# Pharmacokinetics, pharmacodynamics and adverse event profile of GSK2256294, a novel soluble epoxide hydrolase inhibitor


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| Key Words: | epoxide hydrolase, clinical trial, COPD, pharmacokinetics, smokers |



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| Abstract: | Aims: Endothelial-derived epoxyeicosatrienoic acids may regulate vascular tone and are metabolised by soluble epoxide hydrolase enzymes (sEH). GSK2256294 is a potent and selective sEH inhibitor that was tested in two Phase I studies.<br>Methods: Single escalating doses of GSK2256294 2-20 mg or placebo were administered in a randomized crossover design to healthy male subjects or obese smokers; once daily doses of 6 or 18 mg or placebo were administered for 14 days to obese smokers. Data were collected on safety, pharmacokinetics, sEH enzyme inhibition and blood biomarkers. Single doses of GSK2256294 10 mg were also administered to healthy younger males or healthy elderly males and females with and without food. Data on safety, pharmacokinetics and biliary metabolites were collected.<br>Results: GSK2256294 was well-tolerated with no serious adverse events (AEs) attributable to the drug. The most frequent AEs were headache and contact dermatitis. Plasma concentrations of GSK2256294 increased with single doses, with a half-life averaging 25-43 hours. There was no significant effect of age, food or gender on pharmacokinetic parameters. Inhibition of sEH enzyme activity was dose-dependent, from an average of 41.9% on 2 mg (95% confidence interval [CI] -51.8, 77.7) to 99.8% on 20 mg (95% CI 99.3, 100.0) and sustained for up to 24 hours. There were no significant changes in serum VEGF or plasma fibrinogen.<br>Conclusions: GSK2256294 was well-tolerated and demonstrated sustained |

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# Pharmacokinetics, pharmacodynamics and adverse event profile of GSK2256294, a novel soluble epoxide hydrolase inhibitor

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## Abstract

**Aims:** Endothelial-derived epoxyeicosatrienoic acids may regulate vascular tone and are metabolised by soluble epoxide hydrolase enzymes (sEH). GSK2256294 is a potent and selective sEH inhibitor that was tested in two Phase I studies.

**Methods:** Single escalating doses of GSK2256294 2-20 mg or placebo were administered in a randomized crossover design to healthy male subjects or obese smokers; once daily doses of 6 or 18 mg or placebo were administered for 14 days to obese smokers. Data were collected on safety, pharmacokinetics, sEH enzyme inhibition and blood biomarkers. Single doses of GSK2256294 10 mg were also administered to healthy younger males or healthy elderly males and females with and without food. Data on safety, pharmacokinetics and biliary metabolites were collected.

**Results:** GSK2256294 was well-tolerated with no serious adverse events (AEs) attributable to the drug. The most frequent AEs were headache and contact dermatitis. Plasma concentrations of GSK2256294 increased with single doses, with a half-life averaging 25-43 hours. There was no significant effect of age, food or gender on pharmacokinetic parameters. Inhibition of sEH enzyme activity was dose-dependent, from an average of 41.9% on 2 mg (95% confidence interval [CI] -51.8, 77.7) to 99.8% on 20 mg (95% CI 99.3, 100.0) and sustained for up to 24 hours. There were no significant changes in serum VEGF or plasma fibrinogen.

**Conclusions**: GSK2256294 was well-tolerated and demonstrated sustained inhibition of sEH enzyme activity. These data support further investigation in patients with endothelial dysfunction or abnormal tissue repair, such as diabetes, wound healing or COPD.

**What is known about this subject?**

- Soluble epoxide hydrolase (sEH) is a critical enzyme in the metabolism of epoxyeicosatrienoic acids (EETs).

- EETs released from the endothelium exhibit anti-inflammatory effects, regulate vascular tone and may have a cytoprotective role

- GSK2256294 is a potent and selective, orally-available sEH inhibitor

**What this study adds**

GSK2256294 is a well-tolerated and highly effective oral sEH inhibitor irrespective of age, gender or food administration.

## Introduction

Epoxyeicosatrienoic acids (EETs) are one of many vasoactive factors released by the vascular endothelium, including nitric oxide (NO), prostacyclin, and endothelium derived hyperpolarizing factors. EETs are formed by the oxidation of arachidonic acid by cytochrome P450 enzymes. Soluble epoxide hydrolase (sEH, an *EPHX2* gene product) is a critical enzyme in the metabolism of EETs to their corresponding much less active or less available dihydroxyeicosatrienoic acids (DHETs) [1,2]. In addition, *EPHX2* contains a phosphatase domain of unknown function. EETs have demonstrated a wide range of activities including protection of endothelial cell survival and function in both coronary and pulmonary derived cells, as well as anti-inflammatory and pro-resolving functions and protection of organs from damage [3–5]. The mechanism of action of EETs is complex, and may include changes in potassium channel open states, as well as engagement of as yet unidentified receptors or action through peroxisome proliferator-activated receptors [3].

Preclinical data with sEH inhibitors provide evidence for a number of potentially beneficial cardioprotective effects [6–10]. Exogenous EETs and sEH inhibitors are also efficacious in a wide variety of animal models of pulmonary disease such as smoke-induced airway inflammation and airspace enlargement, allergen-induced airway inflammation, bleomycin-induced pulmonary fibrosis and toxin-induced lung injury [11–16]. High levels of sEH activity inversely correlate with low concentrations of the pro-resolving lipoxin A4 in sputum supernatants of patients with severe asthma [17]. It is proposed that the inhibition of sEH will increase the cellular concentration of EETs and thus increase their positive cellular effects.

Clinical studies with sEH inhibitors are limited. The selective sEH inhibitor AR9281 (Arete Pharmaceuticals) was shown to improve endothelial function in animal models [18] and demonstrated inhibition of enzyme activity and a modest decrease in dihydroxy lipids in a clinical study in healthy subjects [19]. A Phase 2 trial to assess effects on blood pressure and glucose metabolism in patients with moderate hypertension and impaired glucose tolerance was terminated (NCT00847899). GSK2256294 is a potent, tight-binding but reversible inhibitor of sEH. It is specific for the hydrolase domain of *EPHX2* and is inactive against the phosphatase domain and has been shown to attenuate cigarette smoke-induced lung inflammation in animal models [20]. We report data from two clinical trials designed to test the safety, pharmacokinetics, and pharmacodynamics of GSK2256294 in healthy subjects.

## Methods

### Clinical Study design

Study 1, First-time-in-human (FTIH, GSK protocol SEH114068, ClinicalTrials.gov identifier NCT01762774). The FTIH study was a single-center, randomized, double-blind, placebo-controlled design comprising four cohorts. Study 1 recruited healthy male non-smokers between 18-65 years of age with body mass index (BMI) 19-25kg/$m^2$ for Cohort 1, and moderately overweight smokers (defined as ≥10 cigarettes/day for at least 1 year prior to the screening visit) with BMI 26 – 35kg/$m^2$ for Cohorts 2-4. Subjects with abnormal liver function, who were on statins, or who had hypertension or other significant cardiac, pulmonary, metabolic, renal, or gastrointestinal conditions, were excluded.

Single escalating doses of GSK2256294 (2, 6, 10, 12, and 20mg) or placebo were administered in a randomised crossover design to Cohorts 1 and 2. Single dosing subjects followed a 4-period dosing schedule with placebo insertion, such that all subjects received placebo and three doses of the active drug randomly over the course of the study. Subjects were assigned to the treatment regimens in accordance with a randomisation schedule that included 4 sequences with 3 subjects assigned to each sequence.

The emerging pharmacokinetic (PK) and pharmacodynamic (PD) data from Cohorts 1 and 2 were modelled with a population exposure-response analysis approach to select appropriate doses for repeat dosing cohorts. Two cohorts of overweight smokers were subsequently recruited for repeat dosing, with one cohort randomised to receive either 6mg or placebo (Cohort 3), and another 18 mg or placebo (Cohort 4). Fifteen subjects were planned for each cohort with 12 randomised to the active arm and 3 to placebo. Data were collected on safety, PK, sEH enzyme inhibition and blood biomarkers. An experimental medicine study in Cohorts 3 and 4, to provide early proof of mechanism of the potential biological effects of sEH inhibition on endothelial function using measurements of forearm blood flow, was also included, but reported separately [21]. The trial received favourable ethical opinion from the London Bloomsbury Ethics Committee (12/LO/1832) as well as regulatory approval by the MHRA.

Study 2, Food effect (GSK protocol SEH117023, ClinicalTrials.gov identifier NCT02006537). The food effect study was open label and performed at a single center. Subjects included healthy males between 18-45 years of age recruited for Cohort 1, and healthy male and females (of non-child bearing potential) aged ≥60 years of age recruited for Cohort 2.

Subjects in Cohort 1 received a single dose of GSK2256294 10mg, and underwent non-invasive bile sampling with the Entero-Test® device, as previously described [22]. Subjects in Cohort 2 were randomized to receive two single doses of GSK2256294 10 mg in both the fed and fasted state. This trial was reviewed and approved by the Aspire Institutional Review Board (Santee, CA, USA).

Both studies complied with the Declaration of Helsinki 2008 and ICH Good Clinical Practice guidelines, and full written informed consent was obtained from all participants before the performance of any study-specific procedures. A complete list of inclusion and exclusion criteria is available on clinicaltrials.gov.

**Safety**

Safety assessments were monitored using adverse event (AE) reporting, clinical laboratory tests, vital signs, ECGs, and physical examinations. In the single dose escalation phase of Study 1, 25-hour continuous Holter and electrocardiographic monitoring were performed from 1hr pre-dose to 24 hours post-dose.

The addition of EETs and/or inhibition of sEH have been linked in preclinical studies to two important activities that pose potential clinical risks. The first set of studies concerns the role of EETs and sEH in VEGF signaling and expression [23,24]; for this reason, we measured VEGF concentrations in the repeat dose cohorts of Study 1 (described below). The second set of studies concerns control of acute pulmonary vasoconstriction and pulmonary hypertension. Mice in which the sEH gene has been deleted develop pulmonary hypertension in response to chronic hypoxia, but sEH inhibition in wild-type mice does not recapitulate the knockout [25]. At this time, a role of the *EPHX2* gene in development of pulmonary hypertension is focused on the phosphatase domain, which is unaffected by GSK2256294 [20]; nevertheless, we measured pulmonary artery pressure using transthoracic echocardiography in the repeat dose cohorts of Study 1.

**Measurement of GSK2256294**

GSK2256294 was extracted from 50 μL of human plasma by protein precipitation using acetonitrile containing an isotopically labelled internal standard ([$^{2}H_{3}{}^{13}C$]-GSK2256294) and extracts were analysed for GSK2256294 by HPLC-MS/MS using a TurboIonSpray™ interface and multiple reaction monitoring. The assay was validated over the range 0.6 to 250 ng/mL of GSK2256294, with calibration correlation coefficients of >0.996, obtained using $1/(x^2)$ weighted linear regression. The assay precision (%CV) was ≤12.9% for within-run and ≤4.7% between-runs, with an accuracy (%bias) between -9.5%≤ bias ≤14.5%.

**Soluble Epoxide Hydrolase Activity Assay**

Blood samples were collected from subjects in Cohorts 1-4 (Study 1), immediately mixed by inversion in NaF/Potassium oxalate containing tubes and stored at 2-8°C for up to 12 hours (if not processed immediately). Whole blood samples were assessed for both sEH activity and non-sEH mediated EET hydrolysis, by preincubating for 20 minutes at room temperature with either 25 mM HEPES/10 µM CHAPS buffer (pH 7-7.6) either alone or with addition of a known potent sEH inhibitor (final concentration 10uM GSK2188931), respectively. The reactions were initiated by the addition of 14,15-EET-deuterated (d11) substrate in HEPES/CHAPS buffer (0.45 µM final concentration) and incubated for 30 minutes at room temperature. The enzymatic reaction was terminated by the addition of zinc sulfate (3.3 mM final concentration) and subsequently diluted with an equal volume of distilled water (~300uL) prior to storage at ca. -70°C. Samples were analysed for the conversion of 14,15-EET-d11 to 14,15-DHET-d11 by LC/MS/MS, using an assay that was validated over the range 0.5 to 500 ng/mL for 14,15-EET-d11 and 0.1 to 100 ng/mL for 14,15-DHET-d11, with calibration correlation coefficients obtained using $1/(x^2)$ weighted linear regression of >0.996 and 0.997, respectively. The assay precision (%CV) was ≤17.5% (14,15-EET-d11) and ≤13.3% (14,15-DHET-d11) for within-run and ≤4.6% (14,15-EET-d11) and ≤13.7% (14,15-DHET-d11) between-runs, with an accuracy (%bias) between -9.2% ≤ bias ≤5.7% (14, 15 EET) and -5.5% ≤ bias ≤22.9% (14, 15 DHET).  The sEH activity was evaluated by the formation of 14,15-DHET-d11, corrected for non-sEH mediated hydrolysis and subsequently the % inhibition of sEH activity following GSK2256294 dose escalation was defined.

**Blood biomarkers**

VEGF was measured using a validated ELISA (performed by Quest Diagnostics, Valencia, CA). Plasma fibrinogen was measured using the modified Clauss method. Detailed methods for the analysis of Leukotoxin/Leukotoxin-diol (LT/LTD) assays are included in the Supplemental material [26].

**Analysis and Statistical methods**

These studies focused primarily on the safety, tolerability, and pharmacokinetics (PK) of GSK2256294, and no formal hypotheses around the pharmacodynamic assessments were tested.

Plasma concentration-time data were analysed by non-compartmental methods with WinNonlin 6.3 and calculations were based on the actual sampling times recorded during the study. Actual elapsed times from dosing were used to estimate all individual plasma PK parameters for evaluable subjects. Based on available data, various PK parameters were estimated following GSK2256294 dose administration in single dose and repeat dose, including maximum observed plasma concentration (Cmax), time to Cmax (tmax), the apparent terminal elimination half-life (t1/2), and the area under the plasma concentration-time curve (AUC). Descriptive statistics (n, arithmetic mean, standard deviation, 90% CI, minimum, median and maximum) were calculated for all PK parameters by treatment. Dose proportionality was assessed by using Power model and Analysis of Variance.

A preliminary interim analysis of the systemic concentrations of GSK2256294 (exposure) and the sEH enzyme inhibition (response) data was conducted to select a starting dose for the repeat dose arm using a population modeling and simulation approach.

## Results

### Safety

Fifty-six male subjects aged 18-65 years inclusive were recruited to take part in Study 1. Fourteen healthy male non-smokers were recruited into cohort 1, and 42 moderately overweight smokers were recruited into cohorts 2 - 4. Forty-eight subjects completed the study as planned. Detailed subject disposition is shown in Figure 1. In study 2, 8 subjects were randomized into Cohort 1 and 18 subjects were randomized into Cohort 2. All subjects completed the study. Demographics for both studies are shown in Table S1 of the online supplement.

Overall, GSK2256294 was well-tolerated with no serious adverse reactions attributed to the drug. One serious adverse event of nephrolithiasis occurred in a subject who received a single dose of 2 mg in Study 1; this subject had a previous history of nephrolithiasis and the event was not considered to be drug-related, although the subject was withdrawn from the study. One additional subject was withdrawn after a vasovagal episode while a blood sample was being obtained, which was not felt to be drug-related. No subjects were withdrawn for adverse events in the repeat dose cohorts.

The most frequent adverse events in study 1 were headache and contact dermatitis in the healthy subjects (Cohort 1), and headache and nasopharyngitis in the obese smokers (cohorts 2-4)(Supplemental Tables S2-S4). The occurrence of AEs was similar between the active and placebo groups, with the exception of contact dermatitis at the site of ECG electrode placement, which occurred in 9 healthy subjects receiving the active drug, and none in the placebo group. The majority of AEs were mild-moderate in severity. In the single dose cohorts, five subjects were noted to have a transient elevation of creatinine at the 12 hour time point after dosing (1 on placebo, 2 each on GSK2256294 2mg or 6 mg). Contemporaneous Cystatin C concentrations in these subjects were normal (data not shown) and creatinine concentrations were within the normal range at the 24 hour time point. No changes in creatinine were noted in the repeat dose cohorts. The subject with nephrolithiasis was noted to have increased hepatic transaminases at the time of his hospitalization. One additional subject had a transient increase in alanine aminotransferase noted after a single dose of 10 mg GSK2256294; no clinically significant changes in transaminases were seen in the repeat dose cohorts. Finally, there were no changes in the pulmonary artery pressures of subjects who received 14 days repeat dosing of GSK2256294 in Study 1.

There were 3 AEs in study 2 and none was reported in more than one person (Table S5). No clinically significant differences in ECG or vital signs were noted in either study.

**Pharmacokinetics and Metabolism**

GSK256294 was well absorbed with maximum systemic concentrations achieved around 1-2 hours and with a dose proportional increase in systemic exposure from 6 mg to 20 mg. The observed half-life was 20-30 hours (Figure 2A). The exposure and t½ were slightly higher in obese smokers as compared to healthy volunteers (Table 1). With once daily repeat dosing, steady state was achieved within 6-8 days and resulted in approximately 2 fold accumulation (Figure 2B).

The preliminary exposure data and enzyme inhibition data from the single dose cohorts in FTIH were utilized to characterize the sEH enzyme inhibition and determine an approximate clinical dose for the repeat dose cohorts. An indirect response model was built to characterize the sustained enzyme inhibition following single dose administration. A population kinetic-pharmacodynamic model (KPD) model adequately characterized the observed sEH enzyme inhibition at different dose levels. The doses rather than systemic drug concentrations provided a better fit of the observed data and this the KPD approach was used [27]. Simulations were performed with the KPD model to predict the probability of 90% or higher level of enzyme inhibition after 2 weeks of once daily dosing at different dose levels. The 6 mg dose presented a very high probability of achieving >90% sEH enzyme inhibition at once daily dosing for 2 weeks. Assuming a less than 2 fold accumulation, the 18 mg dose was then selected to help characterize the safety and tolerability of a high dose of GSK2256294, while maintaining exposures within the stopping criteria based on animal safety studies.

Data from Study 2 demonstrated no impact on systemic exposure of GSK2256294 due to age or gender. There was an approximately 22% increase 1.22 (90% CI 1.10, 1.34) in AUC when GSK2256294 was administered with FDA recommended meal as compared to fasted state with no change in the Cmax. This increase in exposure when GSK2256294 is administered with food is clinically insignificant.

In Study 2, GSK2256294 and five metabolites were detected in the pooled human duodenal bile samples by mass spectroscopy. Unchanged parent was the major drug-related component, with metabolites being formed by oxidation with or without subsequent glucuronidation (data not shown).

**sEH Enzyme inhibition**

. Following single doses of GSK2256294, there was a dose-dependent inhibition from an average of 41.9% on 2 mg (95% CI -51.8, 77.7) to an average of 99.8% on 20 mg (95% CI 99.3, 100.0)(Figure 3A). The duration of inhibition was sustained for up to 24 hours. Near maximal inhibition of sEH enzyme activity (98-99%) was observed for both 6mg and 18 mg following 14 days repeated dosing (Figure 3B). Repeat dose enzyme inhibition at 6 mg/day and 18 mg/day (Cohorts 3 and 4, respectively) was consistent with the single dose inhibition at 6 hr (98-99 %) at both doses, and this was maintained at 24h after repeat dosing.

A large number of the endogenous LT levels were non-quantifiable, as they were below the limit of LC/MS/MS assay detection (LLQs of 250 pg/mL and 50 pg/mL for LT and LTD, respectively). In addition, there were no meaningful changes in the LT/LTD ratio between any of the three treatment arms compared to baseline at any time point (data not shown).

**Blood biomarkers**

In the repeat dosing subjects in Study 1, there were no significant changes in VEGF concentrations from baseline to day 15. The fold change from baseline in the placebo group was 1.06 (95% CI: 0.73, 1.55), and 1.15 (95% CI: 0.85, 1.56), in the 6mg group. There was a trend for a decrease in VEGF in subjects receiving 18mg of GSK2256294 (ratio to baseline 0.77 (95% CI: 0.56, 1.05)).

Adjusted mean values of plasma fibrinogen were within the normal range and were similar across all groups. The average difference at day 15 compared with placebo was negligible. The fold change from baseline in subjects who received 6mg GSK2256294 was 1.06 (95% CI: 0.82, 1.37), and 1.04 (95% CI: 0.81, 1.34) in those who received 18mg GSK2256294.

## Discussion

We report the first studies to assess the safety, pharmacokinetics and pharmacodynamic effects of the sEH inhibitor GSK2256294, in healthy subjects and otherwise healthy overweight smokers. Overweight smokers represent a population with endothelial dysfunction manifested by reduced NO-mediated vasodilatation and impaired fibrinolytic pathways; in this situation, up-regulation of EETs would be expected to play a compensatory role. Preclinical studies of sEH inhibition in models of metabolic syndrome support obesity as a factor regulating this pathway [8,28]. Finally, smoking has a synergistic effect with sEH polymorphisms coding for enhanced sEH enzyme [29], though subjects were not genotyped in this study.

GSK2256294 was rapidly absorbed and demonstrated an approximately dose proportional increase in exposure from 6 mg to 20 mg dose with a half life consistent with once daily dosing. The majority of GSK2256294 was cleared within 72 hrs of dosing, yet very low concentrations of parent compound were detectable in the plasma for up to 3 weeks. While the mechanism for this is unknown, one potential explanation may be that a small amount of GSK2256294 is distributed to a deep compartment and was then eliminated slowly.

GSK2256294 was well tolerated following both single and repeat dosing, and the majority of adverse events were classified as mild to moderate. Transient elevations of creatinine were noted in the single dose cohorts of the FTIH study that were considered possibly drug-related, and occurred primarily in subjects receiving GSK2256294. These events were only noted at a single time point, occurred soon after a meal and only at 2 and 6 mg but not higher doses, and did not re-occur with subsequent dosing, nor were they associated with elevations in serum cystatin C or urinalysis abnormalities. No changes in creatinine were observed in the repeat dose cohorts. The etiology of these transient changes is unknown. One possibility which may explain why this was only seen at lower doses, and not in repeat dosing, is the fact that the 12 hour blood sampling point was only done in Cohorts 1 and 2, i.e. immediately after the evening meal, and that the changes in creatinine were related to dietary intake (a heavy protein meal eaten immediately prior to sampling) as previously reported in the literature [30], although a formal dietary chart was not kept for the trial.

In pre-clinical models, EETs increase the proliferation and survival of endothelial cells [31] as well as induce angiogenesis [32,33]. The critical role for EETs in organ regeneration and tissue repair may be dependent on this effect [34]. Using the same experimental model, investigators also demonstrated that transgenic overexpression of EETs or inhibition of sEH promoted tumor growth and metastasis [35]. In contrast, dual inhibition of cyclo-oxygenase 2 and soluble epoxide hydrolase has demonstrated synergistic anti-angiogenic and anti-cancer activity [36]. The relevance of these models to humans is unknown, but suggests that the regulation of sEH activity and tissue EETs in tumor growth is complex. In the FTIH study, serum VEGF concentrations were not increased following 2 weeks of dosing with GSK2256294, and actually trended lower in subjects who received the higher dose. One might speculate that in presence of an sEH inhibitor, stabilization of EET levels results in enhanced local utilization reducing concentrations of VEGF, although long term studies are necessary to fully assess the potential effects.

More recent studies have suggested that under conditions of low VEGF signaling, EETs may increase the response to VEGF [23,24], thus, offering a novel therapeutic approach to target endothelial apoptosis and subsequent tissue loss, as has been demonstrated in emphysema [37–41]. Our results demonstrate that sEH inhibition with GSK2256294 is well tolerated, with rapid and sustained target inhibition. The results from these studies provide a meaningful rationale for future studies with

GSK2256294, particularly in diseases characterized by endothelial dysfunction or abnormal tissue repair, such as diabetes, wound healing or emphysema.

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**Competing Interests**

AL, JR, NG, RB, SB, RTS and RM are GSK employees and shareholders. JC is employed by Cambridge University Hospitals NHS Foundation Trust and is obligated to spend 50% of his time on GSK clinical trial research; however, he receives no other benefits or compensation from GSK. DN has received consultancy fees from GSK; IW has received educational grants from GSK. LY has no conflicts to declare.

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**Contributions**

AL, JR, NG, RB, RM, DN, IW and JC designed the clinical studies; SB developed the assay methodology; all authors analyzed and/or interpreted the data; AL and LY wrote the first draft; all authors reviewed and approved the manuscript.

## Figure Legends

**Figure 1:** Subject Disposition, Study 1

**Figure 2: Single and Repeat Dose Pharmacokinetics of GSK2256294.** Concentration-time plots following single (A) and repeat (B) doses of GSK2256294. Data are expressed as mean ±SD. HV-healthy volunteer; OS-overweight smoker

**Figure 3: Dose-dependent inhibition of sEH enzyme activity.** Percent inhibition of sEH enzyme activity following single (A) and repeat (B) dosing with GSK2256294. Data are expressed as mean ±SD. HV-healthy volunteer; OS-overweight smoker

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# Pharmacokinetics, pharmacodynamics and adverse event profile of GSK2256294, a novel soluble epoxide hydrolase inhibitor

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**Abstract**

**Aims:** Endothelial-derived epoxyeicosatrienoic acids may regulate vascular toneand are metabolised by soluble epoxide hydrolase enzymes (sEH). GSK2256294 is a potent and selective sEH inhibitor that was tested in two Phase I studies.

**Methods:** Single escalating doses of GSK2256294 2-20 mg or placebo were administered in a randomized crossover design to healthy male subjects or obese smokers; once daily doses of 6 or 18 mg or placebo were also administered for 14 days to obese smokers. Data were collected on safety, pharmacokinetics, sEH enzyme inhibition and blood biomarkers. Single doses of GSK2256294 10 mg were also administered to healthy younger males or healthy elderly males and females with and without food. Data on safety, pharmacokinetics and biliary metabolites were collected.

**Results:** GSK2256294 was well-tolerated with no serious adverse events (AEs) attributable to the drug. The most frequent AEs were headache and contact dermatitis. Plasma concentrations of GSK2256294 increased with single doses, with a half-life averaging 25-43 hours. There was no significant effect of age, food or gender on pharmacokinetic parameters. Inhibition of sEH enzyme activity was dose-dependent, from an average of 41.9% on 2 mg (95% confidence interval [CI] -51.8, 77.7) to 99.8% on 20 mg (95% CI 99.3, 100.0) and sustained for up to 24 hours. There were no significant changes in serum VEGF or plasma fibrinogen.

**Conclusions**: GSK2256294 was well-tolerated and demonstrated sustained inhibition of sEH enzyme activity. These data support further investigation in patients with endothelial dysfunction or abnormal tissue repair, such as diabetes, wound healing or COPD.

### What is known about this subject?

- Soluble epoxide hydrolase (sEH) is a critical enzyme in the metabolism of epoxyeicosatrienoic acids (EETs).

- EETs released from the endothelium exhibit anti-inflammatory effects, regulate vascular tone and may have a cytoprotective role

- GSK2256294 is a potent and selective, orally-available sEH inhibitor

### What this study adds

GSK2256294 is a well-tolerated and highly effective oral sEH inhibitor irrespective of age, gender or food administration.

## Introduction

Epoxyeicosatrienoic acids (EETs) are one of many vasoactive factors released by the vascular endothelium, including nitric oxide (NO), prostacyclin, and endothelium derived hyperpolarizing factors. EETs are formed by the oxidation of arachidonic acid by cytochrome P450 enzymes. Soluble epoxide hydrolase (sEH, an *EPHX2* gene product) is a critical enzyme in the metabolism of EETs to their corresponding much less active or less available dihydroxyeicosatrienoic acids (DHETs) [1,2]. In addition, *EPHX2* contains a phosphatase domain of unknown function. EETs have demonstrated a wide range of activities including protection of endothelial cell survival and function in both coronary and pulmonary derived cells, as well as anti-inflammatory and pro-resolving functions and protection of organs from damage [3–5]. The mechanism of action of EETs is complex, and may include changes in potassium channel open states, as well as engagement of as yet unidentified receptors or action through peroxisome proliferator-activated receptors [3].

Preclinical data with sEH inhibitors provide evidence for a number of potentially beneficial cardioprotective effects [6–10]. Exogenous EETs and sEH inhibitors are also efficacious in a wide variety of animal models of pulmonary disease such as smoke-induced airway inflammation and airspace enlargement, allergen-induced airway inflammation, bleomycin-induced pulmonary fibrosis and toxin-induced lung injury [11–16]. High levels of sEH activity inversely correlate with low concentrations of the pro-resolving lipoxin A4 in sputum supernatants of patients with severe asthma [17]. It is proposed that the inhibition of sEH will increase the cellular concentration of EETs and thus increase their positive cellular effects.

Clinical studies with sEH inhibitors are limited. The selective sEH inhibitor AR9281 (Arete Pharmaceuticals) was shown to improve endothelial function in animal models [18] and demonstrated inhibition of enzyme activity and a modest decrease in dihydroxy lipids in a clinical study in healthy subjects [19]. A Phase 2 trial to assess effects on blood pressure and glucose metabolism in patients with moderate hypertension and impaired glucose tolerance was terminated (NCT00847899). GSK2256294 is a potent, tight-binding but reversible inhibitor of sEH. It is specific for the hydrolase domain of *EPHX2* and is inactive against the phosphatase domain and has been shown to attenuate cigarette smoke-induced lung inflammation in animal models [20]. We report data from two clinical trials designed to test the safety, pharmacokinetics, and pharmacodynamics of GSK2256294 in healthy subjects.

## Methods

### Clinical Study design

Study 1, First-time-in-human (FTIH, GSK protocol SEH114068, ClinicalTrials.gov identifier NCT01762774). The FTIH study was a single-center, randomized, double-blind, placebo-controlled design comprising four cohorts. Study 1 recruited healthy male non-smokers between 18-65 years of age with body mass index (BMI) 19-25kg/m$^2$ for Cohort 1, and moderately overweight smokers (defined as ≥10 cigarettes/day for at least 1 year prior to the screening visit) with BMI 26 – 35kg/m$^2$ for Cohorts 2-4. Subjects with abnormal liver function, who were on statins, or who had hypertension or other significant cardiac, pulmonary, metabolic, renal, or gastrointestinal conditions, were excluded.

Single escalating doses of GSK2256294 (2, 6, 10, 12, and 20mg) or placebo were administered in a randomised crossover design to Cohorts 1 and 2. Single dosing subjects followed a 4-period dosing schedule with placebo insertion, such that all subjects received placebo and three doses of the active drug randomly over the course of the study. Subjects were assigned to the treatment regimens in accordance with a randomisation schedule that included 4 sequences with 3 subjects assigned to each sequence.

The emerging pharmacokinetic (PK) and pharmacodynamic (PD) data from Cohorts 1 and 2 were modelled with a population exposure-response analysis approach to select appropriate doses for repeat dosing cohorts. Two cohorts of overweight smokers were subsequently recruited for repeat dosing, with one cohort randomised to receive either 6mg or placebo (Cohort 3), and another 18 mg or placebo (Cohort 4). Fifteen subjects were planned for each cohort with 12 randomised to the active arm and 3 to placebo. Data were collected on safety, PK, sEH enzyme inhibition and blood biomarkers. An experimental medicine study in Cohorts 3 and 4, to provide early proof of mechanism of the potential biological effects of sEH inhibition on endothelial function using measurements of forearm blood flow, was also included, but reported separately [21]. The trial received favourable ethical opinion from the London Bloomsbury Ethics Committee (12/LO/1832) as well as regulatory approval by the MHRA.

Study 2, Food effect (GSK protocol SEH117023, ClinicalTrials.gov identifier NCT02006537). The food effect study was open label and performed at a single center. Subjects included healthy males between 18-45 years of age recruited for Cohort 1, and healthy male and females (of non-child bearing potential) aged ≥60 years of age recruited for Cohort 2.

Subjects in Cohort 1 received a single dose of GSK2256294 10mg, and underwent non-invasive bile sampling with the Entero-Test® device, as previously described [22]. Subjects in Cohort 2 were randomized to receive two single doses of GSK2256294 10 mg in both the fed and fasted state. This trial was reviewed and approved by the Aspire Institutional Review Board (Santee, CA, USA).

Both studies complied with the Declaration of Helsinki 2008 and ICH Good Clinical Practice guidelines, and full written informed consent was obtained from all participants before the performance of any study-specific procedures. A complete list of inclusion and exclusion criteria is available on clinicaltrials.gov.

**Safety**

Safety assessments were monitored using adverse event (AE) reporting, clinical laboratory tests, vital signs, ECGs, and physical examinations. In the single dose escalation phase of Study 1, 25-hour continuous Holter and electrocardiographic monitoring were performed from 1hr pre-dose to 24 hours post-dose.

The addition of EETs and/or inhibition of sEH have been linked in preclinical studies to two important activities that pose potential clinical risks. The first set of studies concerns the role of EETs and sEH in VEGF signaling and expression [23,24]; for this reason, we measured VEGF concentrations in the repeat dose cohorts of Study 1 (described below). The second set of studies concerns control of acute pulmonary vasoconstriction and pulmonary hypertension. Mice in which the sEH gene has been deleted develop pulmonary hypertension in response to chronic hypoxia, but sEH inhibition in wild-type mice does not recapitulate the knockout [25]. At this time, a role of the *EPHX2* gene in development of pulmonary hypertension is focused on the phosphatase domain, which is unaffected by GSK2256294 [20]; nevertheless, we measured pulmonary artery pressure using transthoracic echocardiography in the repeat dose cohorts of Study 1.

**Measurement of GSK2256294**

GSK2256294 was extracted from 50 µL of human plasma by protein precipitation using acetonitrile containing an isotopically labelled internal standard ([$^{2}H_{3}{}^{13}C$]-GSK2256294) and extracts were analysed for GSK2256294 by HPLC-MS/MS using a TurboIonSpray™ interface and multiple reaction monitoring. The assay was validated over the range 0.6 to 250 ng/mL of GSK2256294, with calibration correlation coefficients of >0.996, obtained using $1/(x^2)$ weighted linear regression. The assay precision (%CV) was ≤12.9% for within-run and ≤4.7% between-runs, with an accuracy (%bias) between -9.5%≤ bias ≤14.5%.

**Soluble Epoxide Hydrolase Activity Assay**

Blood samples were collected from subjects in Cohorts 1-4 (Study 1), immediately mixed by inversion in NaF/Potassium oxalate containing tubes and stored at 2-8°C for up to 12 hours (if not processed immediately). Whole blood samples were assessed for both sEH activity and non-sEH mediated EET hydrolysis, by preincubating for 20 minutes at room temperature with either 25 mM HEPES/10 μM CHAPS buffer (pH 7-7.6) either alone or with addition of a known potent sEH inhibitor (final concentration 10uM GSK2188931), respectively. The reactions were initiated by the addition of 14,15-EET-deuterated (d11) substrate in HEPES/CHAPS buffer (0.45 μM final concentration) and incubated for 30 minutes at room temperature. The enzymatic reaction was terminated by the addition of zinc sulfate (3.3 mM final concentration) and subsequently diluted with an equal volume of distilled water (~300uL) prior to storage at ca. -70°C. Samples were analysed for the conversion of 14,15-EET-d11 to 14,15-DHET-d11 by LC/MS/MS, using an assay that was validated over the range 0.5 to 500 ng/mL for 14,15-EET-d11 and 0.1 to 100 ng/mL for 14,15-DHET-d11, with calibration correlation coefficients obtained using $1/(x^2)$ weighted linear regression of >0.996 and 0.997, respectively. The assay precision (%CV) was ≤17.5% (14,15-EET-d11) and ≤13.3% (14,15-DHET-d11) for within-run and ≤4.6% (14,15-EET-d11) and ≤13.7% (14,15-DHET-d11) between-runs, with an accuracy (%bias) between -9.2% ≤ bias ≤5.7% (14, 15 EET) and -5.5% ≤ bias ≤22.9% (14, 15 DHET). The sEH activity was evaluated by the formation of 14,15-DHET-d11, corrected for non-sEH mediated hydrolysis and subsequently the % inhibition of sEH activity following GSK2256294 dose escalation was defined.

**Blood biomarkers**

VEGF was measured using a validated ELISA (performed by Quest Diagnostics, Valencia, CA). Plasma fibrinogen was measured using the modified Clauss method. Detailed methods for the analysis of Leukotoxin/Leukotoxin-diol (LT/LTD) assays are included in the Supplemental material [26].

**Analysis and Statistical methods**

These studies focused primarily on the safety, tolerability, and pharmacokinetics (PK) of GSK2256294, and no formal hypotheses around the pharmacodynamic assessments were tested.

Plasma concentration-time data were analysed by non-compartmental methods with WinNonlin 6.3 and calculations were based on the actual sampling times recorded during the study. Actual elapsed times from dosing were used to estimate all individual plasma PK parameters for evaluable subjects. Based on available data, various PK parameters were estimated following GSK2256294 dose administration in single dose and repeat dose, including maximum observed plasma concentration (Cmax), time to Cmax (tmax), the apparent terminal elimination half-life (t1/2), and the area under the plasma concentration-time curve (AUC). Descriptive statistics (n, arithmetic mean, standard deviation, 90% CI, minimum, median and maximum) were calculated for all PK parameters by treatment. Dose proportionality was assessed by using Power model and Analysis of Variance.

A preliminary interim analysis of the systemic concentrations of GSK2256294 (exposure) and the sEH enzyme inhibition (response) data was conducted to select a starting dose for the repeat dose arm using a population modeling and simulation approach.

## Results

### Safety

Fifty-six male subjects aged 18-65 years inclusive were recruited to take part in Study 1. Fourteen healthy male non-smokers were recruited into cohort 1, and 42 moderately overweight smokers were recruited into cohorts 2 - 4. Forty-eight subjects completed the study as planned. Detailed subject disposition is shown in Figure 1. In study 2, 8 subjects were randomized into Cohort 1 and 18 subjects were randomized into Cohort 2. All subjects completed the study. Demographics for both studies are shown in Table S1 of the online supplement.

Overall, GSK2256294 was well-tolerated with no serious adverse reactions attributed to the drug. One serious adverse event of nephrolithiasis occurred in a subject who received a single dose of 2 mg in Study 1; this subject had a previous history of nephrolithiasis and the event was not considered to be drug-related, although the subject was withdrawn from the study. One additional subject was withdrawn after a vasovagal episode while a blood sample was being obtained, which was not felt to be drug-related. No subjects were withdrawn for adverse events in the repeat dose cohorts.

The most frequent adverse events in study 1 were headache and contact dermatitis in the healthy subjects (Cohort 1), and headache and nasopharyngitis in the obese smokers (cohorts 2-4)(Supplemental Tables S2-S4). The occurrence of AEs was similar between the active and placebo groups, with the exception of contact dermatitis at the site of ECG electrode placement, which occurred in 9 healthy subjects receiving the active drug, and none in the placebo group. The majority of AEs were mild-moderate in severity. In the single dose cohorts, five subjects were noted to have a transient elevation of creatinine at the 12 hour time point after dosing (1 on placebo, 2 each on GSK2256294 2mg or 6 mg). Contemporaneous Cystatin C concentrations in these subjects were normal (data not shown) and creatinine concentrations were within the normal range at the 24 hour time point. No changes in creatinine were noted in the repeat dose cohorts. The subject with nephrolithiasis was noted to have increased hepatic transaminases at the time of his hospitalization. One additional subject had a transient increase in alanine aminotransferase noted after a single dose of 10 mg GSK2256294; no clinically significant changes in transaminases were seen in the repeat dose cohorts. Finally, there were no changes in the pulmonary artery pressures of subjects who received 14 days repeat dosing of GSK2256294 in Study 1.

There were 3 AEs in study 2 and none was reported in more than one person (Table S5). No clinically significant differences in ECG or vital signs were noted in either study.

**Pharmacokinetics and Metabolism**

GSK256294 was well absorbed with maximum systemic concentrations achieved around 1-2 hours and with a dose proportional increase in systemic exposure from 6 mg to 20 mg. The observed half-life was 20-30 hours (Figure 2A). The exposure and t½ were slightly higher in obese smokers as compared to healthy volunteers (Table 1). With once daily repeat dosing, steady state was achieved within 6-8 days and resulted in approximately 2 fold accumulation (Figure 2B).

The preliminary exposure data and enzyme inhibition data from the single dose cohorts in FTIH were utilized to characterize the sEH enzyme inhibition and determine an approximate clinical dose for the repeat dose cohorts. An indirect response model was built to characterize the sustained enzyme inhibition following single dose administration. A population kinetic-pharmacodynamic model (KPD) model adequately characterized the observed sEH enzyme inhibition at different dose levels. The doses rather than systemic drug concentrations provided a better fit of the observed data and this the KPD approach was used [27]. Simulations were performed with the KPD model to predict the probability of 90% or higher level of enzyme inhibition after 2 weeks of once daily dosing at different dose levels. The 6 mg dose presented a very high probability of achieving >90% sEH enzyme inhibition at once daily dosing for 2 weeks. Assuming a less than 2 fold accumulation, the 18 mg dose was then selected to help characterize the safety and tolerability of a high dose of GSK2256294, while maintaining exposures within the stopping criteria based on animal safety studies.

Data from Study 2 demonstrated no impact on systemic exposure of GSK2256294 due to age or gender. There was an approximately 22% increase 1.22 (90% CI 1.10, 1.34) in AUC when GSK2256294 was administered with FDA recommended meal as compared to fasted state with no change in the Cmax. This increase in exposure when GSK2256294 is administered with food is clinically insignificant.

In Study 2, GSK2256294 and five metabolites were detected in the pooled human duodenal bile samples by mass spectroscopy. Unchanged parent was the major drug-related component, with metabolites being formed by oxidation with or without subsequent glucuronidation (data not shown).

**sEH Enzyme inhibition**

. Following single doses of GSK2256294, there was a dose-dependent inhibition from an average of 41.9% on 2 mg (95% CI -51.8, 77.7) to an average of 99.8% on 20 mg (95% CI 99.3, 100.0)(Figure 3A). The duration of inhibition was sustained for up to 24 hours. Near maximal inhibition of sEH enzyme activity (98-99%) was observed for both 6mg and 18 mg following 14 days repeated dosing (Figure 3B). Repeat dose enzyme inhibition at 6 mg/day and 18 mg/day (Cohorts 3 and 4, respectively) was consistent with the single dose inhibition at 6 hr (98-99 %) at both doses, and this was maintained at 24h after repeat dosing.

A large number of the endogenous LT levels were non-quantifiable, as they were below the limit of LC/MS/MS assay detection (LLQs of 250 pg/mL and 50 pg/mL for LT and LTD, respectively). In addition, there were no meaningful changes in the LT/LTD ratio between any of the three treatment arms compared to baseline at any time point (data not shown).

**Blood biomarkers**

In the repeat dosing subjects in Study 1, there were no significant changes in VEGF concentrations from baseline to day 15. The fold change from baseline in the placebo group was 1.06 (95% CI: 0.73, 1.55), and 1.15 (95% CI: 0.85, 1.56), in the 6mg group. There was a trend for a decrease in VEGF in subjects receiving 18mg of GSK2256294 (ratio to baseline 0.77 (95% CI: 0.56, 1.05)).

Adjusted mean values of plasma fibrinogen were within the normal range and were similar across all groups. The average difference at day 15 compared with placebo was negligible. The fold change from baseline in subjects who received 6mg GSK2256294 was 1.06 (95% CI: 0.82, 1.37), and 1.04 (95% CI: 0.81, 1.34) in those who received 18mg GSK2256294.

## Discussion

We report the first studies to assess the safety, pharmacokinetics and pharmacodynamic effects of the sEH inhibitor GSK2256294, in healthy subjects and otherwise healthy overweight smokers. Overweight smokers represent a population with endothelial dysfunction manifested by reduced NO-mediated vasodilatation and impaired fibrinolytic pathways; in this situation, up-regulation of EETs would be expected to play a compensatory role. Preclinical studies of sEH inhibition in models of metabolic syndrome support obesity as a factor regulating this pathway [8,28]. Finally, smoking has a synergistic effect with sEH polymorphisms coding for enhanced sEH enzyme [29], though subjects were not genotyped in this study.

GSK2256294 was rapidly absorbed and demonstrated an approximately dose proportional increase in exposure from 6 mg to 20 mg dose with a half life consistent with once daily dosing. The majority of GSK2256294 was cleared within 72 hrs of dosing, yet very low concentrations of parent compound were detectable in the plasma for up to 3 weeks. While the mechanism for this is unknown, one potential explanation may be that a small amount of GSK2256294 is distributed to a deep compartment and was then eliminated slowly.

GSK2256294 was well tolerated following both single and repeat dosing, and the majority of adverse events were classified as mild to moderate. Transient elevations of creatinine were noted in the single dose cohorts of the FTIH study that were considered possibly drug-related, and occurred primarily in subjects receiving GSK2256294. These events were only noted at a single time point, occurred soon after a meal and only at 2 and 6 mg but not higher doses, and did not re-occur with subsequent dosing, nor were they associated with elevations in serum cystatin C or urinalysis abnormalities. No changes in creatinine were observed in the repeat dose cohorts. The etiology of these transient changes is unknown. One possibility which may explain why this was only seen at lower doses, and not in repeat dosing, is the fact that the 12 hour blood sampling point was only done in Cohorts 1 and 2, i.e. immediately after the evening meal, and that the changes in creatinine were related to dietary intake (a heavy protein meal eaten immediately prior to sampling) as previously reported in the literature [30], although a formal dietary chart was not kept for the trial.

In pre-clinical models, EETs increase the proliferation and survival of endothelial cells [31] as well as induce angiogenesis [32,33]. The critical role for EETs in organ regeneration and tissue repair may be dependent on this effect [34]. Using the same experimental model, investigators also demonstrated that transgenic overexpression of EETs or inhibition of sEH promoted tumor growth and metastasis [35]. In contrast, dual inhibition of cyclo-oxygenase 2 and soluble epoxide hydrolase has demonstrated synergistic anti-angiogenic and anti-cancer activity [36]. The relevance of these models to humans is unknown, but suggests that the regulation of sEH activity and tissue EETs in tumor growth is complex. In the FTIH study, serum VEGF concentrations were not increased following 2 weeks of dosing with GSK2256294, and actually trended lower in subjects who received the higher dose. One might speculate that in presence of an sEH inhibitor, stabilization of EET levels results in enhanced local utilization reducing concentrations of VEGF, although long term studies are necessary to fully assess the potential effects.

More recent studies have suggested that under conditions of low VEGF signaling, EETs may increase the response to VEGF [23,24], thus, offering a novel therapeutic approach to target endothelial apoptosis and subsequent tissue loss, as has been demonstrated in emphysema [37–41]. Our results demonstrate that sEH inhibition with GSK2256294 is well tolerated, with rapid and sustained target inhibition. The results from these studies provide a meaningful rationale for future studies with

GSK2256294, particularly in diseases characterized by endothelial dysfunction or abnormal tissue repair, such as diabetes, wound healing or emphysema.

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**Competing Interests**

AL, JR, NG, RB, SB, RTS and RM are GSK employees and shareholders. JC is employed by Cambridge University Hospitals NHS Foundation Trust and is obligated to spend 50% of his time on GSK clinical trial research; however, he receives no other benefits or compensation from GSK. DN has received consultancy fees from GSK; IW has received educational grants from GSK. LY has no conflicts to declare.

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**Contributions**

AL, JR, NG, RB, RM, DN, IW and JC designed the clinical studies; SB developed the assay methodology; all authors analyzed and/or interpreted the data; AL and LY wrote the first draft; all authors reviewed and approved the manuscript.

## Figure Legends

**Figure 1:** Subject Disposition, Study 1

**Figure 2: Single and Repeat Dose Pharmacokinetics of GSK2256294.** Concentration-time plots following single (A) and repeat (B) doses of GSK2256294. Data are expressed as mean ±SD. HV-healthy volunteer; OS-overweight smoker

**Figure 3: Dose-dependent inhibition of sEH enzyme activity.** Percent inhibition of sEH enzyme activity following single (A) and repeat (B) dosing with GSK2256294. Data are expressed as mean ±SD. HV-healthy volunteer; OS-overweight smoker

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**Table 1: Selected Plasma GSK2256294 Pharmacokinetic Parameters**

| Treatment | Cmax (ng/mL) | AUC(0-24) (ng.h/mL]) | AUC(0-∞) (ng.h/mL) | t1/2 (h) | tmax (h) |
|---|---|---|---|---|---|
| Cohort 1 (SD) | | | | | |
| 2mg | 29.6 (79.6) | 111 (124) | 1272 (17.9) | 29.9 (11.8) | 0.50 (0.3-1.0) |
| 6mg | 321 (44.7) | 2471 (37.3) | 3376 (37.3) | 25.0 (23.6) | 1.00 (0.5-4.0) |
| 12mg | 836 (33.4) | 6680 (23.4) | 8641 (26.8) | 19.3 (11.0) | 0.63 (0.5-1.0) |
| Cohort 2 (SD) | | | | | |
| 6mg | 333 (29.4) | 3244 (18.4) | 5905 (24.7) | 48.8 (36.3) | 1.00 (0.5-3.0) |
| 10mg | 559 (32.8) | 5267 (34.6) | 9611 (46.9) | 42.7 (52.7) | 1.00 (0.5-2.0) |
| 20mg | 1223 (23.2) | 11464 (31.0) | 17359 (35.8) | 41.4 (35.7) | 1.00 (0.5-4.0) |
| Cohort 3-4 (RD) | | | | | |
| 6mg | 689 (33.2) | 5801 (57.5) | ND | ND | 0.52 (0.5-1.0) |
| 18mg | 1455 (29.1) | 15774 (36.3) | ND | ND | 0.50 (0.5-2.0) |

Selected pharmacokinetic parameters from the FTIH study (Study 1), expressed as geometric mean (coefficient of variance [CV]%), with the exception of tmax, which is expressed as median (range). ND-not determined; SD-single dose; RD-14 day repeat dose

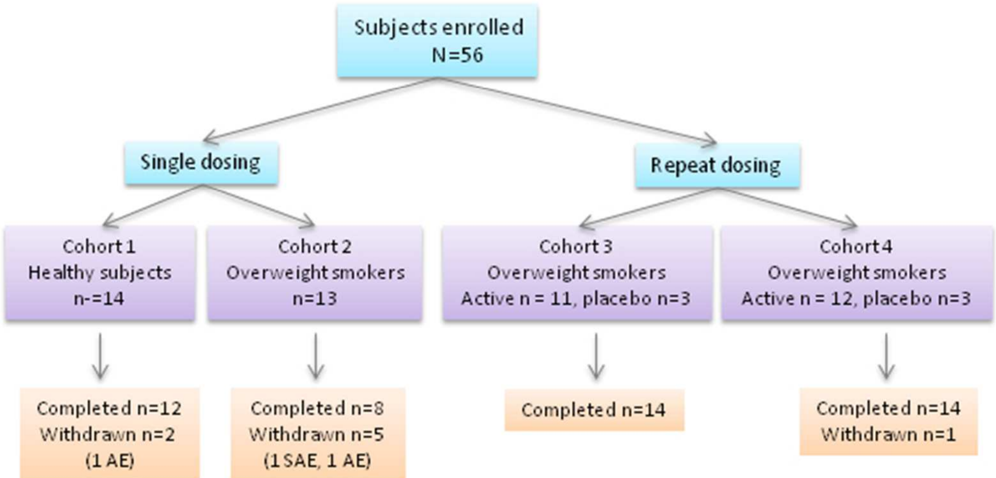


Figure 1
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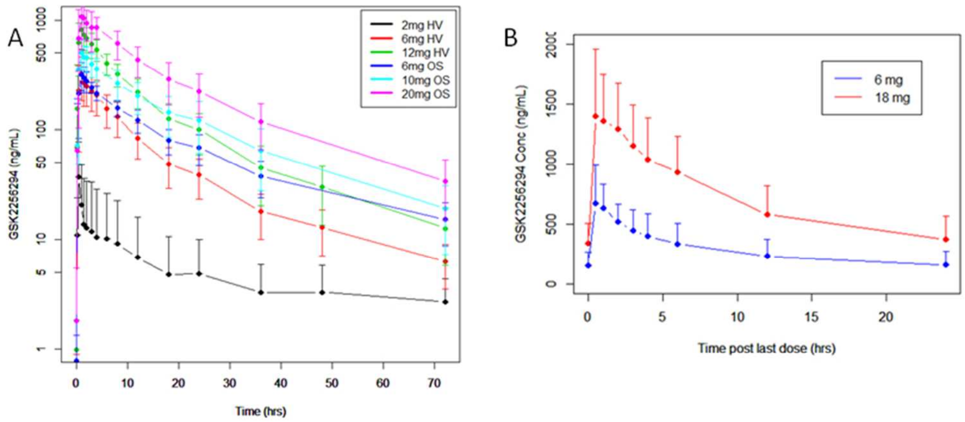


Figure 2: Single and Repeat Dose Pharmacokinetics of GSK2256294. Concentration-time plots following single (A) and repeat (B) doses of GSK2256294. Data are expressed as mean ±SD. HV-healthy volunteer; OS-overweight smoker
222x105mm (96 x 96 DPI)

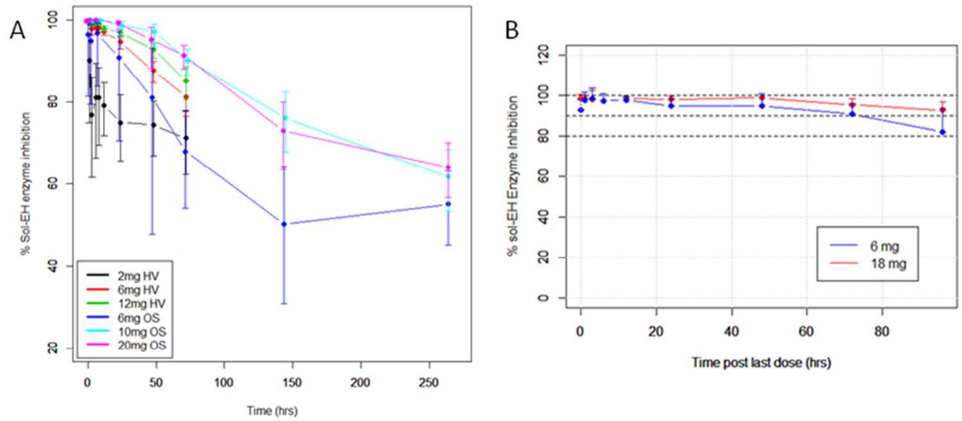


Figure 3: Dose-dependent inhibition of sEH enzyme activity. Percent inhibition of sEH enzyme activity following single (A) and repeat (B) dosing with GSK2256294. Data are expressed as mean ±SD. HV-healthy volunteer; OS-overweight smoker
222x100mm (96 x 96 DPI)

## Supplemental Data

### Leukotoxin/Leukotoxin-diol (LT/LTD) Assays

Blood samples were collected from subjects in Cohorts 3-4, into EDTA containing tubes, placed on crushed ice prior to centrifugation to obtain plasma (ca. 1500 x g for 15 minutes, 4°C) and stored at -20°C or colder (within 1h of blood collection). Samples were analysed for the determination of endogenous levels of LT converted to LTD by LC/MS/MS, with assay LLQs of 250 pg/mL and 50 pg/mL, respectively.

## Table S1: Demography

### Study 1

| Demographics | Single Dose | | | Repeat Dose | | |
|---|---|---|---|---|---|---|
| | Cohort 1 (n=14) | Cohort 2 (n=13) | | GSK2256294 6 mg (n=11) | GSK2256294 18 mg (n=12) | Placebo (n=6) |
| **Age in Years** Mean (SD) | 32.4 (7.92) | 38.9 (9.57) | | 42.9 (10.48) | 42.4 (9.46) | 41.3 (8.04) |
| **Sex** [n (%)] | | | | | | |
| Male: | 14 (100) | 13 (100) | | 11 (100) | 12 (100) | 6 (100) |
| **BMI** (kg/m²) Mean (SD) | 23.15 (1.446) | 30.26 (1.640) | | 30.55 (1.675) | 30.92 (2.265) | 30.55 (2.894) |
| **Height** (cm) Mean (SD) | 178 (5.0) | 181 (4.7) | | 183 (6.0) | 178 (4.3) | 176 (9.0) |
| **Weight** (kg) Mean (SD) | 73.2 (6.57) | 98.9 (8.80) | | 102.8 (9.90) | 98.3 (10.14) | 94.5 (9.24) |
| **Ethnicity** n (%) | | | | | | |
| Not Hispanic or Latino: | 14 (100) | 13 (100) | | 11 (100) | 12 (100) | 6 (100) |
| **Race** n (%) | | | | | | |
| Asian – South East Asian Heritage | 0 | 1 (8) | | 1(9) | 1 (8) | 0 |
| Black or African American | 1 (7) | 0 | | 0 | 2 (17) | 0 |
| White – White/Caucasian/European Heritage | 13 (93) | 12 (92) | | 10 (91) | 9 (75) | 6 (100) |

### Study 2

| Demographics | Cohort 1 (N=8) | Cohort 2 (N=18) |
|---|---|---|
| **Age in Years** [Median (Range)] | 36 (20-40) | 62 (59-75) |
| **Sex** [n (%)] | | |
| Female: | 0 | 8 (44) |
| Male: | 8 (100) | 10 (56) |
| **BMI** (kg/m²) [Median (Range)] | 24.50 (20.40-28.80) | 27.85 (21.50-33.00) |
| **Height** (m) [Median (Range)] | 1.77 (1.66-1.90) | 1.73 (1.58-1.91) |
| **Weight** (kg) [Median (Range)] | 79.3 (61.2-100.0) | 79.3 (64.2-113.0) |
| **Ethnicity** [n (%)] | | |
| Hispanic or Latino: | 2 (25) | 1 (6) |
| Not Hispanic or Latino: | 6 (75) | 17 (94) |
| **Race** [n (%)] | | |
| African American/African Heritage | 5 (63) | 11 (61) |
| Asian – South East Asian Heritage | 0 | 1 (6) |
| White – White/Caucasian/European Heritage | 3 (38) | 6 (33) |

**Supplemental Table 2: Adverse Events reported in Cohort 1, study 1**

| System organ class preferred term, n (%) | Placebo (n=12) | GSK2256294 2 mg (n=12) | GSK2256294 6 mg (n=12) | GSK2256294 12 mg (n=12) |
|---|---|---|---|---|
| **Subjects with Any Adverse Event** | 4 (33) | 9 (75) | 8 (67) | 6 (50) |
| **Skin and subcutaneous tissue disorders** | | | | |
| Dermatitis contact | 0 | 4 (33) | 3 (25) | 1 (8) |
| **Nervous system disorders** | | | | |
| Headache | 2 (17) | 1 (8) | 2 (17) | 3 (25) |
| **General disorders and administration site conditions** | | | | |
| Catheter site erythema | 0 | 0 | 0 | 2 (17) |
| **Investigations** | | | | |
| Blood creatinine increased | 0 | 2 (17) | 1 (8) | 0 |

**Supplemental Table 3: Adverse Events reported in Cohort 2, study 1**

| System organ class preferred term, n (%) | Placebo (n=12) | GSK2256294 2 mg (n=12) | GSK2256294 6 mg (n=12) | GSK2256294 12 mg (n=12) |
|---|---|---|---|---|
| **Subjects with Any Adverse Event** | 7 (64) | 7 (64) | 8 (80) | 6 (75) |
| **Nervous system disorders** | | | | |
| Headache | 3 (27) | 2 (18) | 3 (30) | 2 (25) |
| Somnolence | 0 | 0 | 0 | 2 (25) |
| **Musculoskeletal and connective tissue disorders** | | | | |
| Back pain | 0 | 2 (18) | 0 | 1 (13) |
| **Gastrointestinal disorders** | | | | |
| Flatulence | 0 | 1 (9) | 2 (20) | 0 |
| **General disorders and administration site conditions** | | | | |
| Fatigue | 3 (27) | 0 | 1 (10) | 0 |
| **Infections and infestations** | | | | |
| Nasopharyngitis | 0 | 2 (18) | 0 | 0 |
| **Investigations** | | | | |
| Hepatic enzyme increased | 0 | 2 (18) | 0 | 0 |

**Supplemental Table 4: Adverse Events reported in Cohorts 3 and 4, Study 1**

| System organ class preferred term, n (%) | Placebo (n=6) | GSK2256294 6 mg (n=11) | GSK2256294 18 mg (n=12) |
|---|---|---|---|
| **Subjects with Any Adverse Event** | 5 (83) | 9 (82) | 9 (75) |
| **Nervous system disorders** | | | |
| Headache | 3 (50) | 4 (36) | 5 (42) |
| Dizziness | 0 | 0 | 2 (17) |
| **Musculoskeletal and connective tissue disorders** | | | |
| Back pain | 1 (17) | 2 (18) | 0 |
| Neck pain | 0 | 1 (9) | 2 (17) |
| **Gastrointestinal disorders** | | | |
| Abdominal pain | 0 | 2 (18) | 0 |
| **Infections and infestations** | | | |
| Nasopharyngitis | 0 | 3 (27) | 2 (17) |

**Supplemental Table S5: Adverse events reported in Study 2**

| System Organ Class<br>Preferred Term | GSK2256294 10 mg (N=8) | GSK2256294 10 mg (N=18) |
|---|---|---|
| **Subjects with Any Adverse Event** | 2 (25) | 1 (6) |
| **Gastrointestinal disorders** | | |
| Flatulence | 0 | 1 (6) |
| **General disorders and administration site conditions** | | |
| Catheter site swelling | 1 (13) | 0 |
| **Musculoskeletal and connective tissue disorders** | | |
| Pain in extremity | 1 (13) | 0 |