Synopsis of study report: 378/2004
Location in Module 5:

Study Protocol No.:
BY217/FHP031

Report Version:
0.4

Title of the study:

Anti-inflammatory activity of roflumilast. Study on the efficacy of oral roflumilast (500 µg/d) over 4-weeks on sputum eosinophils in asthma patients. A double-blind, randomized, placebo-controlled, parallel group study (BY217/FHP031)

Coordinating investigator:

Not applicable

Study center(s):

Hôpital Laval, Canada
Hôpital du Sacré-Coeur de Montréal, Canada
St. Joseph's Hospital – McMaster University, Canada
National Jewish Medical & Research Center, USA

Publication (reference):

Not applicable

Studied period (years):
15 March 2001 (first patient in) to 3 September 2004 (last patient out)

Clinical phase:
IIa

Objectives:

To investigate the anti-inflammatory effects of a 4-week treatment with roflumilast 500 µg once daily (od) as vs placebo (parallel group design) in patients with asthma

Methodology:

- Randomized, double-blind, placebo-controlled, parallel group design.

The following measurements were performed during baseline and the treatment period:

Activities	**Baseline (1-2 weeks)**			**Treatment (4 weeks)**					**F**
	B0 –14 d < x < 7 d	**B1*** d -7	**B2** d0	**V0** d0	**V1** d7	**V2** d14	**V3** d21	**V4** d28	
Written informed consent	X								
Physical examination/ Medical history	X							X	X*
Random to treatment				X					
Medication	X		X	X	X	X	X		
ECG, BP, HR, laboratory	X	X*	X					X	
Bronchodilation-Test Reversibility documentation	X X		X X					X	
Lung function: pre Lung function: post	X X		X		X	X	X	X X	
Induced sputum	X		X		X	X	X	X	
Blood withdrawal (biochemical markers)	X		X		X	X	X	X	
Pharmacokinetics (1 sample)			X		X	X	X	X	
Compliance check		X*	X		X	X	X	X	
Questions on Adverse Events		X*	X		X	X	X	X	
Evaluation escape criteria		X*	X		X	X	X	X	
Diary card check		X*	X		X	X	X	X	

X* if applicable; Baseline period (2-weeks) visits B0 – if applicable B1 – and B2; Treatment period (4-weeks) visit V0 – V4; Follow-up (F), if necessary

- After the single-blind baseline period (placebo), patients who met the randomization criteria were randomly assigned to Roflumilast 500 µg od or Placebo in a 1:1 ratio. A stratification based on the sputum eosinophils % at V0 was applied (stratum 1: 2.5 – 9.9 % and stratum 2: ≥ 10 % in a 2:1 ratio).
- Inhaled steroids had to be kept constant 4 weeks prior to the start of the study (B0) and during the entire study. The inhaled corticosteroid dose was limited to maximal 1000 µg BDP or equivalent.
- Asthma is regarded as an eosinophilic inflammation in the airways. The anti-inflammatory effect of treatment with roflumilast 500 µg od vs placebo was evaluated using sputum eosinophils % as surrogate marker for airway inflammation (primary variable).

No. of subjects (total and for each treatment):

In total, 84 asthma patients were enrolled. There were 42 patients who did not meet the inclusion criteria at B0 or the randomization criteria at B2 and these patients were withdrawn before randomization; 42 patients were randomized.

Dataset	Number of patients		
	Total	**Roflumilast 500 µg once daily**	**Placebo**
Full analysis set	42	21	21
Valid cases set	36	16	20

Diagnosis and criteria for inclusion and randomization:

Inclusion criteria

Patients with asthma of either sex, aged 18 to 65 years, who gave their written informed consent and who met the following criteria were included in the study:

- Inflammatory status evaluated as sputum eosinophils at baseline (B0): at least 2.5 %
- Bronchial asthma (symptoms like episodic wheezing, chest tightness, and/or dyspnea)
- Baseline pre-bronchodilator FEV_1 of greater than or equal to 60% of predicted; One of the 4 possibilities were fulfilled:
  - Post-bronchodilator improvement of ≥ 15% (of the pre-bronchodilator FEV_1 value) after receiving 200 µg salbutamol (Canada)/180 µg albuterol (USA) via MDI, measurement after 15- 45 min or
  - Metacholine bronchoprovocation challenge with a 20% fall in FEV_1 (PC_{20}) less than 8 mg/ml or
  - "Historical" reversibility test within the last 6 months
  - "Historical" bronchoprovocation test within the last 6 months
- Capability of producing induced sputum, no prurulent sputum
- Non-smoker or ex-smoker for at least 1 year with a smoking history of no more than *5* pack-years (*5 pack years was changed to 10 pack years in Amendment 2 in Section 9.8.1*)

- No other change in the treatment in the last 4 weeks prior to B0 than as stated in Section 9.4.7
- Patients in a stable clinical state (no exacerbation or history of unresolved sinus or upper airway infection 4-weeks prior to the baseline visit)
- Patients who, with the exception of asthma, did not suffer from any additional disease(s) which might interfere with study related procedures, as assessed by the investigator

Randomization criteria

After a 2-week baseline period, the patients were randomized to treatment with roflumilast or placebo at visit V0 (1:1 allocation to the two treatment groups and a stratification according to a 2:1 ratio), if the following criteria were fulfilled:

- Sputum eosinophilia results at V0 ≥*4%* of total, and in two repeated measurements (B0 and V0) the variability of eosinophils was within a factor of ± 3 as compared to B0 (*≥4% was changed to ≥2.5% in Amendment 2 in Section 9.8.1*)
- FEV_1 (% predicted): pre-bronchodilator FEV_1 ≥*60% of predicted* at visit B2 (was time range reference value for the following visits) when salbutamol/albuterol was withheld for at least 4 h
  (*≥60% was changed to ≥55% of predicted in Amendment 2 in Section 9.8.1*)
- FEV_1 pre-bronchodilator value at visit B2 was within a range of ± 15% of visit B0 value

Test product: Roflumilast

Dose: 500 µg/tablet

Mode of administration: oral administration, once daily, in the morning

Batch No.:

Batch 1: 499110

Batch 2: 101160

Batch 3: 130220

Duration of treatment: 4 weeks

Reference product: Placebo

Dose: Not applicable

Mode of administration: oral administration, once daily, in the morning

Batch No.:

Batch 1: 199112

Batch 2: 410190

Batch 3: 130290

Criteria for evaluation:

Efficacy

Primary variable:

- Percentage of sputum eosinophils of differential cell count (sputum eosinophils %), analyzed as change from baseline, during a 4-week treatment with roflumilast 500 µg od vs placebo

Secondary variables were analyzed in analogy to the primary variable:

- Other sputum cells expressed as percentage: neutrophils %, macrophages %, lymphocytes %, bronchial epithelial cells %
- Absolute numbers of eosinophils, neutrophils, macrophages, lymphocytes, bronchial epithelial cells per milligram of sputum
- Sputum general variables (total cell count, viability, squamous cell contamination)
- Lung function variables from spirometry: FEV_1, FVC, FEF_{25-75}, PEF
- Asthma exacerbations
- Diary variables:
    - PEF (morning, and evening) and %PEF variability
    - Use of rescue medication

Pharmacokinetics

- Evaluation of roflumilast and roflumilast N-oxide as compliance check

Safety

- Adverse Events (AEs)
- Laboratory work-up (clinical chemistry, hematology, urine analysis)
- Physical examination
- ECG (PR-, QRS-, QT-, QTc, QTc-interval and Heart Rate)
- Blood pressure, Pulse rate

Statistical methods:

- The primary variable was the difference in sputum eosinophils % during a 4-week treatment with roflumilast 500 µg od vs placebo, analyzed as change from baseline. The primary variable was tested for superiority of roflumilast 500 µg od vs placebo with an intention-to-treat analysis (ITT-analysis) using a repeated measurement analysis. Sputum eosinophils % was log transformed (using the natural logarithm: ln transformed) prior to

statistical analysis. Changes from baseline (B2) were calculated for all visits V1-V4, using the baseline variable and the stratum variable as covariates. In order to investigate the robustness of results a PP analysis was performed.

- Alpha-level was 2.5% for the primary efficacy variable (one-sided test)
- The secondary efficacy variables were tested in an exploratory manner (ITT and PP analysis).
- All sputum variables were ln transformed prior to a repeated measurements analysis.
- Lung function variables were analyzed using a repeated measurements analysis
- Time to occurrence of an asthma exacerbation was analyzed by the log-rank test
- Morning PEF and evening PEF were evaluated using a repeated measurement analysis. PEF variability and the average of number of puffs of rescue medication per week were evaluated using Wilcoxon tests

Pharmacokinetic analyses of roflumilast and roflumilast N-oxide were performed as compliance check.

All safety variables were analyzed in a descriptive manner.

SUMMARY – CONCLUSIONS

Efficacy results

If not indicated otherwise, all data was evaluated using a repeated measurements analysis.

I OVERALL ANALYSIS

Primary variable

Sputum eosinophils expressed as percentage (primary variable)

The mean percentage of sputum eosinophils varied from 6 to 14% of differential cell count during the treatment period.

For the primary variable, sputum eosinophils % analyzed as difference in change from baseline, the mean ratio of roflumilast vs placebo was 0.57 in ITT analysis ($p = 0.021$ in Table 1). Treatment with roflumilast 500 µg od vs placebo statistically significantly reduced sputum eosinophils % by 43% in the primary ITT analysis.

This reduction was 44% in PP analysis (ratio 0.56, $p = 0.032$).

Inhaled steroids are known to reduce sputum eosinophil %. The reduction in sputum eosinophil % was found in the overall analysis despite the fact that 55% of the patients used inhaled steroids.

Table 1: Sputum eosinophils expressed as percentage of differential cell count (sputum eosinophils %) during a 4-week treatment with roflumilast 500 µg od *vs* placebo (original scale)

Primary Variable[a, b]	Roflumilast 500 µg od		Placebo				Ratio of mean change to baseline (Roflumilast/Placebo)				Change (95% CI)
Eosinophils %	**Mean[a]**	**CV[a]**	**Mean[a]**	**CV[a]**	**N**	**N[d]**	**Mean[a]**	**CV[a]**	**95% CI[a]**	**p-value[a,e]**	
ITT	0.50	0.21	0.87	0.20	40[c]	148	0.57	0.27	(0.34, 0.98)	**0.021**	**-43% (-66, -2)**
PP	0.50	0.25	0.88	0.24	35	127	0.56	0.31	(0.31, 1.04)	0.032	-44% (-69, +4)

[a] Mean = Geometric Mean. All sputum variables including sputum eosinophil % were anti-logged after analysis, thus geometric mean, CV and 95% CI are presented on the original scale, p-values result from log-scale analysis (ln transformed data in repeated measurements)
[b] 0% values were replaced by the lowest observed value before ln transformation
[c] Patient with squamous cells > 20% at the reference value B2 were not evaluated for sputum variables (#1039 and #1041)
[d] Used data points in repeated measurements
[e] Eosinophils % was tested at level α=0.025 (one-sided) in ITT and PP analysis
CI = Confidence Interval; ITT = Intention-to-treat; PP = Per-protocol; CV= Coefficient of Variation

Secondary variables

1) Sputum eosinophils expressed as absolute number

The mean absolute number of sputum eosinophils varied from 143 to 720 cells/mg during the treatment period.

The secondary variable of sputum eosinophils, expressed as absolute number and analyzed as difference in change from baseline, showed a treatment effect during a 4-week treatment with roflumilast 500 µg od *vs* placebo. The mean ratio of roflumilast vs placebo was 0.43 in ITT analysis (p = 0.0089 in Table 2). Treatment with roflumilast 500 µg od vs placebo statistically significantly reduced the sputum eosinophils expressed as absolute number by 57% in ITT analysis (Table 2) and by 65% in PP analysis (ratio 0.35, p = 0.0035 in Table 2).

Table 2: Sputum eosinophils expressed as absolute number during a 4-week treatment with roflumilast 500 µg od *vs* placebo in ITT analysis (original scale)

Secondary Variables[a, b]	Roflumilast 500 µg od		Placebo		Ratio of mean change to baseline (Roflumilast/Placebo)						Change (95% CI) (%)
Eosinophils (Cells/mg)	**Mean[a]**	**CV[a]**	**Mean[a]**	**CV[a]**	**N[c]**	**N[d]**	**Mean[a]**	**CV[a]**	**95% CI[a]**	**p-value[a,e]**	
ITT	0.38	0.27	0.89	0.24	40	147	0.43	0.35	(0.21, 0.86)	**0.0089**	-57% (-79, -14)
PP	0.31	0.30	0.89	0.25	35	126	0.35	0.38	(0.17, 0.74)	**0.0035**	-65% (-83, -26)

[a] Mean = Geometric mean. All sputum variables were anti-logged after analysis, thus geometric mean, CV and 95% CI are presented on the original scale, p-values result from log-scale analysis (ln transformed data in repeated measurements)
[b] 0% values were replaced by the lowest observed value before ln transformation
[c] Patients with squamous cells > 20% at the reference value B2 were not evaluated for sputum variables (#1039 and #1041)
[d] Used data points in repeated measurements
[e] Tested one-sided at $\alpha = 0.025$
CI = Confidence Interval; ITT = Intention-to-treat; CV= Coefficient of Variation

*2) **Other sputum inflammatory cells expressed as percentage (%)***

Macrophages (49-57% of differential cell count during the treatment period) and neutrophils (26-38% of differential cell count during the treatment period) were the predominant inflammatory cells in the sputum of these asthma patients.

The changes for sputum macrophages % (-4%, ratio 0.96, p=0.69), sputum neutrophils % (+19%, ratio 1.19, p=0.27), sputum lymphocytes % (+1%, ratio 1.01, p=0.98) and bronchial epithelial cells % (+29%, ratio 1.29, p=0.34) during treatment with roflumilast *vs* placebo were not statistically significant (ITT analysis). These changes also did not reach statistical significance in the PP analysis.

*3) **Other sputum cells expressed as absolute number***

Treatment with roflumilast 500 µg od vs placebo reduced all other sputum inflammatory cells expressed as absolute number in ITT analysis. These reductions were 28% for macrophages (ratio 0.72, p=0.054), 24% for lymphocytes (ratio 0.76, p=0.16), 9% for neutrophils (ratio 0.91, p=0.36) and 2% for bronchial epithelial cells (ratio 0.98, p=0.47) in ITT analysis; none of these reductions reached statistical significance at $\alpha = 0.025$.

The reductions in absolute numbers of sputum inflammatory cells were more pronounced in PP analysis: 42% for macrophages (ratio 0.58, p=0.0037), 44% for lymphocytes (ratio 0.56, p=0.014), and 29% for neutrophils (ratio 0.71, p=0.09), except for bronchial epithelial cells for which there was an increase (+11%, ratio 1.11, p=0.65).

4) Total cell count = Total non-squamous cells

Treatment with roflumilast 500 µg od vs placebo reduced the total cell count by 24% in ITT analysis (ratio 0.76, p = 0.08) and by 37% in PP analysis (ratio 0.63, p = 0.0061). The total cell count consisted of macrophages (52%), neutrophils (34%), eosinophils (9%), bronchial epithelial cells (3%) and lymphocytes (2%) (percentage at visit V0 in the roflumilast group).

Treatment with roflumilast 500 µg od vs placebo numerically reduced all these cells (see point 1 and point 3). The decrease in total cell count was mainly due to the decrease in macrophages, the predominant sputum inflammatory cell in the sputum of these asthma patients (52%).

5) Lung function and diary data

- FEV1 was increased during roflumilast treatment with +14 mL, and during placebo treatment with +25 mL, whereas FVC was decreased with –14 ml during roflumilast treatment and – 35 ml during placebo treatment. The mean treatment difference for roflumilast vs placebo was not statistically significant for either FEV1 -11 mL (p=0.58), FVC +21ml (p=0.30), or PEF –23 L/s (p=0.55).
- For diary morning PEF the mean treatment difference for roflumilast vs placebo was +14.60 L/min (p=0.12) and for diary evening PEF the mean treatment difference for roflumilast vs placebo was –2.77 L/min (p=0.57).
- There was no treatment difference between roflumilast and placebo for PEF variability and the use of rescue medication during a 4-week treatment with roflumilast vs placebo (Wilcoxon test).
- Note that inhaled steroids are known to increase lung function in asthma patients, and that 55% of the patients used inhaled steroids in a constant dose 4 weeks before and during the study.

6) Asthma exacerbations

There was no difference between roflumilast and placebo regarding asthma exacerbations (p=0.20 in ITT and PP analysis).

II SUBGROUP ANALYSIS

Low sample size in the subgroups yield low power, and therefore the sample size is rather too low to give good evidence of the subgroup outcomes. However, the following hints existed in the subgroup analyses (concomitant use of inhaled steroids and stratum: eosinophil percentage at V0):

1) Subgroup analysis based on concomitant use of inhaled steroids

The subgroup concomitant use of inhaled steroids consisted of:

- 23 patients taking inhaled steroids (ICS) in a constant dose 4 weeks prior and during the study (55% of the patient population) (referred to as subgroup ICS) and
- 19 patients not taking ICS (45% of the patient population) (referred to as subgroup not ICS).

Primary variable

- ICS are known to reduce sputum eosinophils % in asthma patients. The reduction in sputum eosinophils % during treatment with roflumilast vs placebo was similar in both subgroups: -39% in the subgroup ICS and -41% in the subgroup not ICS. Treatment with roflumilast vs placebo statistically significantly reduced sputum eosinophils % in the subgroup not ICS (ratio 0.59, p=0.0074), but not in the subgroup ICS (ratio 0.61, p=0.15)

Secondary variables

- The reduction in sputum eosinophils % during treatment with roflumilast vs placebo was accompanied by a reduction in sputum eosinophils expresses as absolute number in both subgroups: -63% in the subgroup ICS and –43% in the subgroup not ICS. Both reductions, based on the ratios 0.37 and 0.57, did not reach statistical significance
- Treatment with roflumilast vs placebo statistically significantly reduced the total cell count by 40% in the subgroup ICS (ratio 0.60, p=0.021), but not in the subgroup not ICS (+5%, ratio 1.05, p=0.56). This suggests that treatment with roflumilast vs placebo may have an add on effect for patients taking inhaled steroids.
- There were no statistically significant differences for roflumilast vs placebo in both subgroups for lung function and diary PEF

2) Subgroup analysis based on eosinophil percentage at V0 (stratum)

There were 27 patients in the stratum eosinophils 2.5-9.9% (64%) and 15 patients in the stratum eosinophils ≥ 10% (36%).

Primary variable

- Treatment with roflumilast vs placebo reduced, although not statistically significantly, sputum eosinophils % to the same extent in both eosinophil strata (reduction of 43% based on the ratio 0.57 in both strata)

Secondary variables

- Treatment with roflumilast vs placebo reduced, although not statistically significantly, sputum eosinophils expressed as absolute number to the same extent in both eosinophil strata (reduction of 58% and 50% based on the ratio 0.42 and 0.50 in stratum 2.5-9.9% and > 10% respectively)

- The total cell count was reduced to the same extent in both subgroups (reduction of 23% and 19% based on the ratio 0.77 and 0.81 in stratum 2.5-9.9% and > 10% respectively)
- There were no statistically significant differences for roflumilast vs placebo in the two strata for all sputum inflammatory cells, lung function and diary PEF

Safety results:

In total 72 AEs occurred during the treatment period in 34 randomized patients; 17 patients (81%) in the roflumilast group experienced 41 AEs, and 17 patients (81%) in the placebo group experienced 31 AEs. Most AEs were of mild or moderate intensity.

The most frequently reported AEs were headache and nausea during treatment with roflumilast. Headache was reported by 8 patients in the roflumilast group and by 6 patients in the placebo group. Nausea was reported by 6 patients in the roflumilast group and by 1 patient in the placebo group.

In total, 3 serious AEs were reported in this study (anemia and asthma exacerbation during treatment with roflumilast and creatine kinase increased during the baseline period).

Two patients discontinued the study due to the AE “asthma aggravated”, both during roflumilast treatment.

Routine laboratory investigations, physical examination, ECG, blood pressure, and heart rate, measured during treatment, were not affected by the study medication.

Overall Conclusions:

Asthma is regarded as an eosinophilic inflammation in the airways. Overall, superiority of roflumilast 500 µg vs placebo in reducing sputum eosinophils % (primary variable) was demonstrated in this parallel group study (reduction by 43% in the overall analysis) and could be confirmed by reduction in the absolute number of sputum eosinophils per mg of sputum (reduction by 57% in the overall analysis).

Therefore, the anti-inflammatory effect of treatment with roflumilast 500 µg od vs placebo using sputum eosinophils % as surrogate marker for airway inflammation was demonstrated.

Based on the safety data reported in this study, there is no safety concern associated with the use of roflumilast 500 µg od.