Drug product:	Symbicort Turbuhaler	**SYNOPSIS**	
Drug substance(s):	Budesonide/formoterol		
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Effects of high dose inhaled budesonide + formoterol versus placebo and oral prednisolone on biomarkers of airway inflammation in the treatment of exacerbations in non-hospitalised patients with mild to moderate COPD

Publications

- Abstract on primary outcomes has been presented at the American Thoracic Society congress, May 2006, San Diego, USA.
- Abstracts on spin-off results were presented at other congresses.
- Full publication manuscripts are being prepared. To date, the publication manuscript on the primary outcomes is not accepted for publication, a publication manuscript on spin-off results (the safety of sputum induction in COPD patients) has been accepted by Chest.

Study dates

Study dates		**Phase of development**
First subject enrolled	16 January 2001	Therapeutic exploratory (IIA)
Last subject completed	30 January 2005	
Clean File:	23 June 2005	

Objectives

The primary objective was to determine the effect on biomarkers of airway inflammation of a high dose of the inhaled combination product budesonide and formoterol versus placebo in the treatment of acute exacerbations in non-hospitalised patients with mild to moderate COPD. The primary variable was the number of eosinophils (in % of total cells) in induced sputum. The secondary objective was to compare the effects on other biomarkers of airway inflammation of budesonide/formoterol with that of oral prednisolone. Additional aims were to study effects on lung function and clinical symptoms.

Study design: This was a study performed with a parallel design at one single centre. Enrolment (Visit 1) was followed by a run-in period in which (when used) corticosteroid treatment was withheld and baseline data (inflammatory, lung function, symptoms) were collected after a corticosteroid-free and exacerbation-free period of (minimally) 2 months (Visit 2). The first COPD exacerbation thereafter (Visit 3) was treated in a double blind, randomized fashion with either inhaled budesonide/formoterol, with oral prednisolone or with placebo for 14 days in parallel groups using a double dummy technique. Data (inflammatory, lung function, symptoms) were obtained at the start of the exacerbation and after approximately 3, 7 and 14 days (Visit 4 to 6). A telephonic post-study contact with the patient was established after an additional 2 weeks and 10 weeks (Visit 7 and 8) to record adverse events and Treatment Failure or a Relapse of the exacerbation (defined as the need for open corticosteroid treatment within 3 weeks of randomisation and within 3 months respectively). When more than 6 months elapsed from Visit 2 without an exacerbation a second Visit 2 was performed (Visit 2B). Inflammatory data was obtained (on Visit 1 to Visit 6) in sputum samples, obtained after induction with hypertonic saline, in blood and in urine.

Target subject population and sample size

Patients of either sex with Chronic Obstructive Pulmonary Disease (COPD), aged > 40 years, smokers or ex-smokers, at Enrolment a Forced Expiratory Volume in the first second (FEV_1) <85 % of predicted, a FEV_1/Vital Capacity (VC) ratio <88% of predicted (men) or <89% of predicted (women) and at Randomisation an exacerbation of COPD and a FEV_1 <70% of predicted. Approximately 100 patients were to be enrolled in order to study 60 patients during an exacerbation.

Investigational product and comparators: dosage, mode of administration and batch numbers

Patients were treated in a double blind manner with one out of three treatments, either with 1: inhaled budesonide/formoterol, with 2: oral prednisolone or 3: with placebo. The details of the investigational products used are:

- Budesonide/formoterol Turbuhaler (Symbicort® Turbuhaler®), 160 µg budesonide and 4.5 µg formoterol per (delivered) dose, batch numbers AI25, CC28, CM28, DL30, EG32.

- Placebo Turbuhaler®, batch numbers AI18, CC20, CM14, DL15, EG16.

- Prednisolone, 5 mg tablets, batch 00J25026.
- Placebo tablets, batch 00J26001.

Inhalers were produced by AstraZeneca, tablets were prepared by the Hospital Pharmacy of the University Medical Centre Groningen.

The dosing was four times daily 2 inhalations and once daily 6 tablets. The patients received thus either:

1. 1280 µg budesonide and 36 µg formoterol daily (doses expressed as delivered doses, equivalent with 1600 µg budesonide and 48 µg formoterol as metered doses) or:
2. 30 mg prednisolone or:
3. no corticosteroids.

As concomitant treatment all patients received doxycycline 200 mg the first day and 100 mg daily for another 7 days (batch numbers 001096, 01D18MA, 02B20WC) and they could use as needed for symptom relief inhaled terbutaline pMDI, 250 µg per dose (batch numbers 00D21, 02A15) and inhaled ipratropium bromide pMDI 20 µg per dose (batch numbers 00F07, 04A09).

Duration of treatment

14 days.

Criteria for evaluation (main variables)

Efficacy and pharmacokinetics

1. Primary variable: Inflammation: the number of eosinophils in sputum (as % of total sputum cells)
2. Secondary variables:
   - Inflammation: <u>in sputum</u>: Total Cell Count, differential count (other than eosinophils), Eosinophil Cationic Protein (ECP), Myeloperoxidase (MPO), Interleukin (IL)-6, IL-8, Albumine, Leukotriene B_4 (LTB_4), Tumor Necrosis Factor alpha (TNFα), Monocyte Chemoattractant Protein 1 (MCP-1), desmosine and messenger RiboNucleic Acid (mRNA) for a series of proteins [Hemoxygenase-1, TNFα, Tumor Growth Factor beta (TGFβ), Interferon gamma (INFy), IL-5, IL-10, IL-12a, IL-12b, IL-13, chemokine CCL5]. <u>In serum:</u> CRP, albumine, soluble adhesion factor sICAM, TNFα and IL-6. <u>In urine:</u> desmosine, relative to creatinine.

- Lung function **[** FEV_1, Inspiratory VC (IVC), Forced VC (FVC), FEV_1/IVC, specific airway conductance (sGaw) **]**, symptoms **[** Borg Score and Clinical COPD questionnaire (CCQ)-score **]**, diaries **[** Peak Expiratory Flow (PEF), breathing difficulties, coughing, sputum production **]**, Quality of Life (Chronic Respiratory Questionnaire (CRQ).

- Pathogens in sputum: bacterial typing and bacterial count, antibodies to viruses.

- Treatment Failure or Relapse (defined as the need for additional corticosteroid treatment in between Visit 3 and Visit 8. Within 3 weeks of Visit 3 such need for corticosteroid treatment was defined as Treatment Failure, thereafter, it was defined Relapse).

Safety

During the two weeks of double blind treatment: Adverse Events by standard questioning, in blood clinical chemistry, haematology, blood gas analysis, serum cortisol, and Electrocardiogram (ECG).

During the run-in period (from Visit 1 to Visit 3) and during the post-study follow-up (from Visit 6 to Visit 8) only Serious Adverse Events were collected and Adverse Events leading to Discontinuation.

Statistical methods

Changes after two weeks treatment from baseline (thus Visit 6 versus Visit 3) were compared between treatment groups. Continuous data was presented using descriptive statistics (e.g. mean (arithmetic or geometric), median, standard deviation (SD) or coefficient of variation (cv), minimum and maximum). Categorical data was summarised in terms of frequencies (n) and percentages (%). All hypothesis testing was done using two-sided alternative hypotheses. P-values less than 5% were considered statistically significant. In the multiplicative analyses zero values were estimated with the lowest non-zero value obtained in the study. Values prefixed by "<" were set to half the value (i.e., divided by 2). No adjustment for multiplicity was performed, considering the amount of tests performed a number of significant results are expected by chance alone. Change in number of eosinophils was compared between treatments using a multiplicative (ie, log transformation of the response and the covariate) analysis of variance (ANOVA) model with treatment as fixed factor and the measurement from the randomization visit as a covariate. In pairs treatment ratios and 95% confidence intervals were calculated from the model. The ratio between measurements at other visits and the measurement at randomization were described in summary tables. The Last Observation Carried Forward (LOCF) technique was used to impute missing data before and after treatment separately.

Subject population

The study was prematurely interrupted after 4 years. At that time-point 114 patients were enrolled of whom 45 experienced an exacerbation which was studied during double blind treatment. It was planned to enrol minimally 100 patients and complete the study with 60 randomised patients with evaluable data.

Table S1 Subject population and disposition

Population		**Budesonide / formoterol**		**Prednisolone**		**Placebo**	
Number of patients randomised		15		15		15	
Demographic characteristics							
Sex (n and % of subjects)	Male	10	(67)	13	(87)	14	(93)
	Female	5	(33)	2	(13)	1	(7)
Age (years)	Mean	61.4		64.8		64.6	
	Range	48 – 72		47 – 74		49 – 77	
Race (n and % of subjects)	Caucasian	15	(100)	15	(100)	13	(87)
	Oriental	0	(0)	0	(0)	2	(13)
Baseline characteristics (at Visit 1)							
Mean FEV_1 (L) (range)		1.937	1.21 - 2.94	1.875	1.19 - 2.63	1.733	1.11 – 2.55
Mean FEV_1 (% of predicted) (range)		63.5	38 - 77	60.2	34 - 81	57.4	38 – 82
Mean FEV_1 / IVC (%) (range)		46.8	29 - 67	43.8	26 - 65	45.5	23 – 62
Baseline characteristics (at Visit 3)							
Mean FEV_1 (L) (range)		1.564	0.83 - 2.43	1.572	0.81 - 2.48	1.446	1.05- 2.41
Disposition							
N (%) of subjects who	Completed Visit 8	9	(60)	6	(40)	9	(60)
	Discontinued before V8	6	(40)	9	(60)	6	(40)
	Completed Visit 6	14	(93)	15	(100)	14	(93)
N analysed for safety [a]		15		15		15	
N analysed for efficacy (ITT) [b]		15		15		15	

[a] : Number of subjects who took at least 1 dose of study treatment; [b] : Number of subjects who had at least 1 data point after dosing; ITT=Intention to treat; n=Number;

Efficacy and pharmacokinetic results

The **primary** variable for the assessment of efficacy (**sputum % eosinophils**) was determined in 44 subjects. The 45th patient had missing data. After 14 days of budesonide/formoterol and prednisolone treatment sputum % eosinophils decreased (by 57% from 2.6% to 1.1% and by 58% from 2.0% to 0.9% respectively), while during placebo % eosinophils increased slightly (by 24% from 2.7% to 2.9%). There was a difference in the change in % eosinophils between budesonide/formoterol and placebo (relative ratio 0.35, 95% C.I. 0.16 – 0.77, p=0.011). There was also a difference between prednisolone and placebo (relative ratio 0.34, 95% C.I. 0.16 – 0.73, p=0.0072) whereas no difference between budesonide/formoterol and prednisolone was found (relative ratio 1.03 (95% C.I. 0.47 – 2.27, p=0.95).

Concerning **secondary** parameters, there were statistically significant differences between the three treatments in **other inflammatory markers in sputum**. In general, inflammation was more intense at the moment of exacerbation (the Randomisation Visit 3) than after the initial 2-months run-in period with an assessment made in a stable condition (Visit 2) and inflammation gradually decreased during the two weeks of follow-up (to Visit 6). The following changes during treatments (from Visit 3 to Visit 6) differed statistically significant between treatments: during budesonide/formoterol treatment compared to placebo treatment sputum eosinophil count (in 10^6/g sputum) decreased (p=0.015) and mRNA for the expression of IL-5 decreased (the number of PCR-cycles increased, p=0.021).

During budesonide/formoterol treatment compared to prednisolone treatment sputum albumin decreased (p=0.016), the relative level of albumin in sputum/in serum decreased (p=0.015), expression of mRNA for Hemoxygenase-1 increased (lower number of cycles, p=0.022), levels of IL-6 decreased (p=0.039), the levels of IL-8 decreased (p=0.026) and the levels of TNFα decreased (p=0.026).

During prednisolone treatment compared to placebo treatment expression of mRNA for TGFβ decreased (the number of cycles increased, p=0.045), the levels of LTB_4 increased (p=0.030) and the levels of TNFα increased (p=0.0058).

From the other inflammatory markers **in serum,** levels of sICAM were lower during prednisolone treatment than during both budesonide/formoterol treatment (p=0.011) and lower than during placebo treatment (p=0.010).

Lung function and symptoms gradually improved during the 14 days of randomized treatment. **FEV_1** increased with 8.2% during treatment with budesonide/formoterol compared to 2.0% during treatment with prednisolone and 0.5% during placebo treatment (p=0.067 for budesonide/formoterol versus placebo). **Morning PEF,** as averaged over all 14 days of treatment for the exacerbation was lower than in the run-in period under both prednisolone and placebo treatment (-26.8 and –22.2 L/min respectively), while during budesonide/formoterol treatment PEF was slightly higher than n the run-in period (+2.5 L/min). However, the differences between treatments were not statistically significant. **Total**

symptom scores as recorded in the diary were higher during the 14 days of randomized treatment than during the run-in period for all three treatments, especially for placebo treatment. The difference in mean overall total symptom scores between budesonide/formoterol and placebo (as changes from run-in to treated exacerbation) was statistically significant (-1.37, p=0.0097) as was the difference between prednisolone and placebo treatments (-1.03, p=0.048). Symptoms, recorded with the **CCQ** decreased from the start to the end of the treatment period, more during budesonide/formoterol than during prednisolone or placebo treatment. There was a significant difference in mean overall CCQ scores between budesonide/formoterol and prednisolone (-0.65, p=0.016). Quality of life, assessed with **CRQ** (as the change from Visit 2 to Visit 6) improved after budesonide/formoterol and deteriorated slightly after prednisolone and placebo. The difference budesonide/formoterol versus prednisolone was significant (difference -0.54, p=0.036).

There were no differences between treatments in the number of **Treatment Failure and Relapses**, and no difference in the time to Treatment Failure / Relapse.

Analysis of pathogens (bacteria and virus) did not provide unexpected information.

There were no pharmacokinetic results obtained during the present study.

Table S2 Efficacy Results (restricted to the primary parameter; for the secondary parameters only the statistically significant differences compared to budesonide/formoterol)

	budesonide/ formoterol	**predni-solone**	**placebo**	**p-values**
	n=15	**n=15**	**n=15**	
Primary				
Sputum eosinophils (%) V3 → V6	2.6%→ 1.1%	2.0%→ 0.9%	2.7%→ 2.9%	B/F vs Pla: p=0.011 B/F vs Pr: p=0.95 Pr vs Pla: p=0.0072
Secondary				
Sputum eosinophil count (10^6/g) V3 → V6	0.21 → 0.03	0.32 → 0.09	0.21 → 0.19	B/F vs Pla: p=0.012
Sputum mRNA for IL-5 (cycles[a]) V3 → V6	35.89 → 40.44	38.61 → 37.70	37.77 → 38.69	B/F vs Pla: p=0.021
Sputum Albumin (mg/l) V3 → V6	77.1 → 42.7	73.1 → 77.0	71.7 → 57.7	B/F vs Pr: p=0.016
Sputum/serum ration albumin V3 → V6	2.02 → 1.03	1.91 → 1.91	1.74 → 1.41	B/F vs Pr: p=0.015
Sputum mRNA for HO-1	25.23	26.27 →	25.48 →	B/F vs Pr: p=0.022

	budesonide/ formoterol n=15	predni-solone n=15	placebo n=15	p-values
Primary				
(cycles[a]) V3 → V6	→24.97	26.02	24.97	
Sputum IL-6 (pg/ml) V3 → V6	256 → 134	619 → 397	295 → 174	B/F vs Pr: p=0.039
Sputum IL-8 (pg/ml) V3 → V6	1854 → 936	3901 → 3086	1938→ 1308	B/F vs Pr: p=0.026
Sputum TNF-α (pg/ml) V3 → V6	3.78 → 2.24	15.73 → 12.43	5.22 → 2.07	B/F vs Pr: p=0.026
Serum ICAM (ng/ml)	108.4 → 103.5	123.0 → 95.8	118.3 → 120.3	B/F vs Pr: p=0.011
Diary Total Symptom score Run-in → Treatment[b]	4.17 → 4.40	4.97 → 5.18	4.74 → 6.09	B/F vs Pla: p=0.0097
Overall CCQ symptom score V3 → V6	2.50 → 1.50	2.47 → 2.13	2.76 → 2.06	B/F vs Pr: p=0.016
CRQ[c] score V2 – V6	5.05 → 5.48	5.05 → 4.88	4.77 → 4.71	B/F vs Pr: p=0.036

[a]: a higher number of cycles represent a lower quantity of mRNA; [b]: Run-in data are obtained in a stable phase; [c]: a higher CRQ score represents a better status; B/F: budesonide/formoterol; Pla: placebo; Pr: prednisolone

Safety results

The present data describes adverse events obtained after the start of blind treatment in the 45 Randomised patients, thus not only those observed during the 14 days double blind treatment, but also during the run-in period and during the follow-up period of 3 months. It must be noted that events observed or reported during the run-in period of all Enrolled patients were not reported as Adverse Events, unless they constituted a Serious Adverse Event or lead to discontinuation. Of relevance: one Enrolled patient died of lung cancer in the run-in period before being randomised. In the table below the AE data is presented for the 14 days blind treatment period and for the total 3 months' observation period after randomisation.

Table S3 Number (%) of patients who had at least 1 adverse event in any category, and total numbers of adverse events (safety analysis set, only randomised patients)

	budesonide/ formoterol	predni-solone	placebo	All
	n=15	n=15	n=15	n=45
No. of deaths	0	0	0	**0**
No. of SAEs other than death during blind treatment (Visit 3 – Visit 6)	0	0	0	**0**
No. of SAEs other than death in total (Visit 3 – Visit 6)	1	0	0	**1**
No. (%) of patients with an SAE	1 (7%)	0	0	**1 (2%)**
Max no. of SAEs/patient	1	0	0	**1**
No. of other significant AEs	0	0	0	**0**
No. (%) of patients with DAE during blind treatment (Visit 3 – Visit 6)	1 (7%)	0	1(7%)	**2 (4%)**
No. (%) of patients with DAE in total (Visit 3 – Visit 8)	4 (27%)	7 (47%)	6 (40%)	**17 (38%)**
No. of AEs	11	10	17	**38**
Mild	6	3	12	**21**
Moderate	3	6	3	**12**
Severe	2	1	2	**5**
No. (%) of patients with AE	7 (47%)	9 (60%)	11 (73%)	**27 (60%)**
Max no. of AEs / patient	3	2	3	**3**

[a] SAE : Serious Adverse Event; DAE: Discontinuation due to Adverse Event; one patient died in the run-in period, prior to receiving randomised treatment.

Table S4 Number (%) of subjects with the most commonly reported[a] adverse events, sorted by decreasing order of frequency as summarised over all treatment groups (safety analysis set)

	budesonide/ formoterol	prednisolone	placebo	All
Preferred term	**n=15**	**n=15**	**n=15**	**n=45**
Chronic obstructive airways disease exacerbated	5 (33%)	7 (47%)	6 (40%)	**18 (40%)**

Preferred term	budesonide/ formoterol n=15	prednisolone n=15	placebo n=15	All n=45
Dysphonia	1 (7%)	0	1 (7%)	**2 (4%)**
Haemoptysis	0	1 (7%)	1 (7%)	**2 (4%)**

[a] Events with a total frequency of ≥2 and thus ≥4 % across all treatment groups are included in this table

It must be noted that when a patient experienced a worsening of the disease under study (COPD) in the follow-up phase and corticosteroid treatment was deemed necessary, this patient was to be withdrawn and the event was to be coded as an exacerbation, leading to DAE.

Three **laboratory** parameters showed a statistically significant difference (for the change from Visit 3 to Visit 6), all three during prednisolone, compared to placebo: blood leukocyte count increased (+ $3.6x10^9$/L, compared to change during placebo, p<0.001), blood neutrophil count increased (+$2.4x10^9$ /L, p=0.010) and serum Potassium decreased (-0.32 mMol/l, p=0.032). There were no differences between budesonide/formoterol and prednisolone or between budesonide/formoterol and placebo. Prednisolone differed also from placebo concerning effects on **ECG** parameters as changes form Visit 3 to Visit 6): compared to changes during placebo QRS duration decreased (-6.2 ms, p=0.0048) and QTc time decreased (-19 ms, p=0.013). Morning **serum cortisol** decreased more under prednisolone treatment (by 49% from Visit 2 to Visit 6), than under budesonide/formoterol treatment (by 23%) and than during placebo treatment (by 12%). The changes under prednisolone and placebo differed statistically significant (p=0.033).