Synopsis

Identifier: GM2006/00630/00 **Study Number:** INO102141

Title: Interim Report: A two-centre, randomised, double-blind, double-dummy, placebo-controlled, 3-period cross-over study to evaluate the effect of treatment with repeat doses of GW274150 on the allergen-induced late asthmatic response in subjects with mild asthma

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Study period: 05Nov2004 - 28Oct2005

Phase of development: I

Objectives:

Primary Objective

- To evaluate the effect of treatment with repeat oral doses of GW274150 for 14 days on the late asthmatic response (LAR) to inhaled allergen in mild asthmatic subjects compared with placebo.

Secondary Objectives

- To evaluate the effect of treatment with repeat oral doses of GW274150 for 14 days on bronchial hyper-reactivity in response to methacholine challenge following allergen challenge compared with placebo in mild asthmatic subjects.
- To evaluate the effect of treatment with repeat oral doses of GW274150 on bronchial hyper-reactivity in response to adenosine monophosphate (AMP) challenge on Day 10 compared with placebo in mild asthmatic subjects.
- To evaluate the effect of treatment with repeat oral doses of GW274150 for 14 days on the early asthmatic response (EAR) after allergen challenge in mild asthmatic subjects compared with placebo.
- To evaluate the effect of treatment with repeat oral doses of GW274150 on concentrations of exhaled nitric oxide on Days 1, 7, 10, 14 and 15 in subjects with mild asthma compared with placebo.
- To assess the safety and tolerability of repeat oral doses of GW274150 in mild asthmatic subjects compared with placebo.
- To evaluate the effect of treatment with repeat oral doses of GW274150 on established markers of inflammation and iNOS activity in blood, bronchoalveolar lavage (BAL) and bronchial biopsy in mild asthmatic subjects.

Tertiary Objectives

- To evaluate the effect of treatment with repeat oral doses of GW274150 on exploratory markers of inflammation and inducible nitric oxide synthase (iNOS) activity in blood, urine, BAL and bronchial biopsy in mild asthmatic subjects.
- To assess the effect of treatment with repeat oral doses of GW274150 on bronchial mucosal blood flow in mild asthmatic subjects during challenge with AMP on Day 10.

Endpoints:

Primary Endpoints

- Late asthmatic response: minimum forced expiratory volume in 1 second (FEV1) between 4–10 h after allergen challenge on Day 14 of each treatment period.

Secondary Endpoints

- Late asthmatic response: weighted mean FEV1 4–10 h after allergen challenge on Day 14 of each treatment period.
- Provocative concentration of methacholine resulting in a 20% reduction in FEV1 (PC20) on Day 15 of each treatment period.
- Provocative concentration of AMP resulting in a 20% reduction in FEV1 (PC20) on Day 10 of each treatment period.
- Early asthmatic response: minimum FEV1 and weighted mean FEV1 0–2 h after allergen challenge on Day 14 of each treatment period.
- Concentration of exhaled NO pre-dose on Days 1, 7, 10 and 14, post-dose on Day 14 and post-methacholine challenge on Day 15 of each treatment period.
- Incidence of treatment emergent adverse events (AEs).
- Vital signs, electrocardiogram (ECG), FEV1 and safety laboratory parameters.
- Established markers of inflammation in bronchial biopsies, BAL, and blood.

Tertiary Endpoints

- Exploratory markers of inflammation in bronchial biopsies and BAL and markers of iNOS activity in blood (3-nitrotyrosine and tyrosine), urine (3-nitro-4 hydroxyphenylacetic acid, NHPA, and para-hydroxyphenylacetic acid, PHPA) and BAL (3-nitrotyrosine and tyrosine).
- Bronchial mucosal blood flow marker (change in concentration of acetylene with time) during challenge with AMP on Day 10.

Methodology:

This was a two-centre, randomised, double-blind, double-dummy, placebo-controlled, three-period crossover study in subjects with mild asthma. The total duration of the study for each subject was 97–175 days, including screening and follow-up.

Successfully screened subjects underwent three 15-day treatment periods, each separated by a washout period of 14–28 days. Eligible subjects attended the study unit on the morning of Day 1 of the first dosing occasion, having fasted for at least 8 h. Prior to dosing on this visit, subjects were randomised to one of the 6 treatment sequences in the following table:

Period 1	Period 2	Period 3
Placebo	Singulair 10mg	GW274150 90mg
Placebo	GW274150 90mg	Singulair 10mg
Singulair 10mg	Placebo	GW274150 90mg
Singulair 10mg	GW274150 90mg	Placebo
GW274150 90mg	Placebo	Singulair 10mg
GW274150 90mg	Singulair 10mg	Placebo

Each treatment period consisted of once daily, randomised treatment for 14 days, plus one extra day for assessments. A follow-up visit took place 10–14 days after the last dose of study medication.

Eligibility Criteria:

Subjects were eligible for inclusion in the study only if all of the following criteria applied:

1. Males and females aged 18 to 55 years inclusive.
2. Documented history of bronchial asthma, first diagnosed at least 6 months prior to the screening visit and currently being treated only with intermittent short-acting β-agonist therapy by inhalation.
3. Pre-bronchodilator FEV1 >65% of predicted at screening.
4. Documented sensitivity to AMP with an AMP PC20 of less than 100 mg/mL at screening.
5. Documented sensitivity to methacholine with a methacholine PC20 <8 mg/mL or provocative dose of methacholine ≤3.2 mg (PD20) resulting in a 20% fall in FEV1 at screening.
6. No history of smoking within 6 months of the start of the study, and with a total pack year history of ≤10 pack years.
7. Demonstration of a positive wheal and flare reaction (≤3 mm relative to negative control) to at least one allergen from a battery of allergens (including house dust mite, grass pollen and cat dander) on skin prick testing at screening, or within 12 months of study start.
8. Screening allergen challenge demonstrated that the subject experienced both an EAR and LAR. The EAR had to include a fall in FEV1 of ≥20% from the post-saline value, on at least one occasion, between 5 and 30 minutes after the final concentration of allergen. The LAR had to include a fall in FEV1 of ≥15% from the

post-saline value, on at least three occasions, two of which were to be consecutive, between 4 and 10 h after the final concentration of allergen.

9. Able and willing to give written informed consent to take part in the study.
10. Available to complete all study measurements.

Subjects were not eligible for inclusion in this study if any of the following criteria applied:

1. Past or present disease that, as judged by the investigator, could have affected the outcome of this study. These diseases included, but were not limited to, cardiovascular disease, malignancy, hepatic disease, renal disease, haematological disease, neurological disease, endocrine disease or pulmonary disease (including but not confined to chronic bronchitis, emphysema, bronchiectasis or pulmonary fibrosis).
2. Clinically significant abnormalities in safety laboratory analysis at screening.
3. Subject had known history of hypertension or was hypertensive at screening. Hypertension at screening was defined as persistent systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg.
4. Known hypersensitivity to Singulair.
5. Respiratory tract infection and/or exacerbation of asthma within 4 weeks prior to the first dose of study medication.
6. History of life-threatening asthma, defined as an asthma episode that required intubation and/or was associated with hypercapnoea, respiratory arrest and/or hypoxic seizures.
7. Administration of oral, injectable or dermal steroids within 5 weeks or intranasal and/or inhaled steroids within 1 week of the screening visit.
8. Unable to abstain from other medications including non-steroidal anti-inflammatory drugs, anti-depressant drugs, anti-histamines and anti-asthma, anti-rhinitis or hay fever medication, other than short acting inhaled β-agonists and paracetamol (up to 4 g per day) for the treatment of minor ailments, e.g., headache from 48 h before the first dose until the follow-up visit.
9. If, after two concurrent administrations of saline during the AMP, methacholine or allergen challenge at screening the subjects still had a fall in FEV1 of greater than 10%.
10. The subject had participated in a study with a new molecular entity during the previous 3 months.
11. History of being unable to tolerate or complete methacholine, AMP and/or allergen challenge tests.
12. Subject was undergoing allergen desensitisation therapy.
13. There was a risk of non-compliance with study medication or study procedures.
14. History of blood donation (450 mL) within 2 months of starting the clinical study.

15. The subject regularly drank more than 28 units of alcohol in a week if male, or 21 units per week if female. One unit of alcohol was defined as a medium (125 mL) glass of wine, half a pint (250 mL) of beer or one measure (24 mL) of spirits.
16. The subject had a screening QTc value of >430 msec, PR interval outside the range 120 to 200 msec or an ECG that was not suitable for QT measurements (e.g., poorly defined termination of the T-wave).
17. The subject had tested positive for hepatitis C antibody or hepatitis B surface antigen.
18. The subject had tested positive for human immunodeficiency virus antibodies.
19. The subject had a positive drugs-of-abuse test.

Treatment administration:

Each of the three treatment periods consisted of randomised treatment, taken once daily for 14 days. All tablets were swallowed without chewing with at least 240 mL of water. Treatments were administered as follows:

- GW274150 90 mg: three GW274150 30 mg tablets plus one placebo tablet to match Singulair.
- Placebo: three placebo tablets to match GW274150 plus one placebo tablet to match Singulair.
- Singulair 10 mg: one Singulair 10 mg tablet plus three placebo tablets to match GW274150.

In each treatment period, subjects took the medication at home on Days 2–6, 8–9 and 11–13 inclusive. Subjects completed a dosing diary on these days to record the dates, times and amount of dosing. Staff checked the diary during visits to the study unit. On Days 1, 7, 10 and 14, medication was administered under supervision at the study unit.

Criteria for evaluation:

- Efficacy: allergen, methacholine and AMP challenges.
- Pharmacodynamics: exhaled nitric oxide levels.
- Safety: adverse events (AEs), clinical laboratory parameters (clinical chemistry, haematology, urinalysis), forced expiratory volume in 1 second (FEV1), 12-lead electrocardiogram (ECG) and vital signs.
- Biomarkers: blood, bronchoalveolar lavage (BAL) and urine samples, bronchial biopsies and mucosal blood flow.
- Pharmacokinetics: sparse blood samples to determine plasma concentrations of GW274150 and Singulair.

Each subject was to have a bronchoscopy following placebo treatment and was also randomly assigned to have a biopsy following treatment with either GW274150 or Singulair (ratio 2:1).

Statistical Considerations:

Sample Size Assumptions:

It was planned that 28 subjects would be recruited into this study to allow for 22 evaluable subjects (i.e., subjects who completed all treatment periods). The sample size calculations were based primarily on the number of subjects required to detect a 50% attenuation from the change from baseline placebo response for the GW274150 treatment group for minimum LAR following allergen challenge on Day 14. The sample size was also required to detect a difference of two doubling doses comparing GW274150 with placebo PC20 following methacholine challenge on Day 15, and also following AMP challenge on Day 10. All sample size calculations were based upon 90% power and a two-sided 5% significance level.

Hypotheses:

The primary objective of this study was:

- To evaluate the effect of treatment with repeat oral doses of GW274150 for 14 days on the late asthmatic response (LAR) to inhaled allergen in mild asthmatic subjects compared with placebo.

The null and alternative hypotheses for the primary objective were:

$$H0: |\mu_T - \mu_R| = 0$$

$$H1: |\mu_T - \mu_R| \neq 0$$

μ_T = expected mean minimum LAR (i.e. minimum FEV1 between 4-10hrs after allergen challenge) on GW274150 on Day 14

μ_R = expected mean minimum LAR on placebo on Day 14

A difference was to be demonstrated if the two-sided 95% confidence interval for the difference between the treatment groups did not contain zero.

Statistical Methods:

No interim analyses were planned or performed.

Absolute change from baseline LAR endpoints (minimum and weighted mean FEV1 over 4-10 hours) and EAR endpoints (minimum and weighted mean FEV1 over 0-2 hours) were derived. A statistical analysis was performed on each of the absolute change from baseline LAR and EAR endpoints to compare both active treatment groups with placebo. Mixed effects models were fitted with the factors treatment and period fitted fixed effects and subject fitted as a random effect. Day 14 post-saline baseline (highest post-saline FEV1 value) was also fitted as a covariate (fixed effect).

A statistical analysis was performed on the $\log_2$-transformed PC20 values for methacholine (day 15) and AMP (day 10) challenges separately, to compare both active

treatment groups with placebo. A log_2- transformation was applied to the PC20 data as the results are interpreted in terms of a doubling dose difference. Mixed effects models were fitted with the factors treatment and period fitted as fixed effects and subject fitted as a random effect. Log_2 post-saline baseline (highest post-saline FEV1 value) was fitted as a covariate (fixed effect).

Exhaled NO concentrations were summarised. A repeated measures mixed effects model was fitted to trough (pre-dose on days 7, 10, and 14) change from baseline ratios following a log_e-transformation. Period, treatment group, log_e-transformed baseline (pre-dose on day 1), day, treatment group by day interaction term and log_e-transformed baseline by day interaction term were fitted as fixed effects and subject was fitted as a random effect.

The change from baseline ratio exhaled NO $minimum_{(0\text{-}24h)}$ and weighted $mean_{(0\text{-}24h)}$ endpoints were derived and then analysed separately following a log_e-transformation to compare both active treatments with placebo. Mixed effects models were fitted with the factors treatment and period fitted as fixed effects and subject fitted as a random effect. Log_e-transformed baseline was fitted as a covariate (fixed effect).

All safety data (adverse events, vital signs, ECG, laboratory tests and non-challenge FEV_1) were summarised.

Plasma concentrations of GW685698 and Singulair were summarised.

The mucosal blood flow, biomarker and bronchial biopsy listings and summaries are currently unavailable.

Summary:

Number of subjects:

Number of Subjects	Total (N = 28)
Planned, N	28
Randomized, N	28
Completed, n (%)	25 (89)
Total Withdrawn (any reason), n (%)	3 (11)
Withdrawn due to serious adverse event, n (%)	0
Withdrawn due to adverse events, n (%)	2 (7)
Withdrawn due to subject deciding to withdraw, n (%)	1 (4)

Analysis Populations:

The 'All Subjects' population was defined as all subjects randomised to treatment who received at least one dose of study treatment (including placebo). This population was used for all listings, tables and figures with the exception of PK concentration listings and tables. All 28 subjects were included in the 'All Subjects' population.

The 'PK Concentration' population was defined as all subjects from the 'All Subjects' population for whom blood samples were taken for assaying GW274150 and/or Singulair. All 28 subjects were included on the GW274150 PK concentration listing, as this listing included placebo and GW274150 90mg treatment periods (Note: all subjects including those who withdrew received placebo). The 26 subjects who completed the GW274150 treatment periods were included when summarising the GW274150 concentration data (includes the 25 subjects who completed the study plus subject 13 who withdrew). All twenty-five subjects who completed the study were included on the Singulair PK concentration listing and when summarising the Singulair concentration data. None of the subjects who withdrew received Singulair.

Demographics

Demographic parameters are summarised in the following table:

Parameter	Total (N = 28)
Age; years	
Adults (protocol range: 18–55 years):	
Mean (range)	30.6 (21–43)
Sex; n (%)	
Female:	11 (39)
Male:	17 (61)
Race; n (%)	
White – White/Caucasian/European Heritage:	25 (89)
African American/African Heritage:	2 (7)
Asian –Central / South Asian Heritage:	1 (4)
Height; cm	
Mean (range)	173.0 (161–187)
Weight; kg	
Mean (range)	73.28 (54.9–97.0)
Body mass index; kg/m^2	
Mean (range)	24.41 (20.4–32.1)

Efficacy

Allergen Challenge:

The allergen challenge was performed 1 hr post-dose on day 14. The LAR was derived from the FEV1 4-10hr post-inhalation time-points. The minimum FEV1 (4-10hrs) was the primary endpoint, weighted mean FEV1 (4-10hrs) was a secondary endpoint. The following table summarises the results of the statistical analysis performed on both LAR endpoints.

LAR Endpoint	Treatment Comparison	Adjusted Mean Change from Baseline (L)		Treatment Difference (L) (Active – Placebo)	95% CI (Lower, Upper)	% Attenuation from Placebo Response
		Active[a]	Placebo[b]			
Minimum	GW274150 90mg vs Placebo	-1.17	-1.08	-0.09	-0.23, 0.05	-8.3
	Singulair 10mg vs Placebo	-0.90	-1.08	0.18	0.04, 0.32	16.5
Weighted Mean	GW274150 90mg vs Placebo	-0.81	-0.70	-0.11	-0.23, 0.02	-15.2
	Singulair 10mg vs Placebo	-0.52	-0.70	0.19	0.06, 0.31	26.3

a. GW274150 90mg n=25, Singulair 10mg n=25
b. Placebo n=26

The difference between GW27450 90mg and placebo was not statistically significant for either LAR endpoint. However, the difference between Singulair 10mg and placebo was statistically significant for both LAR endpoints. A 50% attenuation from the change from baseline placebo response was considered a priori to be a clinically relevant difference for the minimum LAR endpoint. Neither of the active treatments achieved this response for either LAR endpoint.

The EAR was derived from the FEV1 0 to 2 hour post-inhalation time-points. Minimum FEV1 (0-2hrs) and weighted mean FEV1 (0-2hrs) were both secondary endpoints. The following table summarises the results of the statistical analysis performed on both EAR endpoints.

EAR Endpoint	Treatment Comparison	Adjusted Mean Change from Baseline (L)		Treatment Difference (L) (Active – Placebo)	95% CI (Lower, Upper)	% Attenuation from Placebo Response
		Active[a]	Placebo[b]			
Minimum	GW274150 90mg vs Placebo	-0.91	-0.89	-0.02	-0.20, 0.16	-2.2
	Singulair vs Placebo	-0.52	-0.89	0.37	0.19, 0.55	41.8
Weighted Mean	GW274150 90mg vs Placebo	-0.48	-0.46	-0.02	-0.16, 0.12	-4.7
	Singulair vs Placebo	-0.17	-0.46	0.28	0.15, 0.42	62.2

a. GW274150 90mg n=25, Singulair 10mg n=25
b. Placebo n=26

The difference between GW27450 90mg and placebo was not statistically significant for either EAR endpoint. However, the difference between Singulair 10mg and placebo was statistically significant for both EAR endpoints. A larger percentage attenuation from the change from baseline placebo response was observed for the Singulair 10mg treatment group for both EAR endpoints compared with the LAR endpoints.

Methacholine Challenge:

The methacholine challenge was performed on day 15, 24-26 hours after dosing on day 14. The following table summarises the results of the statistical analysis of methacholine challenge PC20.

Treatment Comparison	Adjusted Geometric Mean (mg/mL)		Doubling Dose Difference	95% CI (Lower, Upper)
	Active[a]	Placebo[b]		
GW274150 90mg vs Placebo	0.283	0.324	-0.19	-0.69, 0.30
Singulair 10mg vs Placebo	0.463	0.324	0.51	0.02, 1.01

a. GW274150 n=25, Singulair n=25
b. Placebo n=26

The doubling dose difference in methacholine challenge PC20 between GW27450 90mg and placebo was not statistically significant. However, the doubling dose difference between Singulair 10mg and placebo was statistically significant. A published study shows that an inhaled corticosteroid results in a difference of at least 2 doubling doses in the methacholine challenge. On this basis a difference of 2 doubling doses was considered a priori to be a clinically relevant difference for the methacholine challenge. Neither of the active treatments achieved this doubling dose difference. In the case of GW274150 versus placebo, the GW274150 group was observed on average to require less concentration of methacoline to achieve a ≥ 20% fall in FEV1 when compared to placebo.

AMP Challenge:

The AMP challenge was performed up to 6 hrs post-dose on day 10. The following table summarises the results of the statistical analysis of AMP challenge PC20.

Treatment Comparison	Adjusted Geometric Mean (mg/mL)		Doubling Dose Difference	95% CI (Lower, Upper)
	Active[a]	Placebo[b]		
GW274150 90mg vs Placebo	6.421	5.242	0.29	-0.32, 0.91
Singulair 10mg vs Placebo	8.156	5.242	0.64	0.00, 1.28

a. GW274150 n=25, Singulair n=22
b. Placebo n=25

The doubling dose difference in AMP challenge PC20 between GW27450 90mg and placebo was not statistically significant. The doubling dose difference between Singulair 10mg and placebo was borderline statistically significant (95% confidence interval just includes 0). Published studies shows that inhaled corticosteroids result in a difference of at least 2 doubling doses in the AMP challenge. On this basis a difference of 2 doubling

doses was considered a priori to be a clinically relevant difference for the AMP challenge. Neither of the active treatments achieved this doubling dose difference.

Pharmacodynamics – Exhaled Nitric Oxide (NO)

Exhaled nitric oxide levels were assessed at pre-dose on days 1 and 7, pre-dose and post-AMP challenge on day 10, pre-dose, 2, 6, 9 and 24hrs post-dose on day 14 and up to 15 mins post-methacholine challenge and 2hrs post-methacholine challenge on day 15.

The following table summarises the results of the repeated measures statistical analysis comparing the trough exhaled NO change from baseline ratios between active and placebo treatment groups on each day.

Day	Treatment Comparison	Treatment Ratio	95% CI (Lower, Upper)
7	GW274150 90mg vs Placebo Singulair vs Placebo	0.27 1.02	0.21, 0.36 0.78, 1.34
10	GW274150 90mg vs Placebo Singulair vs Placebo	0.25 0.86	0.20, 0.33 0.67, 1.11
14	GW274150 90mg vs Placebo Singulair vs Placebo	0.29 0.88	0.22, 0.36 0.70, 1.12

There was statistical evidence of differences between GW274150 90mg and placebo treatment groups on all days trough exhaled NO levels were assessed. On average exhaled NO was reduced by approximately 70% following treatment with GW274150 90mg compared with placebo. There was no statistical evidence of any differences between the Singulair 10mg and placebo treatment groups for any of the days assessed.

The following table summarises the results of the statistical analysis comparing the minimum(0-24hrs) and weighted mean(0-24hrs) exhaled NO change from baseline ratios between active and placebo treatment groups.

Parameter	Treatment Comparison	Treatment Ratio	95% CI (Lower, Upper)
Minimum	GW274150 90mg vs Placebo Singulair vs Placebo	0.12 1.02	0.09, 0.15 0.77, 1.35
Weighted Mean	GW274150 90mg vs Placebo Singulair vs Placebo	0.25 0.90	0.20, 0.31 0.73, 1.12

There was statistical evidence of a difference between GW274150 90mg and placebo treatment groups for both minimum(0-24hr) and weighted mean(0-24hr) day 14 endpoints. There was no statistical evidence of a difference between the Singulair 10mg and placebo treatment groups for either day 14 endpoint.

The following table summarises absolute exhaled NO levels (ppb) at each timepoint assessed.

Day	Planned Relative Time	Placebo (N= 28)		GW274150 90mg (N=26)		Singulair (N=25)	
		n	Geom. Mean (95% CI)	n	Geom. Mean (95% CI)	n	Geom. Mean (95% CI)
1	Pre-dose	28	38.76 (29.96, 50.13)	25	42.88 (32.89, 55.91)	25	42.76 (32.04, 57.07)
7	Pre-dose	26	35.25 (25.88, 48.01)	26	10.78 (8.25, 14.08)	25	38.37 (29.12, 50.55)
10	Pre-dose	26	37.19 (28.64, 48.28)	26	9.78 (7.75, 12.34)	24	35.00 (26.73, 45.82)
	Post-AMP	26	23.08 (16.73, 31.85)	24	2.29 (1.64, 3.19)	24	25.10 (19.32, 32.61)
14	Pre-dose	26	36.12 (27.89, 46.78)	25	10.96 (8.72, 13.78)	25	32.67 (25.00, 42.70)
	2h	25	28.92 (21.84, 38.30)	24	3.41 (2.79, 4.17)	25	32.36 (24.51, 42.73)
	6h	25	28.89 (20.74, 40.25)	25	6.15 (4.81, 7.86)	25	32.00 (24.57, 41.68)
	9h	25	32.47 (24.22, 43.51)	24	8.35 (6.74, 10.36)	25	32.82 (24.68, 43.65)
	24h	25	59.07 (45.20, 77.20)	25	19.64 (15.70, 24.58)	25	52.64 (40.90, 67.74)
15	Post-Methacholine	25	54.78 (41.07, 73.07)	24	16.47 (13.12, 20.68)	25	47.60 (37.44, 60.52)
	2h Post-Methacholine	22	46.06 (30.84, 68.77)	25	15.93 (11.75, 21.59)	23	54.98 (41.35, 73.10)

Exhaled NO levels were reduced on average immediately following methacholine challenge (day 15 (24-26hrs following day 14 dose)) and remained at a similar level at 2hrs post methacholine challenge in the GW274150 90mg treatment group when compared to 24hrs following dosing on day 14. In the singulair 10mg group, exhaled NO levels were on average reduced immediately following methacholine challenge, however at 2 hrs post-challenge the average exhaled NO level was higher than at 24 hrs post-dose on day 14. In the placebo group, exhaled NO levels were on average reduced immediately following methacholine challenge when compared to 24 hrs post-dose on day 14, and reduced further at 2 hrs post-challenge.

Following AMP challenge (performed up to 6 hrs post-dose on day 10), exhaled NO levels were on average reduced in all treatment groups, including placebo, when compared to pre-dose levels on day 10. The average exhaled NO levels were lower following AMP challenge than those observed at 2 hrs post-dose (1hr post allergen challenge) on day 14.

For some subjects the NO analyser's range was set too low and therefore the analyser was unable to read above 101.5ppb. The values of 101.5ppb were included in the summaries and derivation of the exhaled NO parameters. A sensitivity analysis excluding these values was not carried out due to the decision to terminate the

development of the compound for the asthma indication. However, none of the affected results included a day 1 pre-dose timepoint which was used as baseline and none of the affected results occurred during a GW274150 90mg treatment period. Therefore by including the values of 101.5ppb it is thought that no bias has been introduced in favour of GW274150 90mg. It is possible that bias may have been introduced in that placebo and Singulair may be shown to be more effective in reducing NO levels. However it is concluded that any such bias would not affect the overall conclusion concerning GW274150 and its effectiveness on reducing exhaled NO levels.

Safety

Twenty-six out of the 28 subjects randomised received GW274150 90mg. Twenty-five subjects received 90mg for all 14 days, a cumulative dose of 1260mg. One subject missed their dose on day 2 and therefore received 90mg for 13 days, a cumulative dose of 1170mg. Twenty-five out of the 28 subjects randomised received Singulair 10mg. All 25 subjects received 10mg for all 14 days, a cumulative dose of 140mg.

The total number of subjects experiencing at least one adverse event (AE) within a treatment group and the most frequently reported AEs (more than two subjects within a treatment group) are summarised in the following table:

Preferred term	Placebo (N = 28) n (%)	GW274150 90 mg (N = 26) n (%)	Singulair 10 mg (N = 25) n (%)
Subjects with at least 1 AE	23 (82)	15 (58)	15 (60)
Most frequent AEs:			
Headache	8 (29)	7 (27)	6 (24)
Pharnygolaryngeal pain	3 (11)	3 (12)	3 (12)
Fatigue	3 (11)	1 (4)	2 (8)
Cough	1 (4)	3 (12)	1 (4)
Nasal Congestion	0	3 (12)	2 (8)
Vomiting	3 (11)	0	0
Chest pain	1 (4)	0	2 (8)
Diarrhoea	2 (7)	0	1 (4)
Dizziness	2 (7)	0	0
Syncope vasovagal	2 (7)	0	0
Nausea	2 (7)	0	0
Rhinitis	2 (7)	0	0
Chest discomfort	0	0	2 (8)

Adverse events considered to be related to study drug prior to unblinding were reported by 10 subjects (36%) following treatment with placebo, 8 subjects (31%) following treatment with GW274150 90mg, and 7 subjects (28%) following treatment with singulair 10mg. The most frequently reported drug-related AEs were headache and pharyngolaryngeal pain. These were the only drug-related AEs that were reported by more than two subjects within a treatment group.

There were no serious or fatal AEs.

Two subjects were withdrawn from the study due to an AE, both following treatment with placebo. One subject was withdrawn due to a single episode of mild cough and intermittent moderate breathlessness, the other subject was withdrawn due to a single moderate vasovagal episode.

No treatment related trends were observed in safety laboratory parameters. In particular, no trends were observed in the following parameters of particular interest; AST, ALT, CK, amylase and lipase.

No treatment related trends were observed in vital signs parameters, ECG parameters or non-challenge FEV_1 values.

Several isolated QTc parameters were observed greater than 450msec, but no treatment related patterns were observed. One subject was recorded to have an abnormal ECG at several timepoints which were considered clinically significant by the investigator. This was a finding of a junctional rhythm on Day 14 during treatment with GW274150. This was not a new finding in this subject as it had been observed on an ECG a year prior to study start.

Pharmacokinetics:

The following table summarises the median steady state systemic exposure to GW274150 90mg in the asthmatics (n=26). Steady state systemic exposure to GW274150 was achieved on sampling day 10. Peak levels of GW274150 were achieved 0.5h after dosing. These results are consistent with those observed with historical data in healthy volunteers dosed at 90mg at steady state.

Day of sampling	Median peak conc (ng/mL) [Min-Max]	Median trough (ng/mL) [Min-Max]
Day 10	1970 [230 – 3966]	361 [59 – 607]
Day 14	2595 [740 – 4295]	327 [21 – 606]

The following table below summarises the median steady state systemic exposure to Singulair at 10mg in the asthmatics. Steady state systemic exposure to Singulair was achieved on sampling day 14. Peak levels of Singulair were achieved 3h after dosing.

Day of sampling	Median peak conc (ng/mL) [Min-Max]	Median trough (ng/mL) [Min-Max]
Day 10 (n =24)	511 [133 – 1080]	12.7 [NQ – 39.5]
Day 14 (n=25)	498 [167 – 737]	13.2 [NQ – 46.4]

NQ: Not quantifiable, i.e. below quantifiable limit of 5ng/mL

Biomarkers

The results for the biomarker data are not available at present, however they will be included in the full Clinical Pharmacology Study Report.

Conclusions:

- No statistically significant differences were observed between GW274150 90 mg and placebo treatment groups for any of the challenge endpoints. However statistically significant (allergen and methacholine), or borderline statistically significant (AMP) differences, were observed between Singulair 10 mg and placebo for all challenge endpoints.
- There was statistical evidence of reductions in exhaled NO levels following treatment with GW274150 90 mg compared with placebo. However there was no statistical evidence of reductions in exhaled NO levels following treatment with Singulair 10 mg.
- GW274150 90 mg appeared to be well tolerated, showing no notable differences compared with placebo.

Date of Report:

8th November 2006