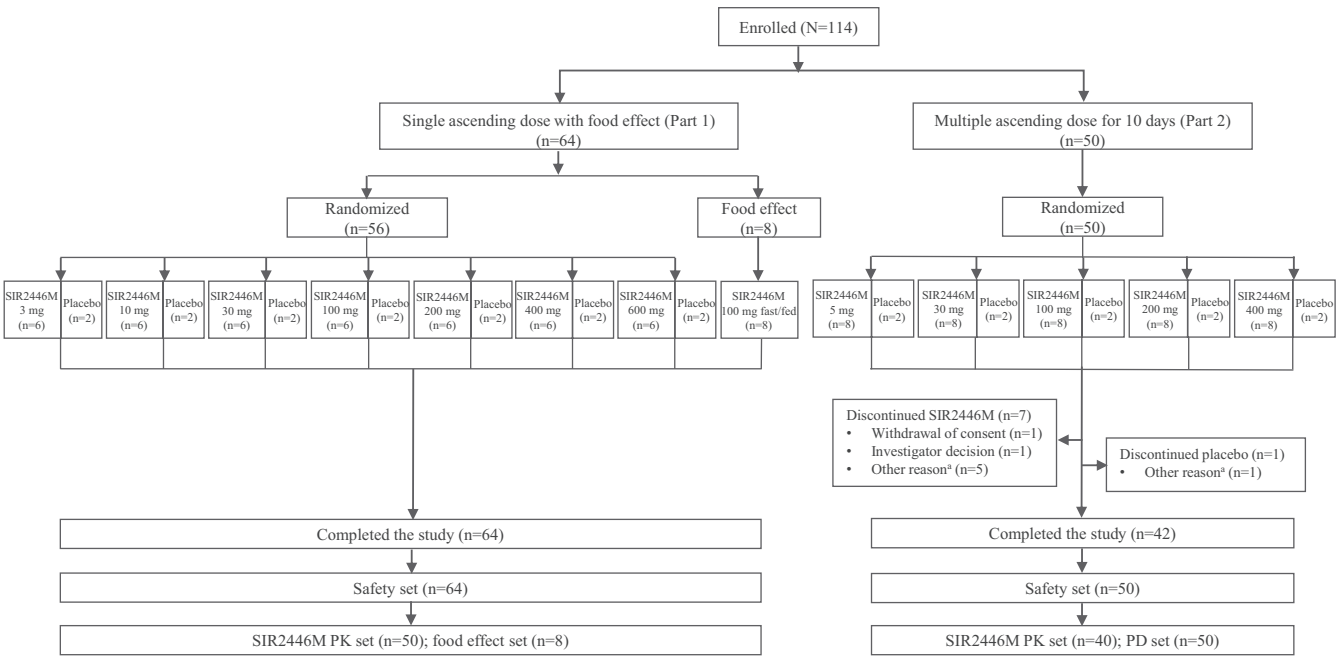

FIGURE 1 Participant disposition. [a]Due to the high frequency of treatment-related rash in participants who had completed dosing in the SIR2446M 400 mg cohort, the sponsor decided that although the stopping criteria in the protocol had not been met, no further dosing was needed for participants who did not complete dosing in the SIR2446M 400 mg cohort and the corresponding placebo cohort.

evaluation of food effect on SIR2446M, which was conducted after the completion of the 200 mg SAD cohort. On day 1, each participant received a single 100-mg SIR2446M tablet under fasted condition; following a 7-day washout period, and on day 8, each participant received another single 100-mg SIR2446M tablet 30 min after the start of a high-fat breakfast (fed condition).

Part 2 included 5 MAD cohorts. Originally, three doses were planned: 30 mg tablet, 200 mg tablet, and 400 mg tablet. Based on Part 1 data, two additional doses of 100 mg tablet and 5 mg capsule were added. Within each cohort, participants were randomized to receive SIR2446M (8 participants per cohort) or a matching placebo (two participants per cohort). Participants received oral doses of SIR2446M or placebo once-daily under fasted condition for 10 days.

Study treatments included two formulations: capsules and tablets. Capsule formulation has enhanced flexibility and was selected for SAD cohorts. Tablet formulation was employed in MAD and food effect cohorts given its potential utility in subsequent clinical development. The inclusion of the lowest MAD cohort of 5 mg was not initially planned and a capsule formulation had to be used because 5 mg tablet was unavailable. Additional methods on dosing and randomization and masking procedures are described in Appendices S1 and S2.

This study was approved by the Bellberry Human Research Ethics Committee and conducted in compliance with the Good Clinical Practice guidelines of the International Council for Harmonization, the Declaration of Helsinki, and applicable local regulations. All participants provided written informed consent before any study procedures were conducted.

Participants

Eligible participants were male or female healthy participants between 18 and 55 years old, body mass index (BMI) of 18–30 kg/m^2, and body weight ≥50 kg. The health of participants was determined by the investigator or delegate based on medical history, full physical examination, clinical laboratory tests, 12-lead electrocardiogram (ECG), and vital signs. Full inclusion and exclusion criteria are listed in Appendices S3 and S4.

Study objectives and assessments

The primary objective was to evaluate the safety and tolerability of SIR2446M after single or multiple escalating doses in healthy participants. The secondary objectives included the characterization of the PK profile of SIR2446M after single or multiple escalating doses under fasted conditions, evaluation of the effect of food on the PK profile of SIR2446M after a single dose under fasted and then fed conditions, and characterization of the PD profile of SIR2446M after multiple escalating doses under fasted condition.

Safety and tolerability were determined by monitoring treatment-emergent adverse events (TEAE), clinical laboratory values, vital signs, physical examinations, and ECG findings throughout the study. Plasma PK parameters included maximum observed plasma concentration (C_{max}), time to reach C_{max} (T_{max}), area under the drug concentration–time curve (AUC), and terminal elimination half-life ($t_{1/2}$). Urine PK parameters are described in Appendix S5. The concentration of SIR2446 was measured using a validated liquid chromatography–tandem mass spectrometry method (Pharmaron [Chengdu] Clinical Services Co. Ltd., Beijing Branch, China) with an analytical range of 2.0–2000 ng/mL for plasma and 1.0–500 ng/mL for urine (Appendix S6). Reduction of phosphorylated RIPK1 (p-RIPK1) and phosphorylated MLKL (p-MLKL) protein levels in peripheral blood mononuclear cells (PBMC) was used as PD markers for inhibition of RIPK1 kinase activity. The PBMC samples were stimulated ex vivo to undergo necroptosis. The levels of p-RIPK1 and p-MLKL were measured by electrochemiluminescence assays on the Meso Scale Discovery (MSD) platform (Pharmaron Clinical Research Service Co. Ltd., China) (Appendix S6).

Statistical analysis

This was a first-in-human phase I safety study and no formal sample size calculations were performed. The sample size was determined empirically as described in Appendix S7.

Safety was assessed in the safety set, including all enrolled participants who received at least one dose of SIR2446M or placebo. The PK profile of SIR2446M was assessed in the PK set, including participants who received at least one dose of SIR2446M and had at least one evaluable PK parameter. The effect of food on the PK profile of SIR2446M was assessed in the food effect set, defined as the subset of participants in the PK set who had at least one primary PK parameter (AUC or C_{max}) during both fasted and fed treatment periods, consumed at least 90% of the meal during the fed period, and had a day-8 predose plasma SIR2446M concentration <5% of C_{max}. The PD profile of SIR2446M was assessed in the PD set, including participants who received at least one dose of SIR2446M or placebo in Part 2 MAD, had at least one evaluable PD result postdose, and had successful ex vivo stimulation to undergo necroptosis at baseline.

TEAEs, PK, and PD parameters were summarized using descriptive statistics by study part and treatment group. Results for the placebo were pooled from all placebo cohorts in Part 1 or Part 2 respectively. TEAEs were coded according to the Medical Dictionary for Regulatory Activities version 24.1. PK parameters were estimated by standard noncompartmental analysis using Phoenix™ WinNonlin® Version 8.3 (Pharsight Corporation, USA). The dose proportionality of SIR2446M was evaluated using a power model, the food effect was analyzed using a mixed effect model, and the relative bioavailability analysis of SIR2446M capsule versus tablet formulation was analyzed using an analysis of variance model (Appendix S8). All statistical analyses were performed using SAS software Version 9.4 (SAS Institute, USA).

RESULTS

Participants

The study was initiated on Nov 24, 2021, and completed on May 01, 2023. A total of 114 participants, 64 in Part 1 and 50 in Part 2, were enrolled and received study treatment (Figure 1). All 114 participants were included in the safety set. Among these, 90 participants were treated with SIR2446M and were included in the PK set, including eight participants in the food effect set, and 50 participants in Part 2 MAD were included in the PD set. All participants in Part 1 completed the study. Forty-two participants in Part 2 completed the study and eight participants discontinued study treatment early for reasons of withdrawal of consent by 1 participant treated with SIR2446M 100 mg, investigator decision for one participant treated with SIR2446M 30 mg, and sponsor decision for five participants treated with SIR2446M 400 mg and one participant with the corresponding placebo (Figure 1). The sponsor's decision for the six participants in the 400 mg cohort was due to a high frequency of rash morbilliform (described below) reported in participants who had completed the dosing. Although the rash was mild and the stopping criteria in the protocol (Appendix S1) had not been met, the sponsor decided that no further dosing was required for the subsequent six participants in this dosing cohort.

SIR2446M and placebo treatment groups were generally balanced at baseline (Table S1). Across all Part 1 and Part 2 dosing cohorts, participants ranged in age from 23.7 to 36.8 years and had a BMI range of 23.1–26.2 kg/m^2. Slightly more men than women were enrolled and most of the participants were white and non-Hispanic (Table S1).

Safety

All participants were dosed as planned in Part 1. The mean (standard deviation) duration of exposure to SIR2446M in Part 2 was 10.0 (0) days for the 5 mg cohort, 9.3 (2.1) days for the 30 mg cohort, 9.5 (1.4) days for the 100 mg cohort, 10.0 (0) days for the 200 mg cohort, and 4.4 (4.7) days for the 400 mg cohort.

In Part 1, 21 (42.0%) participants reported 34 TEAEs in the SIR2446M-treated groups, and 6 (42.9%) participants reported 6 TEAEs in the placebo-treated groups (Table 1, Table S2). The most reported TEAEs (in >4 participants overall) were vascular access site pain, headache, and medical device site reaction. The food effect cohort showed no substantial differences in the frequency of TEAEs under fasted versus fed conditions.

In Part 2, 30 (75.0%) participants reported 87 TEAEs in the SIR2446M-treated groups, and five (50.0%) participants reported 15 TEAEs in the placebo-treated groups (Table 1, Table S2). The most frequently reported TEAEs (in >4 participants overall) were headache, vascular access site pain, rash morbilliform, and vascular access site bruising. The incidence of rash morbilliform trended upward with the increasing dose of SIR2446M (Table 1). The skin rash occurred 2–3 days after the last administration of the study drug, was mild or moderate in severity, and resolved within a few days with or without pharmacological intervention.

Overall, SIR2446 was safe and well tolerated in all study parts. All TEAEs were mild or moderate. There were no deaths or serious TEAEs reported in either Part 1 or Part 2, and no participants discontinued treatment or study due to a TEAE (Table 1). There were no clinically significant changes in laboratory measurements, vital signs, ECGs, or physical examinations.

SIR2446M pharmacokinetics

In Part 1 SAD, SIR2446M was rapidly absorbed (median T_{max}, 2.0–3.0 h) following a single oral dose of 3–600 mg of SIR2446M under fasted conditions (Table 2, Figure 2a). The mean $t_{1/2}$ was similar across doses and ranged from 10.7 to 16.2 h. For the food effect cohort treated with 100 mg of SIR2446M (Figure 2b), the median T_{max} was 4.0 h after a high-fat meal, compared with 2.5 h under fasted condition (Table 2). Based on geometric mean ratios, C_{max}, AUC from 0 to the last quantifiable measurement (AUC_{0-last}), and AUC from zero to infinity (AUC_{0-inf}) were decreased by ~26%, 16%, and 15%, respectively, under fed versus fasted conditions (Table S3). These results suggested a very limited food effect for SIR2446M. Renal excretion of unchanged SIR2446 was low, with the mean cumulative

TABLE 1 Adverse events in the safety set.

	Single ascending dose with food effect (Part 1; $n=64$)											Multiple ascending dose (Part 2; $n=50$)						
	SIR2446M											SIR2446M						
					Food effect ($n=8$)													
TEAE, *n* (%) # of event	3 mg ($n=6$)	10 mg ($n=6$)	30 mg ($n=6$)	100 mg ($n=6$)	100 mg fasted	100 mg fed	200 mg ($n=6$)	400 mg ($n=6$)	600 mg ($n=6$)	Pooled SIR2446M ($n=50$)	Pooled placebo ($n=14$)	5 mg ($n=8$)	30 mg ($n=8$)	100 mg ($n=8$)	200 mg ($n=8$)	400 mg ($n=8$)	Pooled SIR2446M ($n=40$)	Pooled placebo ($n=10$)
Any TEAE	1 (16.7) 1	1 (16.7) 1	3 (50.0) 3	1 (16.7) 1	2 (25.0) 3	3 (37.5) 3	5 (83.3) 6	3 (50.0) 11	3 (50.0) 5	21 (42.0) 34	6 (42.9) 6	4 (50.0) 8	7 (87.5) 20	6 (75.0) 25	6 (75.0) 19	7 (87.5) 15	30 (75.0) 87	5 (50.0) 15
Drug-related TEAE	0	0	1 (16.7) 1	0	0	1 (12.5) 1	2 (33.3) 2	2 (33.3) 2	0	6 (12.0) 6	0	0	1 (12.5) 1	4 (50.0) 10	3 (37.5) 4	4 (50.0) 4	12 (30.0) 19	1 (10.0) 1
Serious TEAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TEAE leading to death	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TEAE leading to treatment or study discontinuation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TEAE reported by ≥2 participants in pooled SIR2446M cohort during either Part 1 or Part 2, *n* (%) # of event																		
Vascular access site pain	0	0	0	0	1 (12.5) 1	1 (12.5) 1	1 (16.7) 1	2 (33.3) 3	2 (33.3) 2	7 (14.0) 8	0	0	3 (37.5) 4	3 (37.5) 5	1 (12.5) 2	1 (12.5) 1	8 (20.0) 12	3 (30.0) 4
Headache	0	0	0	1 (16.7) 1	0	1 (12.5) 1	1 (16.7) 1	2 (33.3) 2	0	5 (10.0) 5	0	1 (12.5) 1	3 (37.5) 3	4 (50.0) 8	2 (25.0) 2	1 (12.5) 1	11 (27.5) 15	1 (10.0) 2
Medical device site reaction	0	1 (16.7) 1	1 (16.7) 1	0	0	0	0	1 (16.7) 1	0	3 (6.0) 3	2 (14.3) 2	0	0	0	1 (12.5) 1	1 (12.5) 1	2 (5.0) 2	1 (10.0) 1
Contusion	0	0	0	0	1 (12.5) 1	0	0	1 (16.7) 1	0	2 (4.0) 2	0	0	0	0	0	0	0	0
Rash morbilliform	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (12.5) 1	2 (25.0) 2	3 (37.5) 3	6 (15.0) 6	0
Vascular access site bruising	0	0	0	0	0	0	0	0	0	0	0	1 (12.5) 1	0	0	3 (37.5) 4	1 (12.5) 1	5 (12.5) 6	1 (10.0) 1
Contact dermatitis	0	0	0	0	0	0	0	1 (16.7) 1	0	1 (2.0) 1	0	0	1 (12.5) 1	0	2 (25.0) 2	1 (12.5) 1	4 (10.0) 4	0

TABLE 1 (Continued)

	Single ascending dose with food effect (Part 1; $n=64$)											Multiple ascending dose (Part 2; $n=50$)						
	SIR2446M											SIR2446M						
					Food effect ($n=8$)													
TEAE, *n* (%) # of event	3 mg ($n=6$)	10 mg ($n=6$)	30 mg ($n=6$)	100 mg ($n=6$)	100 mg fasted	100 mg fed	200 mg ($n=6$)	400 mg ($n=6$)	600 mg ($n=6$)	Pooled SIR2446M ($n=50$)	Pooled placebo ($n=14$)	5 mg ($n=8$)	30 mg ($n=8$)	100 mg ($n=8$)	200 mg ($n=8$)	400 mg ($n=8$)	Pooled SIR2446M ($n=40$)	Pooled placebo ($n=10$)
Abdominal pain	0	0	0	0	1 (12.5) 1	0	0	0	0	1 (2.0) 1	0	0	1 (12.5) 1	2 (25.0) 2	0	0	3 (7.5) 3	1 (10.0) 1
Dizziness	1 (16.7) 1	0	0	0	0	0	0	0	0	1 (2.0) 1	0	1 (12.5) 1	2 (25.0) 2	0	0	0	3 (7.5) 3	0
Constipation	0	0	0	0	0	0	1 (16.7) 1	0	0	1 (2.0) 1	0	1 (12.5) 1	0	0	1 (12.5) 1	0	2 (5.0) 2	1 (10.0) 1
Arthralgia	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (12.5) 1	1 (12.5) 1	0	2 (5.0) 2	0
Back pain	0	0	0	0	0	0	0	0	1 (16.7) 1	1 (2.0) 1	1 (7.1) 1	0	0	1 (12.5) 1	1 (12.5) 1	0	2 (5.0) 2	0
Diarrhea	0	0	0	0	0	0	0	0	0	0	1 (7.1) 1	0	1 (12.5) 1	1 (12.5) 1	0	0	2 (5.0) 2	0
Petechiae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2 (25.0) 2	0	2 (5.0) 2	0
Rash	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (12.5) 1	1 (12.5) 1	2 (5.0) 2	0

Abbreviation: TEAE, treatment-emergent adverse event.

TABLE 2 Plasma PK parameters for SIR2446M in the PK set.

Single ascending dose with food effect (Part 1; $n=50$)									
					Food effect				
	3 mg	10 mg	30 mg	100 mg	100 mg fasted	100 mg fed	200 mg	400 mg	600 mg
Parameter	$n=6$	$n=6$	$n=6$	$n=6$	$n=8$	$n=8$	$n=6$	$n=6$	$n=6$
C_{max} (ng/mL)	50.7 (20.9)	193.1 (51.0)	677.8 (25.2)	3143.8 (31.1)	2812.3 (11.7)	2072.7 (41.7)	7414.9 (17.2)	14,488.0 (15.8)	23,172.0 (26.0)
T_{max} (h) median (range)	2.5 (2.0–10.0)	2.5 (1.0–4.0)	3.0 (2.0–4.0)	2.5 (1.0–6.0)	2.5 (1.0–4.0)	4.0 (2.0–8.0)	2.0 (1.0–2.0)	2.5 (1.0–4.0)	2.5 (1.0–4.0)
AUC_{0-24} (h ng/mL)	404.1 (27.7)	1469.2 (41.9)	5651.4 (19.4)	20,991.0 (27.0)	19,140.0 (21.4)	15,434.0 (28.0)	47,745.0 (24.8)	108,830.0 (26.5)	201,840.0 (41.2)
AUC_{0-inf} (h ng/mL)	586.1 (32.8)	1941.4 (46.0)	7172.0 (20.2)	24,513.0 (28.5)	22,492.0 (23.4)	19,215.0 (26.6)	53,971.0 (25.1)	127,660.0 (27.9)	238,420.0 (42.8)
$t_{1/2}$ (h) mean (SD)	16.0 (3.2)	16.2 (3.7)	14.9 (4.6)	12.1 (2.2)	13.4 (5.2)	14.8 (3.2)	10.7 (1.7)	12.4 (4.0)	11.4 (2.1)

Multiple ascending dose (Part 2; $n=40$)					
Day 1					
	5 mg	30 mg	100 mg	200 mg	400 mg
Parameter	$n=8$	$n=8$	$n=8$	$n=8$	$n=8$
C_{max} (ng/mL)	67.1 (22.1)	797.5 (49.5)	3553.2 (33.3)	7165.9 (28.4)	13,232.0 (22.5)
T_{max} (h) median (range)	2.0 (1.0–4.0)	2.5 (1.0–4.0)	2.5 (0.5–4.0)	2.0 (1.0–4.0)	3.0 (2.0–6.0)
$AUC_{0-\tau}$ (h ng/mL)	516.6 (9.6)	4607.5 (38.7)	22,318.0 (43.6)	46,825.0 (35.2)	114,170.0 (35.1)
Day 10					
	5 mg	30 mg	100 mg	200 mg	400 mg
Parameter	$n=8$	$n=7$	$n=7$	$n=8$	$n=3$
C_{max} (ng/mL)	78.0 (31.5)	968.0 (40.6)	4361.0 (21.6)	9073.1 (37.4)	15,534.0 (17.8)
T_{max} (h) median (range)	2.5 (1.0–4.0)	2.0 (1.0–3.0)	1.0 (0.5–3.0)	2.5 (0.5–24)	2.0 (1.0–3.0)

TABLE 2 (Continued)

Day 10						
	5 mg	30 mg	100 mg	200 mg	400 mg	
Parameter	$n = 8$	$n = 7$	$n = 7$	$n = 8$	$n = 3$	
$AUC_{0-\tau}$ (h ng/mL)	748.9 (20.1)	5776.5 (32.0)	31,692.0 (36.1)	74,934.0 (42.5)	101,520.0 (35.7)	
AR_{Cmax}	1.2 (25.2)	1.3 (27.1)	1.2 (36.9)	1.3 (26.8)	1.2 (10.8)	
AR_{AUC}	1.5 (18.6)	1.3 (14.9)	1.4 (20.0)	1.6 (22.3)	1.2 (12.4)	
$t_{1/2}$ (h) mean (SD)	18.8 (6.7)	15.2 (2.8)	14.0 (2.7)	14.3 (2.4)	14.3 (6.2)	

Note: Data are geometric mean (CV%), except where otherwise indicated.

Abbreviations: AR, accumulation ratio; AUC_{0-24}, area under the plasma concentration–time curve from time 0 to 24 h; AUC_{0-inf}, area under the plasma concentration–time curve from time 0 to infinity; $AUC_{0-\tau}$, area under the plasma concentration–time curve over a dosing interval tau (τ); C_{max}, maximum plasma concentration; CV, coefficient of variation; PK, pharmacokinetic; SD, standard deviation; $t_{1/2}$, terminal elimination half-life; T_{max}, time to reach maximum plasma concentration.

fraction excreted unchanged in the urine ranging from 0.6% to 1.0% for 30 to 200 mg SAD cohorts, and the renal clearance was minimal at approximately 0.02 to 0.04 L/h (Table S4), suggesting the urine was not the main route of excretion of SIR2446.

In Part 2, SIR2446M was rapidly absorbed following multiple oral doses of 5–400 mg of SIR2446M for 10 consecutive days of once-daily dosing under fasted conditions (Table 2, Figure 2c,d). Median T_{max} was similar after the first dose on day 1 (2.0–3.0 h) and at steady state on day 10 (1.0–2.5 h). On day 10, the mean $t_{1/2}$ after repeated dosing was approximately 14.0–18.8 h across the 5 MAD cohorts, comparable to $t_{1/2}$ after single doses in Part 1. After 10 days of administration, no marked accumulation of SIR2446 was observed when comparing the systemic exposure (C_{max} and AUC over a dosing interval tau [$AUC_{0-\tau}$]) on day 1 versus day 10, with a mean accumulation ratio (AR) ranging from 1.2 to 1.6.

The dose proportionality of plasma PK parameters (C_{max}, AUC_{0-last}, AUC_{0-inf} [SAD], and $AUC_{0-\tau}$ [MAD]) was evaluated after administration of SIR2446M single (3–600 mg) or multiple escalating doses (5–400 mg) under fasted conditions. Based on the power model, the 90% CIs associated with estimated slopes were slightly above 1, indicating there were no major deviations from dose proportionality across the tested doses (Table S5).

The analysis of the relative bioavailability between the capsule and tablet revealed that the two formulations were not exactly the same as the 90% CIs for the geometric mean ratios of AUCs and C_{max} were slightly outside the acceptable range (Appendix S8). However, the geometric mean ratios of systemic exposure for capsule: tablet were at 85%–123%, indicating only a minor difference (Table S6).

Pharmacodynamics

To evaluate the PD effect of SIR2446M, the levels of p-RIPK1 and its downstream protein p-MLKL were measured after ex vivo stimulated necroptosis in PBMC extracted from the MAD participants at each timepoint and normalized to the predose levels of each participant. At 3 h postdose on day 1, SIR2446M demonstrated a strong inhibitory effect on p-RIPK1 in all dosing cohorts, with 85% mean inhibition observed at 5 mg and ~90% mean inhibition at 30–400 mg. A sustained inhibition of up to 95% was achieved during the rest of the 10-day treatment period for all dosing regimens. Partial to complete loss of inhibition was observed for 5 mg at 48 and 72 h post-last dose on day 10. For placebo treatment, the mean p-RIPK1 reduction was about 20% (Figure 3a). A similar PD effect of SIR2446M was observed when p-MLKL levels were measured. The 5 mg

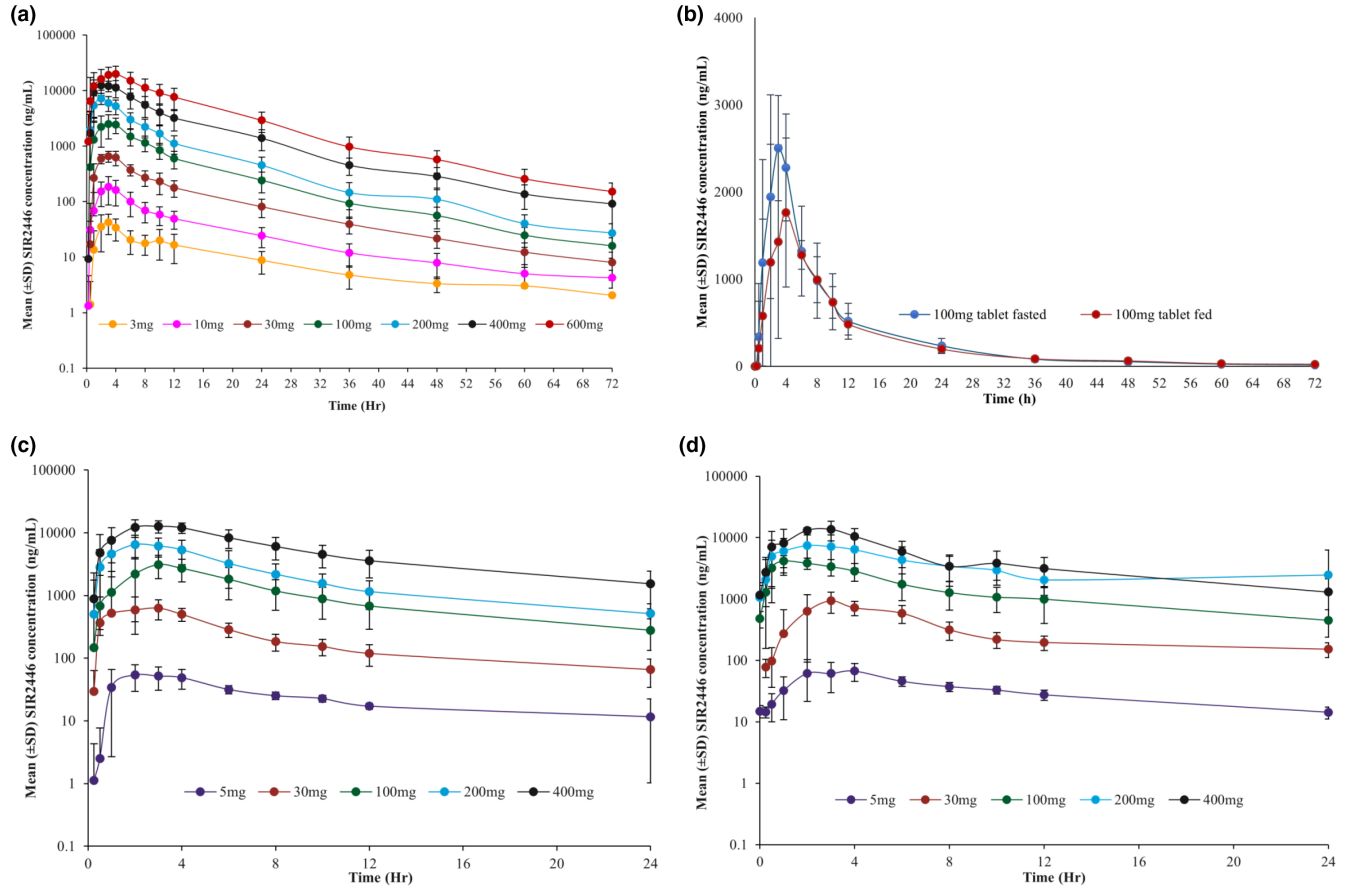

FIGURE 2 Mean (±SD) plasma concentration–time profiles of SIR2446M in the PK set: (a) single ascending dose; (b) food effect; (c) multiple ascending dose day 1; and (d) multiple ascending dose day 10. (a, c, d) represent log-linear scale and (b) represents linear scale. Concentrations below the lower limit of quantification (BLOQ) prior to the first measurable concentration were set to zero. BLOQ concentrations observed after the first measurable concentration were treated as missing. Hr, hour; PK, pharmacokinetic; SD, standard deviation.

cohort showed 80% mean inhibition of p-MLKL at 3 h postdose on day 1, 31%–83% mean inhibition during the rest of the treatment period, and recovery of p-MLKL levels to baseline at 48 and 72 h post-last dose on day 10. The 30–400 mg cohorts demonstrated >95% inhibition of p-MLKL throughout the entire 10-day treatment period, compared with the mean reduction of 2% with placebo treatment (Figure 3b).

DISCUSSION

This first-in-human study demonstrated that SIR2446M was safe and well tolerated following single and multiple oral ascending doses in healthy adult participants. SIR2446M was rapidly absorbed without marked accumulation. Renal excretion of unchanged SIR2446 was minimal. There were no major deviations from dose proportionality for systemic exposure, and a high-fat meal mildly reduced the exposure. SIR2446M demonstrated an effective peripheral PD effect with an overall 90% target engagement achieved at repeated 30–400 mg dosing.

The safety results of this study indicated that inhibition of RIPK1 with SIR2446M was not associated with substantial safety concerns in healthy participants and no new or unexpected safety signals were identified compared with the previously reported RIPK1 inhibitors in similar healthy populations.[15,20–22] All TEAEs in this study were mild or moderate. There were no deaths, serious TEAEs, or clinically significant changes in laboratory measurements or vital signs. The frequency of overall TEAEs was mostly balanced between SIR2446M and placebo treatment groups. The most common TEAEs after a single dose or multiple doses of SIR2446M were vascular access site pain, headache, and rash morbilliform. Most cases of rash morbilliform occurred 1–4 days after the completion of treatment and were considered drug eruptions by a referred dermatologist. They were all mild or moderate and resolved within a few days with or without treatment. The occurrence of rash was more

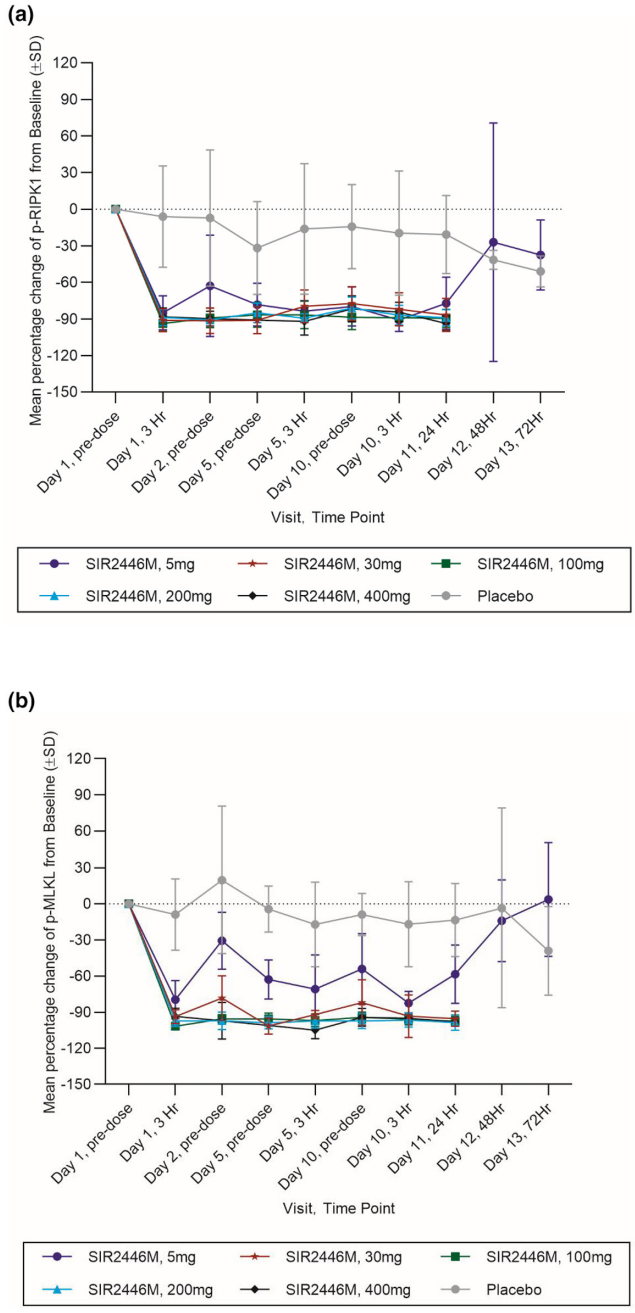

FIGURE 3 Mean (±SD) percentage change from baseline in (a) p-RIPK1 levels and (b) p-MLKL protein levels on a non-linear scale in the multiple ascending dose PD set. Hr, hour; PD, pharmacodynamic; p-MLKL, phosphorylated mixed lineage kinase domain-like; p-RIPK1, phosphorylated-receptor-interacting protein kinase 1; SD, standard deviation.

frequent in higher MAD dose groups, particularly within the 400 mg cohort, and only sporadically reported in lower dose cohorts (none in the 5 and 30 mg cohorts and only one case in the 100 mg cohort). Since lower doses of SIR2446M were sufficient to achieve a high level of target engagement, the higher doses (e.g., 400 mg) were considered beyond potential therapeutic doses. Therefore, skin rash was deemed less clinically significant in the further development of lower doses of SIR2446M, but it will be closely monitored in future studies. Notably, the most frequently reported TEAEs with the previously reported RIPK1 inhibitors GSK2982772[22] and GDC-8264[20] in studies of healthy participants also included headache and skin reaction of contact dermatitis. Other previously tested RIPK1 inhibitors DNL104[21] and DNL747[15] showed significant off-target toxicity, but they appeared to be compound-specific and not on-target effects of RIPK1 inhibition.

SIR2446M exhibited a favorable PK profile. It was rapidly absorbed with a median T_{max} of 1–3 h after single or multiple doses under fasted conditions. The plasma elimination half-lives ranged from 11 to 19 h with no dose effect. A high-fat meal led to a slight delay in absorption and a mild reduction in exposure, but the food effect was unlikely to have significant clinical implications because SIR2446M at low doses of 5–30 mg once-daily for 10 days already had a notable inhibitory effect on the activity of RIPK1 and the impact of fluctuations in drug exposure on the treatment effect of SIR2446M was likely negligible. It was observed that the systemic exposure to SIR2446 increased slightly more than the dose proportionality, as the dose level increased from 3 to 600 mg, a 200-fold increase in dose resulted in about 400-fold increase in AUC. The estimated slopes and the associated 90% CIs were slightly higher than 1. Given the wide range of doses (3–600 mg for SAD and 5–400 mg for MAD), it was concluded that the deviation from dose proportionality was not significant. ARs for $AUC_{0-\tau}$ after multiple doses were limited to 1.2–1.6 across different doses. This accumulation was minimal and consistent with the mean $t_{1/2}$ of 14–19 h after repeated dosing. These favorable PK properties of SIR2446M should predictably facilitate its dosing in future studies. SIR2446M capsules and tablet formulations, though not exactly the same in bioavailability, showed similar exposure levels. Given that the tablet formulation is uniform in weight and drug content and convenient for shipping, dispensing, and dosing, the SIR2446M tablet formulation was considered suitable for further development.

SIR2446M treatment was associated with robust inhibition of RIPK1 activity. In ex vivo stimulated PBMC extracted from participants treated with SIR2446M at 30–400 mg MAD, rapid and almost complete (≥90%) inhibition of p-RIPK1 and p-MLKL was observed as early as 3 h post-dose on day 1 and sustained throughout the 10-day treatment period. The effective 90% target engagement achieved with SIR2446M 30–400 mg confirmed the strong inhibitory effect of SIR2446M. At 5 mg MAD, inhibition of p-RIPK1 and p-MLKL also occurred, though less potent and more variable, and by 48–72 h after the last dose, p-RIPK1 and p-MLKL levels returned to baseline, indicating a reversible

inhibitory effect. Based on preclinical information and evidence from other RIPK1s inhibitors, such as GSK2982772 and DNL-747, it is suggested that high levels (e.g., >90%) of RIPK1 inhibition may be needed to exhibit clinical efficacy.[15–18,22] We intended to maximize the probability of success in future patients' studies by aiming high target engagement. In this study, the compound shows robust inhibition of RIPK1 activity at 30–400 mg MAD. But most of all, real efficacious doses will be determined through future dose–response studies.

This study had limitations typical of first-in-human phase I studies. The study design, including sample size, was determined empirically without formal hypothesis testing. A cohort size of 6–8 participants is considered reasonable to observe common TEAEs associated with the study treatment,[23] but rare safety signals would require a larger sample size to detect in the later stage clinical studies. Slightly more men than women were enrolled, but it may have a minimal impact on study results because with the small cohort size, the imbalance in sex ratio was random across different dose cohorts (e.g., balanced in some and more men or more women in others). In addition, this study included only healthy participants, who are not expected to have an active RIPK1 pathway. The safety profile of SIR2446M in healthy participants may not necessarily be the same as that in participants with active RIPK1 pathway.

In summary, single and repeated doses of SIR2446M were generally safe and well tolerated and exhibited a favorable PK profile and effective target engagement in healthy adult participants. The results from this first-in-human study support further clinical development of SIR2446M for the treatment of patients with degenerative and inflammatory diseases.

AUTHOR CONTRIBUTIONS

All authors wrote the manuscript. A.L.A.S., Y.S., F.X., Y.M., and L.S. designed the research. A.L.A.S., J.D.G., Y.S., F.X., Y.M., and L.S. performed the research. A.L.A.S., Y.S., H.D., F.C., Y.M., and L.S. analyzed the data.

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CONFLICT OF INTEREST STATEMENT

Yang Shen, Huajun Deng, Fenchao Xue, Yongfen Ma, and Linan Song are employees of Sironax, Ltd (Sironax). All other authors declared no competing interests for this work.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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