2 Synopsis

Title of the study: Efficacy of oral roflumilast (500 µg/day) over 15 days on sputum eosinophils in asthma after allergen provocation. A randomized, placebo-controlled, double-blind crossover study

Three centers in Italy:

- University of Modena & Reggio Emilia, Modena;
- University of Pisa, Pisa;
- University of Ferrara, Ferrara.

Publication (reference): Not applicable

Studied period: 12-Sep-2002 (first patient in) to 01-Jun-2004 (last patient out)

Clinical phase: Phase IIa

Objectives:
The primary objective of the study was to investigate the effect of roflumilast (500µg/d) over 15-d on allergen-provoked sputum eosinophils in atopic asthmatics as compared with placebo.

Secondary objectives were the efficacy of roflumilast to suppress early (EAR) and late asthmatic response (LAR) as well as safety and efficacy.

Methodology:
The study was a randomized, placebo-controlled, double-blind, 2-period crossover design with a washout period of at least 4 weeks to evaluate the efficacy of a 15-d treatment with roflumilast on allergen-provoked sputum eosinophils in patients with atopic asthma. The study was divided into 4 phases: Screening, Randomization/Treatment Period 1 (Visit V0, V1 and V2), Washout, Treatment period 2 (Visit V3, V4 and V5), Post-study Visit. Patients received one tablet (roflumilast 500 µg or placebo) orally each morning after breakfast with at least 200 mL of tap water for 15 days in each treatment period. There was a washout period between treatment periods (approximately 4 to 6 weeks).

No. of patients (total and for each treatment) planned and analyzed:

The study was terminated early. The sample size was planned to be at least 12 evaluable patients with atopic asthma. A total of 22 patients were screened; 11 patients were randomized and took at least one dose of study medication. All 11 patients received placebo; 10 patients crossed over and started treatment with roflumilast 500 µg. A higher number of patients was allocated to the treatment sequence placebo- roflumilast 500 µg (7 patients) than the treatment sequence roflumilast 500 µg - placebo (4 patients).

Diagnosis and main criteria for inclusion:

Patients with a history of stable atopic bronchial asthma according to American Thoracic Society (ATS) criteria, age between 18 and 65 years, who had given their written informed consent and met the following conditions were included into the study:

- positive skin prick testing at screening using a standard battery of common aeroallergens (such as Italian grass pollen, cat hair, house dust mite, and parietaria officinalis);
- forced expiratory volume in 1 second (FEV_1) at least 70% of predicted;
- positive reversibility test (ie FEV_1 increase by at least 12% of the baseline value following 200 to 400 µg salbutamol at 15-30 min after inhalation). Test should have been performed within the 6 months before study. The documentation had to be available.

  or

  positive reversibility test performed as described above during a period of seasonal asthma at screening;

  or

  metacholine challenge was performed and proven hyperreactivity to metacholine was required. Provocative concentration that led to a 20% reduction in FEV_1 (PC_{20}-FEV_1) ≤16 mg/mL. The metacholine challenge test was performed in case that no positive reversibility test was documented (within 6 months before study) or the patient had no seasonal asthma during the screening;
- sputum eosinophilia at screening (≥2.0% of total nonsquamous cells in cytospins) and doubling of sputum eosinophils 24 h after allergen challenge (≥ 4.0%);
- dual response to allergen challenge (ie ≥25% and ≥15% reduction in FEV_1 from the baseline value during the EAR and LAR, respectively);
- capability of producing induced sputum;
- except of the history of asthma stated as healthy based on a screening examination including a medical history, physical examination including examination of nose and throat, blood pressure (BP), electrocardiogram (ECG), and clinical laboratory results.

Test product, dose, mode of administration, batch no.:

Roflumilast 500 µg: one tablet once daily (od), orally; batch no. 300150 and 320190.

Reference product, dose, mode of administration, batch no.:

Placebo: one tablet od, orally; batch no. 200130 and 320230.

Duration of treatment:

Two 15-d treatment periods separated by a washout period (approximately 4 to 6 weeks).

Criteria for evaluation:

Primary variable

The primary efficacy variable was the reduction in sputum eosinophils (%) before and 24 h after allergen challenge for a 15-d treatment with roflumilast vs placebo.

Secondary variables

Of the following planned secondary efficacy variables only PD_{20}-FEV_1 allergen was evaluated:

- PD_{20}-FEV_1 allergen as index for EAR;
- allergen-provoked EAR as measured by a decrease in FEV_1 from post-saline value between 10 min and 1 h after allergen challenge for a 15-d treatment with roflumilast vs placebo;
- allergen-provoked LAR as measured by a decrease in FEV_1 from post-saline value between 3 h and 8 h after allergen challenge for a 15-d treatment with roflumilast vs placebo;
- change of lung function for a 14-d treatment with roflumilast vs placebo;
- peak expiratory volume (PEF) and rescue medication as documented in diary for a 14-d treatment with roflumilast vs placebo.

Safety

AEs, standard laboratory, vital signs, and physical examination.

Statistical methods:

It was planned to calculate for each treatment period, the increase in sputum eosinophils % (primary variable) occurring as a consequence of allergen challenge and the reduction in

sputum eosinophils % after a 15-d treatment with roflumilast vs placebo. These values were planned to be tested for a difference between roflumilast and placebo. According to the Study Protocol superiority was to be assessed by a nonparametric 95% confidence interval. In addition to the confidence interval, a point estimate was planned to be given for the treatment difference.

Because of the early termination of the study, all variables (primary, secondary and safety variables) were evaluated descriptively and given as individual data only. Individual efficacy data were evaluated as follows:

- An anti-inflammatory effect of roflumilast was assessed as a decrease in mean allergen-provoked **sputum eosinophils (%)** per patient 24 h after allergen challenge following a 15-d treatment with roflumilast compared with the same value following a 15-d treatment with placebo;
- A protective effect of roflumilast on the EAR was assessed as no **PD_{20}-FEV_1 allergen** or an increase in PD_{20}-FEV_1 allergen with roflumilast compared with placebo.

SUMMARY – CONCLUSIONS

Demography and baseline characteristics

Due to early termination of the study, 11 randomized patients instead of the scheduled 12 patients in the Study Protocol were included in the study. At the screening examination, demographic and other baseline characteristics were documented for each patient. There were 2 females and 9 males included in the study. The age ranged from 18 to 31 years. All patients were of Caucasian origin. There were 10 non-smokers and 1 ex-smoker.

STUDY RESULTS

Efficacy results:

Primary variable

The mean sputum eosinophils % by patient is given in the Table below. Three patients showed a decrease in mean sputum eosinophils (%) by patient 24 h after allergen challenge following a 15-d treatment with roflumilast vs placebo, whereas six patients did not show such an anti-inflammatory effect of roflumilast. For two patients an anti-inflammatory effect could not be shown because of missing values for sputum eosinophils % with roflumilast (#203 and #303).

Mean sputum eosinophils (%) by patient before and 24 h after allergen challenge for the screening and a 15-day treatment with roflumilast 500 µg once daily vs placebo in a double-blind, two-period crossover study

Treatment Sequence / Patient ID	Mean sputum eosinophils (%) before and 24 h after provocation by period							
	Screening			Period 1/Roflumilast		Period 2/Placebo		Result[a]
	Before	After	Inclusion	Before	After	Before	After	
Roflumilast 500 µ od – Placebo								
107	3.0	24.0	yes	12.5	59.0	1.5	56.0	No effect
111	5.1	11.9	yes	12.3	6.1	7.3	6.5	Effect
204	6.0	13.7	yes	8.0	2.2	3.0	-	Effect[b]
206	4.4	17.0	yes	32.4[c]	31.6	6.6	2.5	No effect
	Screening			**Period 2/Roflumilast**		**Period 1/Placebo**		**Result**
	Before	**After**	**Inclusion**	**Before**	**After**	**Before**	**After**	
Placebo – Roflumilast 500 µg od								
105	5.8	14.5	yes	9.1	2.3	9.0	4.5	No effect
108	3.5	38.5	yes	34.0[c]	40.0	22.5	14.8	No effect
203	4.9	11.8	yes	-	31.9[e]	4.5	21.5	NA/No effect[e]
205	2.5	5.1	yes	0.2[d]	13.6	2.9	29.5	No effect
207	3.7	7.4	yes	13.2	8.2	2.2	7.0	Effect
303	2.0	5.0	yes	-	-	3.0	-	NA[f]
304	2.0	4.5	yes	0.0[d]	1.7	3.0	0.0	No effect

[a] An anti-inflammatory effect was assessed as a decrease in mean sputum eosinophils (%) by patient 24 h after allergen challenge after a 15-d treatment with roflumilast compared with the same value after a 15-d treatment with placebo.
[b] For Patient #204 the effect was seen with roflumilast. No data for placebo treatment was available (protocol violator).
[c] The baseline value is unusually high. These values were excluded in the additional analysis by the University of Pisa.
[d] The baseline value is unusually low.
[e] The high percentage of sputum eosinophils after treatment with roflumilast suggests no anti-inflammatory effect of roflumilast.
[f] Patient #303 discontinued the study due to the AE asthma exacerbation.
NA = not applicable, od = once daily

Secondary variables

Early asthmatic response as evaluated by PD_{20}-FEV_1

Five patients showed a protective effect of roflumilast vs placebo on allergen challenge as assessed by PD_{20}-FEV_1. One patient (#205) demonstrated a partial protective effect of roflumilast on allergen challenge. This patient had a lower PD_{20}-FEV_1 with placebo than with roflumilast. Two patients did not show such an effect. For three patients the protective effect of roflumilast could not be assessed by PD_{20}-FEV_1, because there was no placebo response or the response under placebo was not measured.

Additional efficacy analysis at the University of Pisa:

Note that the calculations below (mean data) have been performed by Prof. Paggiaro and coworkers at the University of Pisa based on the individual data of 10 patients provided in the Appendix of the Clinical Study Report. Patient #303 had no measurements after treatment for roflumilast and placebo and was not evaluated.

Early asthmatic response to allergen

The cumulated dose (CD) of allergen and the PD_{20}-FEV_1 are two indexes of EAR to allergen. The allergen challenge test after a 15-d treatment with roflumilast or placebo reached the cumulated dose of allergen able to induce a fall of $FEV_1 \geq 20\%$ in the screening test; if there was no significant response (FEV_1 fall greater than 20%), an additional dose was administered.

The table below compares the **Cumulated Dose (CD)** of allergen in the 3 allergen challenge tests (screening, after a 15-d treatment with roflumilast 500 µg od and placebo). CD allergen after roflumilast was significantly greater than in the other challenge tests (screening and after placebo). For the evaluation of **PD_{20}-FEV_1** allergen there was a significant difference between the 3 allergen challenge tests. In summary, in the allergen challenge tests after a 14-d treatment with roflumilast, patients reached a CD and PD_{20}-FEV_1 greater than in the allergen challenge tests at screening and after a 14-d treatment with placebo. This indicates a significant protection of roflumilast on EAR.

Comparison between two indexes of early asthmatic response to allergen at screening and after a 15-day treatment with roflumilast vs placebo (n = 10)

	EAR (geometric mean and range)	
	CD Allergen	**PD_{20}-FEV_1 Allergen[a]**
Screening test	0.492 [0.100,3.2] * (p=0.02)	0.291 [0.077,1.830] * (p=0.02)
Roflumilast 500 µg	0.985 [0.100,3.2]	0.488 [0.164,2.927]
Placebo	0.492 [0.050,3.2] * (p=0.04)	0.177 [0.032,1.587] * (p=0.02)

[a] conventionally negative response was translated as 3.2
* $p < 0.05$ vs roflumilast (non-parametric Wilcoxon paired t-test)
CD = cumulated dose, EAR = early asthmatic response, PD_{20}-FEV_1 a provocative dose that led to a 20% reduction in forced expiratory volume in 1 second (FEV_1)

Late Asthmatic Response to Allergen

LAR was measured as maximal % reduction of FEV_1 between 3 and 7 to 8 h after performance of the allergen challenge test. No significant difference between the 3 allergen challenge tests was found.

Comparison between indexes of late asthmatic response to allergen at screening and after a 15-day treatment with roflumilast vs placebo (n = 10)

	LAR (mean ± SD (SE)) [a]	
	FEV_1 during LAR	**Maximal % fall of FEV_1**
Screening test	3.06 ± 0.70 (0.22) p=0.39	-23.0 ± 8.0 (2.4) p=0.33
Roflumilast 500 µg	3.25 ± 0.77 (0.24)	-18.8 ± 15.0 (4.8)
Placebo	3.30 ± 0.79 (0.25) p=0.69	-12.7 ± 19.9 (6.3) p=0.21

[a] LAR was measured as maximal % reduction in FEV_1 between 3 and 7 to 8 h after performance of the allergen challenge test.
* $p < 0.05$ vs roflumilast was not reached (non-parametric Wilcoxon paired t-test)
FEV_1 = forced expiratory volume in 1 s; LAR = late asthmatic response, SD = standard deviation, SE = standard error.

Allergen-provoked Sputum Eosinophils (Eight Patients)

Statistical analysis for sputum eosinophils was performed for 8 out of 10 patients, thus excluding 2 patients with out-of-order baseline values for sputum eosinophils % (34.0% for Patient #108 and 32.4% for Patient #206). Median and range of sputum eosinophils % before and 24 h after allergen challenge showed no statistical difference between a 15-d treatment with roflumilast vs placebo and screening. However, there was a clear trend as there was no increase after allergen challenge following a 15-d treatment with roflumilast, whereas there was an increase after a 15-d treatment with placebo.

Comparison between sputum eosinophils % measured before and after allergen challenge test at screening and after a 15-day treatment with roflumilast vs placebo (n = 8)

	Sputum eosinophils % (median and range) [a, b]		
	Before allergen	**24 h after allergen**	**p-value**
Screening test	4.3 [2.0, 6.0]	11.9 [4.5, 38.5]	p=0.01
	Day 1	**Day 15**	
Roflumilast 500 µg	9.1 [0.0, 34.0]	7.1 [1.7, 40.0]	p=0.16
Placebo	3.8 [2.2, 22.5]	7.0 [0.0, 29.5]	p=0.09

[a] Patients were selected at the screening with mean sputum eosinophils ≥ 2% by patient before allergen challenge and more than double ≥ 4% 24 h after allergen challenge (all patients underwent all treatments).
[b] Two patients with out-of-order measures for baseline sputum eosinophils % were removed (34.0% for 108 and 32.4% for 206
* $p < 0.05$ vs roflumilast was not reached (non-parametric Wilcoxon paired t-test)

Safety results:

A total of 7 patients (64% of all patients) reported 14 treatment-emergent AEs in this randomized, placebo-controlled, double-blind, 2-period crossover study, 4 (40%) patients with roflumilast and 4 (36%) patients with placebo. None of the events were classified as serious AEs. No death occurred during treatment; one patient receiving placebo withdrew from the study due to an AE (see Table below).

Treatment-emergent AEs (safety set)

	n (%)[a]	
	Roflumilast 500 µg od (N = 10)	**Placebo (N = 11)**
AEs	4 (40.0)	4 (36.4)
SAEs	0 (0.0)	0 (0.0)
AEs with causality[b] suggested by the investigator	1 (10.0)	0 (0.0)
AEs leading to discontinuation	0 (0.0)	1 (9.1)

[a] Percentages are based on the total number of patients in a treatment group.
[b] AEs assessed by the investigator as "likely" ore "definitely" related to the study medication.
AE = adverse event, N = number of patients in each treatment group, n = number of patients with events, SAE = serious adverse event

All reported AEs were mild in intensity. All AEs resolved completely in all patients. The most frequently reported treatment-emergent AEs were headache, asthenia and nausea; these AEs were experienced each by two patients while receiving roflumilast.

There were no clinically relevant changes in laboratory values, vital signs, ECG, and physical examination over time in individual patients.

Physical examination, BP, PuR and ECG did not reveal any pathological findings.

Conclusions:

The anti-inflammatory effect of roflumilast was evaluated by assessment of allergen-provoked sputum eosinophils, EAR (evaluated as PD_{20}-FEV_1) and LAR.

There seems to be a positive effect of roflumilast on EAR (as expressed by total allergen dose or PD_{20}-FEV_1). A potential effect on LAR might have been diminished by a higher total allergen dose during roflumilast as compared to placebo. Also there seems to be an effect on allergen-provoked sputum eosinophils (%) with roflumilast vs placebo. However, due to the observed variability the number of patients is far too low to draw a final conclusion.

This study did not raise any safety concern for a 15-d treatment with roflumilast 500 µg od in patients with asthma.

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