Synopsis

Identifier: GM2007/00457/00 **Study Number:** IPR109764

Title: A randomised, single-blind, placebo-controlled 5-way crossover trial of single doses of intranasal GSK256066 in subjects with seasonal allergic rhinitis (SAR).

Investigator: [redacted]

Study center: [redacted] Germany.

Publication: None at the time of this report.

Study period:

28Mar2007 - 16May2007

Phase of development: IIa

Objectives:

The primary objective was:

- To explore the effects of GSK256066 on novel markers of phosphodiesterase type 4 (PDE4) inhibition in nasal lavage and scrape samples in mild to moderate allergic rhinitic subjects and determine a dose response relationship.

The secondary objectives were:

- To explore the safety, tolerability and pharmacokinetics of single doses of GSK256066 in mild to moderate allergic rhinitic subjects.
- To explore the effects of GSK256066 on additional markers of PDE4 inhibition in nasal lavage.

The primary endpoint was:

- Effect of GSK256066 on ribonucleic acid (RNA) levels indicative of PDE4 inhibition in nasal scrape samples and on protein biomarkers of PDE4 inhibition in lavage samples.

The secondary endpoints were:

- Clinical safety parameters: Forced expiratory volume in one second (FEV_1), vital signs, electrocardiogram (ECG) changes, adverse events (AE).
- Laboratory safety parameters.
- Plasma concentrations of GSK256066 and active metabolite GSK614917 and derived plasma pharmacokinetic parameters: Area under the plasma drug concentration versus time curve ($AUC_{0\text{-last}}$,), maximum observed plasma drug

concentration (C_{max}), time to maximum observed plasma drug concentration (t_{max}), time to last observed plasma drug concentration (t_{last}). Nasal lavage concentrations of GSK256066.

- Effects of GSK256066 on total vasodilator stimulated phosphoprotein (VASP) protein levels, phospho157 VASP and phospho239 VASP (pVASP) in lavage cells.

Methodology:

Allergic rhinitis is a very common disorder, affecting 15 to 20% of the population in industrialised countries. Selective PDE4 inhibitors offer an appealing molecular basis for anti-inflammatory activity in allergic airway disease. The PDE4 isoenzyme is responsible for the degradation of intracellular cyclic adenosine monophosphate (cAMP) and is selectively expressed in many inflammatory cells including eosinophils, mast cells, T-lymphocytes, monocytes and basophils. As PDE4 inhibitors reduce late-phase inflammation and vascular leakage, they are potential candidates for the treatment of allergic conditions such as rhinitis and bronchial asthma.

GSK256066 is a potent and highly selective PDE4 inhibitor, currently in development by GlaxoSmithKline for the treatment of allergic rhinitis, asthma and chronic obstructive pulmonary disease.

Other markers indicative of PDE4 inhibition such as VASP protein levels and phospho157 VASP were also measured in this study, in lavage cells, following positive data in an enabling study which showed increases in such protein levels in subjects with allergic rhinitis following a single intranasal dose of salbutamol. Nasal lavage data from earlier studies showed that pVASP157 is the best marker and not pVASP239. pVASP239 was therefore not collected or analysed as planned.

This study was planned as a dedicated pharmacodynamics study to evaluate dose response in rhinitic subjects at doses i.e. 50 μg and 200 μg where clinical efficacy had been observed in the environmental challenge chamber studies and to interrogate the lower end of the anticipated therapeutic range for predictive efficacy.

Finally, this study would also yield important insights into the feasibility of using messenger RNA (mRNA) markers in this way, beyond its important contribution to the PDE4 programme.

This was a randomised, single blind, placebo-controlled five-way crossover trial of single doses of intranasal GSK256066 and placebo in subjects with allergic rhinitis.

Subjects underwent Screening 7 to 28 days prior to study start.

There were five treatment periods, with at least a 3 day washout between each treatment. Subjects attended the Unit on the morning of dosing and stayed until all study procedures were completed (approximately 4 hours).

Each subject underwent a Follow-up visit at least 7 days (and no more than 14 days) after their last treatment.

The total duration of the study per subject was approximately 8 to 9 weeks (up to 4 weeks Screening and 3 weeks dosing (including washout) plus 1 to 2 weeks Follow-up). A Time and Events table is provided as Attachment 1.

Number of subjects:

Number of Subjects	Total
Planned, N	32
Randomised, N	32
Completed, n (%)	30(94)
Total Withdrawn (any reason), n (%)	2(6)
Withdrawn due to Adverse Events, n (%)	2(6)

Source Data: Table 9.1

Diagnosis and main criteria for inclusion:

Healthy adult females and males aged 18 to 50 years inclusive with body mass index (BMI) less than 29.0 kg/m² and having a positive history of SAR were considered for this study. The eligible subjects were non-smokers, had a positive skin prick test (wheal ≥4 mm) and a positive radioallergosorbent test for grass pollen at or within the 12 months preceding the screening visit with a baseline FEV_1>80% predicted and a baseline FEV_1(maximum recorded value)/Forced vital capacity(maximum recorded value)>70% predicted. Subjects with any structural nasal abnormalities or nasal polyposis, a history of frequent nosebleeds, recent nasal surgery and recent (within 2 weeks) or ongoing upper respiratory tract infection were excluded from the study.

Treatment administration:

Subjects were assigned to study treatment in accordance with the randomisation schedule. The treatment administration is summarised in the following table.

Summary of Treatment Administration

Drug	Dose	Form/Route	Frequency/Duration	Batch Number
GSK256066 1μg	1 μg (1 puff of 0.5 μg per nostril)	Aqueous nasal spray/Intranasal	Single dose	071132752
GSK256066 10 μg	10 μg (1 puff of 5 μg per nostril)	Aqueous nasal spray/Intranasal	Single dose	071132755
GSK256066 50μg	50 μg (1 puff of 25 μg per nostril)	Aqueous nasal spray/Intranasal	Single dose	061128579
GSK256066 200μg	200 μg (1 puff of 100 μg per nostril)	Aqueous nasal spray/Intranasal	Single dose	071131146
Placebo	1 puff per nostril	Aqueous nasal spray/Intranasal	Single dose	051112513 and 061120948

Criteria for evaluation:

Pharmacodynamics: Nasal scrape samples and samples from nasal lavage were analysed to explore the effects of GSK256066 on novel RNA markers indicative of PDE4 inhibition and on novel protein biomarkers under investigation including pVASP.

Safety: Adverse event monitoring, laboratory safety tests (haematology, biochemistry and urinalysis), 12-lead ECG, cardiac telemetry, FEV_1, vital signs, nasal examination, drug screen and β- human chorionic gonadotrophin pregnancy test in females.

Pharmacokinetic: The pharmacokinetics (PK) of GSK256066 and GSK614197 were assessed in plasma by determining $AUC_{(0\text{-last})}$, C_{max}, t_{last} and t_{max}. Nasal lavage samples were analysed for GSK256066.

Statistical methods:

This study emphasises on estimation, therefore no formal sample size calculation was performed. In addition, the impact of the sample size on the hypothesis of a 1.5 fold change (FC) was investigated. A 1.5 FC was used in this study as this level of change was observed for serine/threonine protein kinase SNF1 like kinase (SNF1LK) in the other environmental chamber study. Based on the maximum estimate of within subject standard deviation of 0.194, 30 subjects were to give 92% power to detect a 1.5 FC in mRNA gene expression between GSK256066 and placebo at the 5% two-sided significance level.

The primary objective was to identify the major source of variation among each nasal scrape sample via principal component scores obtained from a principal component analysis. These scores were included as a covariate for 'sample quality' in the analysis model to investigate how GSK256066 on novel markers of PDE4 inhibition affects RNA expression/abundance.

Each gene of interest had been analysed using analysis of variance. A mixed effect model was fitted with treatment, period and sample quality indicator as fixed effects and subject as a random effect. An estimate of the treatment ratios for each dose of GSK256066 was calculated for each non-housekeeper gene along with adjusted means (least square means) and the associated 95% confidence intervals (CI).

If a signal was seen for any of these genes, then a dose response analysis was performed using a linear model approach on treatment pairs. An estimate of the slope and 95% confidence intervals were calculated.

For the secondary objective, the effects of GSK256066 on protein levels in lavage cells for each analyte (VASP, pVASP, cAMP response element-binding protein (CREB) and phosphorylated CREB (pCREB)) were displayed in box plots, histograms and cumulative density function plots, and then analysed using a sign rank test on the treatment difference.

All safety and tolerability endpoints (adverse events, vital signs, ECG and laboratory tests) were listed and summarised. No formal statistical analyses had been carried out.

To assess the pharmacokinetics of GSK256066 and GSK614917, linear and semi-logarithmic individual plasma concentration-time profiles and mean and median profiles were plotted. Plasma concentrations of GSK256066 and GSK614917 were listed and summarised. All derived pharmacokinetic plasma parameters $AUC_{0\text{-last}}$, C_{max}, t_{max} and t_{last} were listed and summarised.

Full details of the statistical methods are provided in the Reporting and Analysis Plan (Attachment 2).

Summary:

Demographics:

Demographics		Total N=32
Sex, n (%)	Males Females	25(78) 7(22)
Age, years	Mean Range	35.7 18-50
Height, cm	Mean Range	177.3 164-191
Weight, kg	Mean Range	75.1 57.0-94.6
BMI, kg/m^2	Mean Range	23.9 18.96-28.58
Race	White – White/Caucasian/European Heritage	32(100)
Ethnicity	Not Hispanic or Latino	32(100)

Source Data: Table 9.3

Pharmacodynamics:

Nasal lavage and scrapes were taken 2 -3 hour post morning dose; bilateral nasal lavage was conducted before the scrape. Nasal scrape samples were taken from alternate nostrils. Nasal scrape samples and samples from nasal lavage were analysed to explore the effects of GSK256066 on novel RNA markers indicative of PDE4 inhibition and on novel protein biomarkers under investigation including pVASP.

Transcriptome Data Analyses

The results of statistical analysis of mRNA raw abundance data from nasal scrape samples are summarised in the following table.

Summary of Results of Statistical Analysis of mRNA Raw Abundance Data

mRNA	Comparison Test / Ref	Ratio (SE logs)	95% CI of Ratio	P-value
CREM	GSK256066 1 μg/Placebo	1.14 (0.037)	(0.96, 1.35)	0.1351
	GSK256066 10 μg/Placebo	1.19 (0.037)	(1.01, 1.41)	0.0383
	GSK256066 50 μg/Placebo	1.20 (0.037)	(1.02, 1.43)	0.0325
	GSK256066 200 μg/Placebo	1.42 (0.037)	(1.20, 1.68)	< 0.0001
DUSP1	GSK256066 1 μg/Placebo	1.45 (0.049)	(1.16, 1.82)	0.0015
	GSK256066 10 μg/Placebo	1.55 (0.049)	(1.24, 1.93)	0.0002
	GSK256066 50 μg/Placebo	1.74 (0.050)	(1.38, 2.17)	< 0.0001
	GSK256066 200 μg/Placebo	1.75 (0.049)	(1.40, 2.18)	< 0.0001
FOSL2	GSK256066 1 μg/Placebo	1.49 (0.043)	(1.22, 1.81)	0.0001
	GSK256066 10 μg/Placebo	1.52 (0.043)	(1.25, 1.85)	< 0.0001
	GSK256066 50 μg/Placebo	1.68 (0.043)	(1.38, 2.05)	< 0.0001
	GSK256066 200 μg/Placebo	1.55 (0.043)	(1.28, 1.89)	< 0.0001
IRS2	GSK256066 1 μg/Placebo	1.55 (0.064)	(1.16, 2.07)	0.0034
	GSK256066 10 μg/Placebo	1.55 (0.063)	(1.17, 2.07)	0.0029
	GSK256066 50 μg/Placebo	1.74 (0.064)	(1.30, 2.33)	0.0003
	GSK256066 200 μg/Placebo	1.56 (0.063)	(1.17, 2.08)	0.0027
NR4A2	GSK256066 1 μg/Placebo	1.54 (0.092)	(1.01, 2.34)	0.0448
	GSK256066 10 μg/Placebo	1.87 (0.091)	(1.24, 2.83)	0.0033
	GSK256066 50 μg/Placebo	2.16 (0.092)	(1.42, 3.30)	0.0004
	GSK256066 200 μg/Placebo	2.03 (0.091)	(1.34, 3.08)	0.0010
PDE4A	GSK256066 1 μg/Placebo	1.03 (0.072)	(0.74, 1.43)	0.8718
	GSK256066 10 μg/Placebo	1.18 (0.071)	(0.86, 1.63)	0.3078
	GSK256066 50 μg/Placebo	1.19 (0.072)	(0.86, 1.66)	0.2909
	GSK256066 200 μg/Placebo	1.38 (0.071)	(0.99, 1.91)	0.0539
RGS1	GSK256066 1 μg/Placebo	1.06 (0.081)	(0.74, 1.54)	0.7393
	GSK256066 10 μg/Placebo	1.21 (0.080)	(0.84, 1.74)	0.2967
	GSK256066 50 μg/Placebo	1.37 (0.081)	(0.95, 1.98)	0.0934
	GSK256066 200 μg/Placebo	1.37 (0.080)	(0.95, 1.97)	0.0919
SNF1LK	GSK256066 1 μg/Placebo	2.72 (0.056)	(2.11, 3.51)	< 0.0001
	GSK256066 10 μg/Placebo	3.04 (0.055)	(2.37, 3.90)	< 0.0001
	GSK256066 50 μg/Placebo	3.28 (0.056)	(2.54, 4.23)	< 0.0001
	GSK256066 200 μg/Placebo	3.32 (0.055)	(2.59, 4.27)	< 0.0001

Source Data: Table 10.3

The majority of the genes showed a statistically significant effect between all doses of GSK256066 and placebo: dual specificity phosphatase 1(DUSP1), fos-like antigen 2(FOSL2), insulin receptor substrate 2 (IRS2), nuclear receptor subfamily 4, group A, member 2 (NR4A2) and SNF1LK. The highest effect was seen in SNF1LK mRNA with the ratios of 2.72, 3.04, 3.28 and 3.32 for treatment ratios 1 μg/Placebo, 10 μg/Placebo, 50 μg/Placebo and 200 μg/Placebo respectively.

cAMP responsive element modulator (CREM) showed a statistically significant effect between all doses of GSK256066 except for the 1 μg dose. PDE4 A and regulator of G-protein signalling 1(RGS1) had no significant effect on any of the doses.

Although there is an effect between placebo and doses of GSK256066 for most of the genes, the large over lapping CI suggest a large amount of variability in the data and that there is not a relationship between increasing doses.

The results of the dose response statistical analysis of mRNA raw abundance data are summarised in the following table.

Summary of Results of Dose Response Statistical Analysis of mRNA Raw Abundance Data

mRNA	Treatment Relationship	Slope Log10 (Analyte) vs Dose	95% CI of Slope
CREM	Placebo & GSK256066 1 μg	-0.0306	(-0.0889, 0.0277)
	Placebo & GSK256066 10 μg	-0.0029	(-0.0087, 0.0028)
	Placebo & GSK256066 50 μg	-0.0011	(-0.0020, -0.0001)
	Placebo & GSK256066 200 μg	0.0001	(-0.0003, 0.0005)
	GSK256066 1 μg & GSK256066 10 μg	0.0000	(-0.0058, 0.0058)
	GSK256066 1 μg & GSK256066 50 μg	-0.0005	(-0.0011, 0.0002)
	GSK256066 1 μg & GSK256066 200 μg	0.0002	(-0.0001, 0.0006)
	GSK256066 10 μg & GSK256066 50 μg	-0.0004	(-0.0017, 0.0009)
	GSK256066 10 μg & GSK256066 200 μg	0.0003	(-0.0001, 0.0007)
	GSK256066 50 μg & GSK256066 200 μg	0.0004	(-0.0001, 0.0010)
DUSP1	Placebo & GSK256066 1 μg	0.0795	(-0.0132, 0.1722)
	Placebo & GSK256066 10 μg	0.0081	(0.0023, 0.0138)
	Placebo & GSK256066 50 μg	0.0021	(0.0006, 0.0037)
	Placebo & GSK256066 200 μg	0.0004	(0.0001, 0.0008)
	GSK256066 1 μg & GSK256066 10 μg	0.0003	(-0.0071, 0.0077)
	GSK256066 1 μg & GSK256066 50 μg	0.0007	(-0.0006,0.0021)
	GSK256066 1 μg & GSK256066 200 μg	0.0001	(-0.0003, 0.0005)
	GSK256066 10 μg & GSK256066 50 μg	0.0006	(-0.0011, 0.0023)
	GSK256066 10 μg & GSK256066 200 μg	0.0001	(-0.0003, 0.0004)
	GSK256066 50 μg & GSK256066 200 μg	-0.0001	(-0.0008, 0.0005)

Continued

Summary of Results of Dose Response Statistical Analysis of mRNA Raw Abundance Data (Continued)

mRNA	Treatment Relationship	Slope Log10 (Analyte) vs Dose	95% CI of Slope
FOSL2	Placebo & GSK256066 1 μg	0.0751	(0.0072, 0.1430)
	Placebo & GSK256066 10 μg	0.0062	(0.0017, 0.0108)
	Placebo & GSK256066 50 μg	0.0016	(0.0002, 0.0031)
	Placebo & GSK256066 200 μg	0.0001	(-0.0002, 0.0005)
	GSK256066 1 μg & GSK256066 10 μg	-0.0018	(-0.0080, 0.0045)
	GSK256066 1 μg & GSK256066 50 μg	0.0001	(-0.0007, 0.0010)
	GSK256066 1 μg & GSK256066 200 μg	-0.0003	(-0.0006, 0.0000)
	GSK256066 10 μg & GSK256066 50 μg	0.0004	(-0.0011, 0.0020)
	GSK256066 10 μg & GSK256066 200 μg	-0.0002	(-0.0005, 0.0001)
	GSK256066 50 μg & GSK256066 200 μg	-0.0004	(-0.0007, 0.0000)
IRS2	Placebo & GSK256066 1 μg	0.1145	(0.0187, 0.2103)
	Placebo & GSK256066 10 μg	0.0082	(-0.0007, 0.0171)
	Placebo & GSK256066 50 μg	0.0021	(0.0001, 0.0041)
	Placebo & GSK256066 200 μg	0.0002	(-0.0005, 0.0009)
	GSK256066 1 μg & GSK256066 10 μg	-0.0034	(-0.0171, 0.0104)
	GSK256066 1 μg & GSK256066 50 μg	0.0001	(-0.0023, 0.0026)
	GSK256066 1 μg & GSK256066 200 μg	-0.0002	(-0.0008, 0.0003)
	GSK256066 10 μg & GSK256066 50 μg	0.0007	(-0.0017, 0.0031)
	GSK256066 10 μg & GSK256066 200 μg	-0.0002	(-0.0008, 0.0004)
	GSK256066 50 μg & GSK256066 200 μg	-0.0004	(-0.0012, 0.0004)
NR4A2	Placebo & GSK256066 1 μg	0.0908	(-0.0890, 0.2706)
	Placebo & GSK256066 10 μg	0.0161	(0.0029, 0.0294)
	Placebo & GSK256066 50 μg	0.0040	(0.0011, 0.0068)
	Placebo & GSK256066 200 μg	0.0008	(0.0000, 0.0016)
	GSK256066 1 μg & GSK256066 10 μg	0.0070	(-0.0130, 0.0271)
	GSK256066 1 μg & GSK256066 50 μg	0.0023	(-0.0011, 0.0056)
	GSK256066 1 μg & GSK256066 200 μg	0.0004	(-0.0007, 0.0015)
	GSK256066 10 μg & GSK256066 50 μg	0.0008	(-0.0029, 0.0046)
	GSK256066 10 μg & GSK256066 200 μg	0.0000	(-0.0008, 0.0008)
	GSK256066 50 μg & GSK256066 200 μg	-0.0000	(-0.0010, 0.0010)
SNF1LK	Placebo & GSK256066 1 μg	0.3379	(0.2419, 0.4338)
	Placebo & GSK256066 10 μg	0.0364	(0.0290, 0.0438)
	Placebo & GSK256066 50 μg	0.0076	(0.0060, 0.0092)
	Placebo & GSK256066 200 μg	0.0018	(0.0014, 0.0022)
	GSK256066 1 μg & GSK256066 10 μg	0.0022	(-0.0081, 0.0126)
	GSK256066 1 μg & GSK256066 50 μg	0.0007	(-0.0009, 0.0023)
	GSK256066 1 μg & GSK256066 200 μg	0.0002	(-0.0002, 0.0005)
	GSK256066 10 μg & GSK256066 50 μg	0.0002	(-0.0023, 0.0027)
	GSK256066 10 μg & GSK256066 200 μg	-0.0000	(-0.0004, 0.0003)
	GSK256066 50 μg & GSK256066 200 μg	-0.0001	(-0.0006, 0.0004)

Source Data: Table 10.4

From the dose response analysis all genes except for CREM had positive slopes between placebo and at least two doses of GSK256066, with confidence intervals that excluded zero, suggesting there were some dose response relationships when comparing placebo with doses of GSK256066.

For treatment comparisons that did not include placebo (i.e. comparison made between 1 μg, 10 μg, 50 μg and 200 μg) all genes resulted in slopes with 95% confidence intervals that included zero, indicating there was no evidence to suggest a dose response relationship between varying doses of GSK256066.

For the SNF1LK gene, the slopes were significant (the confidence intervals excluded zero) for comparisons made between placebo and all doses of GSK256066. The placebo and 1 μg comparison had a slope of 0.3379 with 95% confidence intervals of 0.2419 and 0.4338. For placebo compared with the 10 μg, 50 μg and 200 μg doses, the slopes were much smaller than for the 1 μg dose 0.0364, 0.0076 and 0.0018 respectively, indicating that the maximum dose response had been reached at the 1 μg dose. For FOSL2 and IRS2 the largest significant slopes were also observed between placebo and the 1 μg dose, with slopes of 0.0751 and 0.1145 respectively, suggesting a maximum dose response (of small magnitude as the slopes were fairly flat, particularly for FOSL2) had been reached at the 1 μg dose.

There were no significant slopes for any of the treatment comparisons looked at for the CREM gene.

Although the majority of the genes had a significant treatment effect between all doses of GSK256066 and placebo in the transcriptome analysis, there was no evidence of a dose response relationship between varying doses of GSK256066. For comparisons made between placebo and doses of GSK256066 there was evidence to suggest that the maximum dose response had been reached at the 1 μg dose for the SNF1LK gene, and on a smaller magnitude for FOSL2 and IRS2. All other slopes were not significant or too small to be considered clinically meaningful.

Protein Expression Data

The results of statistical analysis of protein expression data from nasal lavage samples are summarised in the following table.

Summary of Results of Statistical Analysis of Protein Expression Data

Analyte	Treatment Difference	n	Median	Mean	SD	Sign Rank Statistic	P-Value
CREB	GSK256066 1 μg -Placebo	25	0.1000	-2.6402	15.03799	-23.5	0.538158
	GSK256066 10 μg - Placebo	29	-0.5747	-1.6965	15.34759	-33.0	0.438270
	GSK256066 50 μg - Placebo	26	0.5617	-0.2063	14.97070	4.5	0.906525
	GSK256066 200 μg - Placebo	29	-0.3150	-3.6663	14.74383	-40.0	0.371913
VASP	GSK256066 1 μg -Placebo	30	0.0000	-0.5545	7.79598	17.5	0.647226
	GSK256066 10 μg - Placebo	31	0.0000	-0.3816	9.00754	2.5	0.950846
	GSK256066 50 μg - Placebo	29	0.3000	0.0256	9.24118	21.0	0.534998
	GSK256066 200 μg - Placebo	31	0.0000	-1.3371	7.54590	-10.0	0.815273
pCREB	GSK256066 1 μg -Placebo	30	-0.3755	-1.2526	4.61665	-48.5	0.224767
	GSK256066 10 μg - Placebo	31	0.0000	-0.8228	3.16845	-32.5	0.491866
	GSK256066 50 μg - Placebo	30	-0.2534	-1.3175	3.90084	-55.0	0.191599
	GSK256066 200 μg - Placebo	30	-0.2921	-1.4366	3.79880	-69.5	0.059804
pVASP	GSK256066 1 μg -Placebo	27	0.0000	-2.8053	10.88217	-27.0	0.329983
	GSK256066 10 μg - Placebo	29	0.0000	-1.6602	12.30724	16.0	0.657293
	GSK256066 50 μg - Placebo	27	0.0000	0.1560	4.73976	-4.0	0.898317
	GSK256066 200 μg - Placebo	29	0.0000	-1.5165	12.70748	7.5	0.844794

Source Data: Table 10.6

For each of the analytes CREB, VASP, pCREB and pVASP, there was no evidence to support the average difference between placebo and any dose of GSK256066 as being significantly different. The largest sign rank statistic was seen for the pCREB analyte, when looking at the 200 μg dose – placebo, this was a negative difference of -69.5 with a marginally significant p-value of 0.0598.

SNF1LK Protein Biomarker Results

Nasal lavage cytospins were stained with a SNF1LK specific monoclonal antibody by indirect immunofluorescence. There were no differences in intensity of SNF1LK immunostaining between the different treatments. Mean intensities of staining for SNF1LK were low. There were no differences in nuclear/cytoplasmic ratio of SNF1LK immunostaining between the different treatments.

Safety:

There were no deaths, non-fatal serious adverse events or pregnancies reported in this study.

Twenty-four out of 32 subjects (75%) who received the study drug reported a total of 48 AEs. Headache was the most frequently reported AE across all the treatment groups. Thirty AEs were considered to be drug-related by the Investigator. All the AEs except two were mild in intensity. [redacted] The event of nasal necrosis

was considered to be drug-related by the Investigator. There were no AEs of severe intensity. [redacted] The adverse events are summarised in the following table.

Summary of Number of Subjects Reporting At Least One Adverse Event

Most Frequent Adverse Events	Placebo N=31	GSK256066 1 µg N=30	GSK256066 10 µg N=32	GSK256066 50 µg N=30	GSK256066 200 µg N=31
	n (%)	n (%)	n (%)	n (%)	n (%)
Any AE	7(23)	6(20)	6(19)	8(27)	10(32)
Any AE related to investigational product	5(16)	6(20)	1(3)	7(23)	5(16)
Most Common AEs:					
Headache	3(10)	1(3)	2(6)	3(10)	4(13)
Dysgeusia	0	1(3)	0	0	0
Rhinits	2(6)	0	0	0	1(3)
Nasopharyngitis	0	0	0	0	2(6)
Sinusitis	0	0	1(3)	0	0
Epistaxis	0	0	0	1(3)	2(6)
Throat irritation	1(3)	0	0	0	1(3)
Nasal necrosis	0	1(3)	0	0	0
Pharyngolaryngeal pain	0	0	0	1(3)	0
Eye pruritis	2(6)	1(3)	0	0	0
Ocular hyperaemia	0	1(3)	0	0	1(3)
Photophobia	0	0	0	0	1(3)
Diarrhoea	1(3)	0	0	1(3)	0
Constipation	0	0	0	0	1(3)
Dry mouth	0	1(3)	0	0	0
Gingivitis	0	0	1(3)	0	0
Fatigue	0	1(3)	0	1(3)	1(3)
Acne	0	0	0	1(3)	0
Heat rash	0	0	1(3)	0	0
Supraventricular tachycardia[1]	0	0	0	0	1(3)
Skin laceration	0	0	0	0	1(3)
Listless	0	0	1(3)	0	0
Post menopausal haemorrhage	1(3)	0	0	0	0

Source Data: Table 13.2 and Table 13.3

1. [redacted]

Summary of Number of Subjects Reporting At Least One Drug-Related Adverse Event

Drug-related Adverse Events	Placebo N=31	GSK256066 1 µg N=30	GSK256066 10 µg N=32	GSK256066 50 µg N=30	GSK256066 200 µg N=31
	n (%)	n (%)	n (%)	n (%)	n (%)
Any AE related to investigational product	5(16)	6(20)	1(3)	7(23)	5(16)
Headache	3(10)	1(3)	1(3)	3(10)	2(6)
Dysgeusia	0	1(3)	0	0	0
Epistaxis	0	0	0	1(3)	2(6)
Throat irritation	0	0	0	0	1(3)
Nasal necrosis	0	1(3)	0	0	0
Pharyngolaryngeal pain	0	0	0	1(3)	0
Eye pruritis	1(3)	1(3)	0	0	0
Ocular hyperaemia	0	1(3)	0	0	0
Diarrhoea	1(3)	0	0	1(3)	0
Dry mouth	0	1(3)	0	0	0
Fatigue	0	1(3)	0	1(3)	1(3)
Supraventricular tachycardia[1]	0	0	0	0	1(3)
Rhinitis	0	0	0	0	1(3)
Nasopharyngitis	0	0	0	0	1(3)

Source Data: Table 13.3

1. [redacted]

Concomitant Medications

Four subjects received concomitant medications (Table 9.4). [redacted] These medications were considered not likely to affect the outcome of the study or safety of the subject.

Clinical Laboratory Evaluation

Laboratory data including haematology (Table 13.4), clinical chemistry (Table 13.5) and urinalysis (Table 13.7) were listed and summarised.

Haematology values of potential clinical concern were reported for 4 subjects (Table 13.16). [redacted]

[redacted] None of these values was considered by the Investigator to be clinically significant.

Clinical chemistry values of potential clinical concern were reported for 4 subjects (Table 13.17). [redacted] None of these values were considered by the Investigator to be clinically significant.

The urinalysis dipstick and pH results showed no abnormalities of clinical concern (Table 13.7).

Troponin levels were normal for all the subjects (Table 13.6)

Electrocardiography

ECG variables evaluated included heart rate, PR interval, QRS duration, QT interval, QTcB and QTcF. [redacted]

ECG findings are summarised in Table 13.8. [redacted] This AE was considered to be drug-related by the Investigator and the Subject was withdrawn from the study. [redacted]

No corrected or uncorrected QT intervals >500 msec were observed in this study. No corrected or uncorrected QT intervals extended beyond 60 msec compared to baseline (Table 13.12 and Table 13.13).

Vital Signs

Vital signs measurements comprised heart rate, systolic and diastolic blood pressure. No clinically significant abnormalities in vital signs were recorded.

Pulmonary function Tests:

Pulmonary function test results were within the normal range.

Pharmacokinetics:

All 32 mild to moderate allergic rhinitic subjects who received at least one dose of the active investigational product provided at least one sample for plasma PK analysis

following single doses of 1, 10, 50 and 200 µg GSK256066. Serial blood samples for PK analyses of GSK256066 and its metabolite GSK614917 were collected at pre-dose, 0.25 h, 0.5 h, 1 h, 2 h, 3 h and 4 h post-dose. [redacted]

Following a single dose of either 1 or 10 µg GSK256066, the vast majority of samples were non-quantifiable for both GSK256066 and GSK614917.

Individual plasma GSK256066 and GSK614917 concentration-time profiles were plotted on linear and semi-logarithmic scales (Figure 11.1 and Figure 11.2 respectively).

Plasma GSK256066 and GSK614917 concentration-time data are summarised in Table 11.1 and Table 11.2, respectively.

The PK parameters for GSK256066 and GSK614917 are summarised and presented in Table 11.3 and Table 11.4, respectively.

Derived GSK256066 PK parameters are summarised in the following table.

Summary of Derived GSK256066 Parameters AUC and C_{max}

GSK256066 Parameter	Dose µg	N	n[1]	Geometric Mean	95% Confidence Interval	%CV
$AUC_{(0\text{-}last)}$ [pg.h/mL][2]	10	31	4	7.7	(3.3, 18.0)	58
	50	30	18	14.7	(11.2, 19.3)	59
	200	31	29	41.9	(30.7, 57.1)	97
C_{max} [pg/mL]	1	30	3	6.3	(0.3, 144.4)	198
	10	31	10	5.1	(2.7, 9.6)	110
	50	30	29	5.9	(4.7, 7.5)	70
	200	31	30	20.4	(14.3, 29.1)	121

Source Data: Table 11.3

1. Number of subjects in which the PK parameter could be derived
2. $AUC_{(0\text{-}last)}$ was not calculable for any subject at 1 µg GSK256066 dose

Summary of Other Derived GSK256066 Pharmacokinetic Parameters

GSK256066 Parameter	Dose µg	N	n[1]	Median	Range
t_{last} [h]	1	30	3	3.00	[0.25 - 3.98]
	10	31	10	2.00	[0.52 - 4.00]
	50	30	29	3.02	[2.00 - 4.37]
	200	31	30	4.00	[1.03 - 4.08]
t_{max} [h]	1	30	3	3.00	[0.25 - 3.98]
	10	31	10	2.00	[0.50 - 4.00]
	50	30	29	2.00	[1.00 - 3.03]
	200	31	30	2.00	[1.00 - 4.05]

Source Data: Table 11.3

1. Number of subjects in which the PK parameter could be derived

Derived GSK614917 PK parameters are also summarised in the following table.

Summary of Derived GSK614917 Parameters AUC and C_{max}

GSK614917 Parameter	Dose µg	N	n[1]	Geometric Mean	95% Confidence Interval	%CVb
$AUC_{(0\text{-}last)}$ [pg.h/mL][2]	200	25	18	12.7	(9.5, 16.9)	63.0
C_{max} [pg/mL][3]	10	25	3	2.7	(0.9, 8.2)	47.6
	50	25	6	2.4	(2.0, 3.0)	20.1
	200	25	24	5.2	(4.1, 6.6)	62.4

Source Data: Table 11.4

1. Number of subjects for which the PK parameter could be derived
2. $AUC_{(0\text{-}last)}$ was not calculable in any subject at the 1, 10 or 50 µg GSK256066 dose
3. C_{max} was not calculable for any subject at the 1µg GSK256066 dose

Summary of Other Derived GSK614917 Pharmacokinetic Parameters

GSK614917 Parameter	Dose µg	N	n[1]	Median	Range
t_{last} [h][2]	10	25	3	2.00	[0.52 - 3.98]
	50	25	6	2.51	[1.97 - 4.00]
	200	25	24	4.00	[2.98 - 4.08]
t_{max} [h][2]	10	25	3	2.00	[0.52 - 3.98]
	50	25	6	2.01	[1.97 - 4.00]
	200	25	24	3.00	[2.00 - 4.05]

Source Data: Table 11.4

1. Number of subjects for which the PK parameter could be derived
2. t_{max} and t_{last} could not be determined for any subject at the 1µg GSK256066 dose

Nasal Lavage Concentrations

Nasal lavage samples were taken 2 -3 hour post morning dose and analysed for GSK256066.

Quantifiable concentrations of GSK256066 were observed in nasal lavage samples (Figure 11.9). Summary data are summarised and presented in Table 11.5 and in. Placebo nasal lavage samples were analysed in error and summary data are also presented in. Twelve out of 32 subjects had measurable drug concentrations in placebo samples. [redacted] This suggests that a carry-over effect from previous treatment is unlikely.

GSK256066 Nasal Lavage Pharmacokinetic Concentration Data

Treatment	N	n	Geometric Mean	95% Confidence Interval	%CV
Placebo	31	31	24.0	(10.1, 57.2)	261.2
GSK256066 1 μg	30	30	124.7	(81.3, 191.4)	165.1
GSK256066 10 μg	32	32	541.1	(375.4, 779.8)	130.3
GSK256066 50 μg	30	30	1046.1	(685.0, 1597.6)	161.8
GSK256066 200 μg	31	31	2226.6	(1469.1, 3375.0)	161.7

Source Data: Table 11.5
NB: Raw concentration data-No correction for lavage dilution was made.

Pharmacogenetics:

During the course of the study, 29 subjects provided blood samples for pharmacogenetic analysis. The pharmacogenetic results will be reported in a separate study report if applicable.

Conclusions:

- The majority of the genes from the transcriptome analysis showed a statistically significant effect between all doses of GSK256066 and placebo: DUSP1, FOSL2, IRS2, NR4A2 and SNF1LK. The highest effect was seen in the SNF1LK gene.
- There was no evidence of a dose response relationship between varying doses of GSK256066. For comparisons made between placebo and doses of GSK256066 there was evidence to suggest that the maximum dose response had been reached at the 1 μg dose for the SNF1LK gene, and on a smaller magnitude for FOSL2 and IRS2. All other placebo/GSK256066 dose pair wise comparisons were not significant or too small to be considered meaningful.
- For each of the analytes CREB, VASP, pCREB and pVASP, there was no evidence to support the average difference between placebo and any dose of GSK256066 as being significantly different.
- Intranasal GSK256066 appeared to be generally well tolerated up to a dose of 200 μg demonstrating an acceptable safety profile at the dose tested in healthy male and female subjects with SAR.
- At the lower doses of GSK256066, plasma concentrations of either GSK256066 or its metabolite, GSK614917, were unquantifiable in the majority of subjects.
- High variability in the PK of GSK256066 at the higher doses was observed.
- Quantifiable levels of GSK256066 were observed in nasal lavage samples obtained 2-3 hours post-dose.

Date of Report:

November 2007.