SUMMARY

ZENECA PHARMACEUTICALS

FINISHED PRODUCT: ACCOLATE™

ACTIVE INGREDIENT: Zafirlukast

Trial title (number): A Double-blind, Placebo-controlled Trial to Determine the Effects of Zafirlukast (80 mg bd) and Beclomethasone Dipropionate (200 μg bd), Given for 7 Days, on the Cellular Responses in the Airways to Inhaled Allergen Challenge (9188IL/0109).

Clinical phase: IIIB

First patient recruited: 21 February 1997
Last patient completed: 9 October 1998
Zeneca approval date: 17 May 2000

Publications: Macfarlane AJ, Manning P, Ryan M, Naya I, Harris A, Pavord ID, et al. Zafirlukast and low dose beclomethasone protect against early (EAR) and late systemic reactions (LAR) and allergen induced eosinophilia in mild atopic asthma. European Respiratory Journal 1999;14 (Suppl 30):121s.

OBJECTIVES

Primary objective: To determine whether zafirlukast (80 mg bd) and beclomethasone dipropionate (BDP); (200 μg bd), given for 7 days, affect the inflammatory response of the airways to inhaled allergen challenge in patients who demonstrate a late asthmatic reaction.

Secondary objectives: To determine the effects of zafirlukast (80 mg bd) and BDP (200 μg bd), given for 7 days, on the early and late asthmatic response to standardised doses of inhaled allergen.
To determine the safety of zafirlukast (80 mg bd).

METHODS
Design: This was a randomised, double-blind, double-dummy, placebo-controlled, parallel-group trial, conducted in 4 centres, in adult patients with mild asthma. The trial involved 3 treatment groups: zafirlukast 80 mg bd; BDP 200 μg bd; and placebo. The total duration of the trial was to be 6 weeks. Prospective patients were observed during a 5-week screening period, during which an inhaled allergen challenge was performed (beginning of the 2nd week). The subsequent 4 weeks before randomisation were to allow the airways to return to their baseline condition. Eligible patients were then allocated to receive 1 of the 3 treatments for 8 days. Patients were to be assessed at entry to the trial, during the screening period, at the beginning of the randomised period, and on Days 7 and 8 during the randomised period.
Population: A total of 60 patients (20 per treatment group) were required to complete the trial.
Key inclusion criteria: Male or female aged between 18 to 50 years inclusive; non-smokers, or ex-smokers who had smoked ≤10 pack years and who had stopped smoking at least 6 months before screening; a diagnosed history of asthma; currently receiving short-acting β_2-agonist pro re nata (prn) as their only treatment for asthma; a forced expiratory volume in 1 second (FEV_1) ≥70% of the predicted value (at least 6 hours after β_2-agonist use); a positive skin reaction to at least 1 common allergen (wheal size ≥3 mm); able to demonstrate a late asthmatic reaction (ie, ≥15% fall in FEV_1) 4 to 8 hours after inhaled allergen challenge; able to demonstrate at least a doubling in the eosinophil count (eosinophils must be ≥5% of the total non-squamous cells) in an induced sputum sample collected 24 hours after an inhaled allergen challenge.
Key exclusion criteria: Overnight hospitalisation for asthma in the 3 months before screening; evidence of respiratory disease other than reversible airways obstruction; lower or upper respiratory tract infection in the 6 weeks before entry or during the screening period; use of inhaled or oral corticosteroids in the 3 months before entry; use of cromones, antihistamines, theophylline, non-steroidal anti-inflammatory drugs (NSAIDs), oral β_2-agonists or anticholinergic drugs in the month before entry; use of long-acting β_2-agonists in the 2 weeks before entry; clinically important electrocardiogram (ECG) abnormalities; changes in asthma therapy during the screening period.
Dosage: The oral dosages used in this trial were zafirlukast 80 mg bd and placebo bd. Each 80-mg-bd dose was composed of 2 zafirlukast 40-mg tablets, and each placebo dose was composed of 2 placebo tablets. The inhaled dosages used in this trial were BDP 200 μg bd and matching placebo, supplied as metered-dose inhalers (MDIs). Each BDP 200-μg-bd dose was administered as 100 μg/puff taken as 2 puffs twice a day, and placebo MDI taken as 2 puffs twice a day. Formulation and batch numbers were: zafirlukast 40 mg tablets, F11401 (batch numbers 28125/95 and 37509K96); placebo to zafirlukast tablets, F7173 (batch numbers 36277K96 and 36267D96); BDP MDI 100 μg, F8213 (batch number 37019J96); placebo to BDP MDI, F8215 (batch number 38206F96).

In this report the treatment groups that received zafirlukast 80 mg bd or BDP 200 μg bd are labelled and referred to as the zafirlukast and BDP groups, respectively.

Key assessments:

Efficacy: The comparisons of interest were zafirlukast versus placebo and BDP versus placebo. The primary endpoint for the analysis of efficacy was the total eosinophil cell count (also known as MBP^+ cells) in bronchial biopsy samples taken on Day 8 of the randomised period (ie, collected 24 hours after an allergen challenge that was performed on Day 7). The secondary endpoints for the analysis of efficacy were: in bronchial biopsy samples taken on Day 8 of the randomised period (see above) - counts of activated eosinophils, mast cells, lymphocytes (total, CD4, CD8, CD25), and neutrophils; in bronchoalveolar lavage (BAL) samples taken on Day 8 (see above) – counts of total eosinophils, activated eosinophils, mast cells, total lymphocytes and neutrophils. Data for BAL histamine levels were not available as centres did not retain the relevant lavage fraction. Also, the number of activated eosinophils in the BAL was not a protocolled secondary endpoint but was prospectively identified as such and documented accordingly in the statistical analysis plan (SAP).

Further secondary endpoints on Day 7 of randomised period were the maximum percentage (%) fall in FEV_1 (1 to 2 hours and 4 to 8 hours after the challenge for the early and late responses, respectively); changes in FEV_1 in response to the challenge, measured as the area under the curve for FEV_1 recorded 0 to 2 hours after the challenge ($FEV_1AUC_{0\text{-}2}$ [for the early response]), and the area under the curve for FEV_1 recorded 2 to 12 hours after the challenge ($FEV_1AUC_{2\text{-}12}$ [for the late response]); and the time to recovery from the late response.

All endpoints, apart from the time to recovery, were analysed using analysis of covariance (ANCOVA) applying log-transformations where appropriate, to compare the treatment groups in a "per-protocol" population. Where there was evidence to suggest a deviation from normality, a non-parametric analysis (ANCOVA on ranked observations) was used to confirm the results of ANCOVA. Where results of parametric and non-parametric analyses differed, the non-parametric approach was preferred. No formal statistical analysis was performed on the time to recovery. Results of the analysis were presented in terms of means, standard deviations (SD) and least squares means (lsmeans) for each treatment group. For the comparisons specified, the differences between the lsmeans, the 95% confidence intervals (CI) and the associated p-value were also presented. Where a log-transformation was applied, the results were presented in terms of geometric least squares means (glsmeans) and the coefficient of variation (CV). For the comparisons specified, the ratio of the glsmeans (ie, treatment effect), with the 95% CI and associated p-value were presented. Where a non-parametric approach was used, data were summarised for each treatment group using the median rather than the mean and SD.

Safety: Safety was assessed by the recording of adverse events, physical examinations and routine clinical laboratory tests. Safety results were tabulated and summarised without formal statistical analysis.

RESULTS:

Demography: In total, 120 patients entered the initial screening period of the trial. Of these, 58 patients (41 male, 17 female), aged between 18 and 48 years, were eligible for, and chose to

enter the randomised treatment period and received 1 of the 3 treatments (ie, 8 days treatment taken over a period of 9 days): 19 received zafirlukast; 20 received BDP; and 19 received placebo. Their mean (SD) percentage predicted FEV_1 at baseline was 93.11 (14.3), and all patients had a documented known allergen that precipitated asthma. The treatment groups were comparable with respect to these demographic characteristics.
Of the 58 patients who received trial treatment, 2 patients were withdrawn from the trial: 1 from the zafirlukast group who was withdrawn before the 2nd bronchoscopy on the recommendation of the anaesthetist, and 1 from the placebo group due to an adverse event.
Efficacy: The primary endpoint in this trial was the effect of 7 days of treatment with Zafirlukast or BDP, compared with placebo, on the total eosinophil cell count in bronchial biopsy samples which were taken on Day 8 of the randomised period (ie, 24 hours after an allergen challenge that was performed on Day 7). Because allergen challenge is known to affect total eosinophils in both the bronchial tissue and in the BAL fluid, results from the primary endpoint are presented together with the secondary endpoint: total eosinophil cell count in the BAL. In the ANCOVA analysis of total eosinophil cell counts in the biopsy and BAL samples, there was evidence of non-normality in the form of outlying data. Therefore, a non-parametric analysis (ANCOVA on ranked observations) was performed and considered as the primary analysis of treatment effect; the results of which are summarised in Table I.

Table I Non-parametric analysis of post-challenge, post-treatment, bronchial biopsy (cells/mm) and BAL (%) total eosinophil cell counts

Treatment	n[a]	Value reported	Before treatment[b]	After treatment[c]	Comparison	Rank p-value
Bronchial biopsy cell counts:						
Zaf 80 mg bd	17	Median	10.70	12.49	Zaf 80 mg bd - placebo	0.1645
BDP 200 µg bd	17	Median	14.72	8.03	BDP 200 µg bd - placebo	0.0033
Placebo	15	Median	11.37	20.96	NA	
BAL cell counts:						
Zaf 80 mg bd	15	Median	3.00	2.00	Zaf 80 mg bd - placebo	0.0283
BDP 200 µg bd	16	Median	1.65	3.00	BDP 200 µg bd - placebo	0.7617
Placebo	13	Median	2.00	4.00	NA	

[a] Refers to the number of patients who completed the biopsy and BAL assessments at Visit 5 (Day 0) and at Visit 7 (Day 8).
[b] Before the 1st dose of randomised treatment at Visit 5 (Day 0).
[c] Samples taken at Visit 7 (Day 8) of the randomised period (ie, 24 hours after the challenge performed at Visit 6 [Day 7]).
BAL Bronchoalveolar lavage; BDP Beclomethasone dipropionate; n Number of patients; NA Not applicable; Zaf Zafirlukast.

The effect of the challenge, performed after 7 days of treatment with placebo, was an approximate doubling in total eosinophil cell counts in both the biopsy and BAL, compared with pre-treatment cell counts. This magnitude of increase in cell counts after the challenge, was not observed in the bronchial biopsies after 7 days of randomised treatment with either active therapy, and not observed in BAL after 7 days of treatment with zafirlukast (see Table 1).
In the biopsy, while the median total eosinophil cell count was 40% lower after treatment with zafirlukast (12.49 cells/mm), compared with placebo (20.96 cells/mm), the difference was not statically significant (p=0.1645). The median total eosinophil cell count was 8.03 cells/mm after treatment with BDP, compared with placebo, and was statistically significant (p=0.0033). In the BAL, the median total eosinophil cell count was 4.0% after treatment with placebo, compared

with 2.0% after treatment with zafirlukast, the difference being statically significant (p=0.0283), and 3.0% after treatment with BDP, the difference not being statistically significant (p=0.7617). These results suggest that the anti-inflammatory effects of zafirlukast and BDP may arise through different mechanisms, with eosinophil survival in the tissue appearing to be more effectively reduced by BDP, whilst eosinophil migration into the tissue and the lumen of the airways appearing to be more effectively reduced by zafirlukast. However, given that the design of this trial did not include a pre-treatment biopsy taken before and after the challenge, and post-treatment biopsy taken before and after the challenge, we cannot assess which part of the treatment difference was due to suppressing eosinophil recruitment in response to the challenge, as opposed to changing baseline levels unrelated to the effects of the challenge (ie, it is possible that the groups may not have been comparable before the 2nd challenge).

Overall, no consistent pattern of effect of the challenge on biopsy or BAL secondary cell counts was observed for placebo. The statistically significant increased level of neutrophils (p=0.0006) in the biopsy sample after the challenge for BDP, compared with placebo, was unexpected, although the inhibition of neutrophil apoptosis caused by steroid treatment has been previously reported (Wenzel et al 1997). Also, it is possible that in a trial where so many statistical comparisons are being made, some comparisons may reach significance by chance alone. For other biopsy and BAL secondary cell counts, there was evidence (although not statistically significant), that both the zafirlukast and BDP treatments provided a protective effect, compared with placebo.

Original time windows for the early and late responses (1 to 2, and 4 to 8 hours, respectively), were found to be inappropriate for most patients and analysis of these endpoints was repeated using the windows of 0 to 2 hours for the early response, and 2 to 12 hours for the late response. Results of the early and late responses using the original time windows are presented in Section 4.11 of the statistical report.

Zafirlukast, when compared with placebo, significantly attenuated the early response to the inflammatory effects on lung function induced by the challenge, reducing the drop in FEV_1 (p=0.0478), and providing a significant protective effect on $FEV_1AUC_{0\text{-}2}$ (p=0.0221). BDP also significantly attenuated the drop in FEV_1 (p=0.0430) during the early response, but did not provide a significant protective effect on $FEV_1AUC_{0\text{-}2}$ (p=0.1209). Compared with placebo, both treatments significantly attenuated the late asthmatic response that is associated with inflammatory effects on lung function - each treatment significantly attenuated the maximum fall in FEV_1 (p=0.0021 and p<0.0001 for zafirlukast and BDP, respectively) – and significantly decreased the $FEV_1AUC_{2\text{-}12}$ (p=0.0022 and p<0.0001, respectively).

The protection in LAR offered by zafirlukast *and* the magnitude of the changes observed in the total eosinophil cell count in both the biopsy and BAL samples, suggest that zafirlukast confers clinically meaningful protection against inflammatory changes due to inhaled allergen challenge.

Safety: Adverse events in each treatment group are summarised in Table II.

Table II Overview of adverse events

Category[a]	Zaf 80 mg bd		BDP 200 µg bd		Placebo	
	N of AEs	N of pts (%)	N of AEs	N of pts (%)	N of AEs	N of pts (%)
Patients at risk	NA	19 (100)	NA	20 (100)	NA	19 (100)
All adverse events	7	4 (21.1)	8	5 (25.0)	12	6 (31.6)
Adverse events associated with death	0	0 (0)	0	0 (0)	0	0 (0)
Adverse events reported as serious[b]						
not leading to withdrawal	0	0 (0)	2	1 (5.0)	0	0 (0)
leading to withdrawal	0	0 (0)	0	0 (0)	0	0 (0)
Other adverse events leading to withdrawal	0	0 (0)	0	0 (0)	3	1 (5.3)
Other adverse events	7	4 (21.1)	6	5 (25.0)	9	5 (26.3)

[a] Adverse event categories are mutually exclusive: events are counted in 1 category only. Patient categories are not mutually exclusive; patients may have adverse events in more than 1 category.
[b] A serious adverse event was defined as an adverse event that was fatal, was life-threatening, caused or prolonged hospitalisation, caused disability or incapacity, required medical intervention to prevent permanent impairment or damage, was a cancer, congenital abnormality or overdose.
BDP Beclomethasone dipropionate; NA Not applicable; N Number; Pts Patients; Zaf Zafirlukast.

The incidence of adverse events in each group was similar. One patient had 2 serious adverse events (nausea and dizziness) which resolved, without treatment, 2 hours after onset. One patient (placebo group) was withdrawn due to abnormally elevated clinical laboratory parameters which were not considered by the investigator to be related to trial treatment but secondary to the patient's weight-lifting exercise activities. No other clinically significant changes in haematological, biochemical or ECG measurements were observed across the 3 groups.