Effectiveness of Voriconazole In the Treatment of *Aspergillus fumigatus* Associated Asthma (EVITA^3)

Joshua Agbetile MD, Michelle Bourne RGN, Abbie Fairs PhD, Beverley Hargadon RGN, Dhananjay Desai MD, Clare Broad, Joseph Morley BSc, Peter Bradding DM FRCP, Christopher E. Brightling PhD FRCP, Ruth H. Green DM, FRCP, Pranabashis Haldar DM MRCP, Catherine H. Pashley PhD, Ian D. Pavord DM FRCP, Andrew J. Wardlaw PhD FRCP.

Institute for Lung Health, Department of Infection, Immunity and Inflammation, University of Leicester, Department of Respiratory Medicine University Hospitals of Leicester NHS Trust, Glenfield Hospital, Groby Road, Leicester UK LE3 9QP.

Funding

The study was investigator led, but funded by Pfizer. We also used the resources of the Leicester National Institute for Health Research (NIHR) respiratory biomedical research unit.

Correspondence

Professor Andrew Wardlaw

Institute for Lung Health

Glenfield Hospital

Groby Road

Leicester LE3 9QP

Email: aw24@le.ac.uk Tel:44 (0)116 2583841

Abstract

Background: IgE sensitisation to *Aspergillus fumigatus* and a positive sputum fungal culture are common in refractory asthma. It is not clear whether these patients would benefit from anti-fungal treatment.

Objectives: To determine if a three-month course of voriconazole improved asthma related outcomes in people with asthma who are IgE sensitised to *A. fumigatus.*

Methods; Asthmatics IgE sensitised to *A fumigatus* with a history of at least two severe exacerbations in the previous twelve months were treated for three months with voriconazole two hundred milligrams twice daily, followed by observation for nine months, in a double blind, placebo controlled, randomised design. Primary outcomes were improvement in quality of life at the end of the treatment period and a reduction in the number of severe exacerbations over the twelve months of the study.

Results: 65 patients were randomised. 59 patients started treatment (32 voriconazole and 27 placebo), and were included in an intention to treat analysis. 56 patients took the full three months of medication. There was no significant difference in the number of severe exacerbations between the voriconazole and placebo groups (1.25 vs 1.52/patient/year; mean difference 0.27; 95% CI 0.24 to 0.31) respectively, quality of life (change in AQLQ 0.44 vs 0.35, mean difference between groups 0.08; 95% CI 0.07-0.09), or in any of our secondary outcome measures between the two groups.

Conclusion: We were unable to show a beneficial effect of three months treatment with voriconazole in people with moderate to severe asthma who were IgE sensitised to *A fumigatus* on either the rate of severe exacerbations, quality of life or other markers of asthma control.

Clinical Implications. A short course of voriconazole does not benefit people with asthma associated with allergy to *A .fumigatus*. Previously reported benefits of itraconazole in this group of patients could be due to pharmacokinetic effects on endogenous and exogenous corticosteroids.

Capsule summary. Allergy to *A. fumigatus* is common in refractory asthma and many of these patients grow the mould in their sputum. However treatment with voriconazole for three months did not improve asthma outcomes in these patients.

Key words: refractory; asthma; exacerbations; *Aspergillus fumigatus;* ABPA; mould; eosinophils; voriconazole; quality of life. SAFS

Abbreviations:

ACQ: Asthma control questionnaire

A. fumigatus: Aspergillus fumigatus

AQLQ: Asthma quality of life questionnaire

COPD: Chronic Obstructive pulmonary disease

EVITA[3]: **E**ffectiveness of **V**oriconazole In the **T**reatment of ***A****spergillus fumigatus* **A**ssociated **A**sthma

FEV_1: Forced expiratory volume in one second

HRCT: High resolution computerised tomography

Pc20: Provocational concentration causing a 20% fall in FEV_1

SAFS: Severe asthma with fungal sensitisation

SPT: Skin prick test

VAS: Visual analogue score

Acknowledgements

We would like to thank all the patients who took part in this study. We would like to thank Anna Murphy who gave advice on the interactions of voriconazole with other treatments and Maria Shelley who helped with the randomisation. We would like the thank the consultant physicians Dr R Reddy, Dr S.F Hussain, Dr S Malik, Dr B Richardson, Dr A Jeffrey, Dr J Naylor, Dr I Wahedna, and Dr C Whale who referred patients into the study.

Introduction.

It is well recognised that colonisation of the airways with filamentous fungi (moulds) together with raised specific IgE can occur in asthma (and cystic fibrosis), where it is associated with a distinct syndrome called allergic bronchopulmonary mycosis (ABPM) (1, 2). The main moulds associated with this condition are *A. fumigatus* and related thermotolerant members of the *Aspergillus* genera causing allergic bronchopulmonary aspergillosis (ABPA) (3, 4). The classical clinical features of ABPM are fleeting lung shadows, proximal bronchiectasis and a cough productive of viscid mucus. These are associated with laboratory findings of a raised total IgE, a raised fungal specific IgE (and/or a positive SPT), and IgG and a peripheral blood eosinophilia.

Up to 50% of patients with refractory asthma have been reported as being IgE sensitised to fungi (5). However most patients who are IgE sensitised to *A. fumigatus* do not fulfil all the criteria for ABPA. They often have levels of total IgE below the accepted ABPA threshold (>410IU/L or 1000ng/ml although some authorities use >1000IU/L) (6-9), concentrations of specific IgG in the normal range, absence of proximal bronchiectasis and no evidence of fleeting shadows. We have shown in a cross-sectional study ~60% of people with moderate to severe asthma who are IgE sensitized to *A. fumigatus*, but without ABPA, have a positive sputum culture for the mould suggesting airway colonisation is commonly associated with sensitisation (10, 11). Patients with either sensitisation or a positive sputum culture have a lower post-bronchodilator FEV_1 than matched asthmatics and in patients who have both sensitisation and a positive sputum culture, compared to those who have neither, the mean difference in post-bronchodilator FEV_1 is 20% predicted suggesting a relationship between both lung damage and fungal allergy and infection (10, 11). A positive sputum culture and sensitisation have also been associated with increased incidence of bronchiectasis(12).

If, as is thought to be the case in patients with ABPA, persistent colonisation of the bronchial tree with *A. fumigatus* is contributing to the clinical picture in asthmatics with allergy to *A. fumigatus* without ABPA, it raises the question whether treatment with anti-fungal therapy would be of benefit in this group of patients? Most descriptions of the use of anti-fungals in ABPA in asthma and cystic fibrosis have been limited to case reports. There have been two significant placebo controlled studies of anti-fungal treatment for ABPA identified in a Cochrane review both of which reported benefits of itraconazole (9, 13, 14). The only other randomised study of anti-fungal treatment in asthma was by Denning *et al*. who treated 58 people with severe asthma and fungal sensitisation (SAFS: a terms coined by the authors to describe people with severe asthma and fungal IgE who do not meet the criteria for ABPA) (15) with itraconazole 200mg twice daily for 32 weeks and observed a significant improvement compared with placebo in AQLQ (16).

One problem with interpreting studies that have used itraconazole is that it can markedly enhance the effects of both endogenous and exogenous corticosteroids (17). Thus the improvements seen in the above studies could be due to a pharmacokinetic effect on corticosteroid bioavailability rather than anti-fungal activity. This pharmacokinetic effect has not been reported to occur with voriconazole. It is generally considered that voriconazole is at least as effective as itraconazole in the treatment of invasive infections of *A. fumigatus* and is regarded as first line therapy in many centres. (18). We therefore undertook a study of voriconazole in people with asthma who were sensitised to *A. fumigatus* to determine if this improved their asthma control.

Methods:

Patients

Subjects (all over 18 years), were recruited during 2010 and 2011 mainly from the respiratory clinics at Glenfield Hospital, although ten subjects were referred into the study from other hospitals in the East Midlands, UK. The inclusion criteria were a clinical diagnosis of asthma with at least historical evidence of variable airflow obstruction (short term variability in FEV_1 of >12% or Pc20<8mg/ml), evidence of IgE sensitisation to A.fumigatus (raised specific IgE of >0.35 IU/L or a SPT of >2mm greater that the negative control), and at least two severe exacerbations (defined as requiring a minimum of 3 days of high dose oral corticosteroids for their asthma), in the previous 12 months. Exclusion criteria were pregnancy, a diagnosis of COPD, a medical condition that would increase the likelihood of an adverse reaction to voriconazole and treatment with an anti-fungal agent in the twelve months prior to entry into the study.

Study Design

This was an investigator led, single centre, double blind, placebo controlled, randomised, parallel group study conducted between 2010 and 2012. The funding agency Pfizer provided the drug and placebo but had no role in the accrual or analysis of the data. Ethical approval from the Leicestershire Ethics Committee (UKCRN ID 7763) and the UK Medical and Health Products Regulatory Agency (MHRA: 09/H0402/63) was obtained and each patient gave written informed consent. The clinical trials registration numbers were ISRCTN42366088 and EudraCT 2009-011452-21. The visit schedule together with the investigations undertaken at each visit is shown in supplementary Table 1S. At a baseline visit demographic details were collected including smoking history, treatment and exacerbation history. If a HRCT scan had been undertaken for routine clinical purposes the presence or absence of bronchiectasis on the radiology report was recorded. Spirometry was performed, and quality

of life measurements recorded. Blood was drawn for total IgE, specific IgE and IgG to *A. fumigatus*, a full blood count and routine biochemistry, serum cortisol and cystic fibrosis genotyping. Sputum was obtained either spontaneously or by induction for a cell differential and fungal culture. Skin prick test to a panel of aeroallergens including *A. fumigatus* was undertaken. After a run in period of up to one month to ensure the subjects condition was stable and to allow measurement of the sputum differential and fungal culture which was used for randomisation the subjects were started on treatment at visit two. Treatment was given for three months during which subjects were seen at monthly intervals. They were then seen bimonthly until the end of the study. Investigations were repeated at each visit according to the schedule in Figure 1S. Voriconazole levels were measured at visits three or four, one or two months after starting treatment. Exacerbations were treated either by their personal physician or by the study team and managed according to standard clinical practice. Chronic asthma treatment was not altered during the period of the study. Randomisation was in blocks of three with the use of the minimisation method using the criteria of sputum eosinophil count, the number of exacerbations in the previous twelve months and sputum fungal culture for *A. fumigatus* (19*)*. Voriconazole was given at a dose of 200mg twice daily with the drug and matched placebo provided by Pfizer. The two primary outcome measures were the change in the Juniper asthma quality of life questionnaire (AQLQ) from baseline to the end of the treatment period and the number of severe exacerbations, defined as above, over the twelve months of the study. Secondary outcomes measures were the modified Juniper asthma control questionnaire (ACQ 6 which excludes FEV_1), a combined visual analogue score (VAS) based on three 100mm visual analogue scales (VAS) which measured symptoms of cough, breathlessness and wheeze , a nasal polyp questionnaire (20), post-bronchodilator FEV_1, sputum eosinophil and neutrophil count, peripheral blood eosinophil count, total IgE and *A. fumigatus* specific IgE and IgG.

Investigations.

Clinical investigations and measurement of the sputum differential and fungal culture were undertaken as previously described and detailed in the on line supplementary appendix (21, 22). Measurement of the total IgE and specific IgE and IgG were undertaken in the routine University Hospitals of Leicester immunology laboratory using the ImmunoCAP system. Serum for voriconazole levels was sent to the Health Protection Agency mycology reference centre in Bristol.

Statistics

The study was powered on severe exacerbations. 25 patients were required in each group assuming two exacerbations per patient per year in the placebo group and one exacerbation per patient per year in the voriconazole arm ($\alpha = 0.05$, $\beta=0.02$). The exacerbation data was analysed using negative binomial regression. Those patients who took at least one week of treatment were analysed on an intention to treat basis. For the quality of life data the mean ACQ6, mean AQLQ and mean VAS are shown. Baseline scores were compared with post-treatment data collected at visit five and error bars represent the standard error of the mean. Within-group data was analysed using paired t-tests; between group comparisons were analysed separately at baseline and visit five using unpaired t-tests. Statistical software packages used for various analyses included PASW statistics 18 and GraphPad Prism, version 4 (GraphPad Software).

Results

Recruitment

The details of recruitment are shown in the CONSORT diagram (Figure 1). About a third of patients contacted and screened agreed to take part. 65 patients were randomised. Six dropped out before taking any drug because they changed their mind about participating in the study between the screening visit and taking the first study medication and took no further part in the study. 59 patients were therefore entered into the analysis. Of these, three in each group did not complete the course of treatment although four of these continued in the study.

Baseline demographics and investigations

Baseline details of the patient demographics and investigations are shown in Table 1. The active and placebo groups were generally well matched with no significant differences between them, although there was a trend towards more men in the placebo group. The patients in both groups had moderate to severe disease with a requirement for high doses of corticosteroids and a substantial degree of fixed airflow obstruction and bronchiectasis. Two patients fulfilled all the criteria for a diagnosis of ABPA.

Primary outcomes

There was no significant difference in the total number of severe exacerbations or in the number of subjects exacerbating between the two groups over the twelve-month period of the study (Figure 2). The voriconazole group had a mean of 1.16 exacerbations per subject over the twelve months of the study compared to 2.5 in the twelve months prior to the study. There were a mean of 1.4 exacerbations in the placebo group compared to 3.0 in the previous 12 months. This represented a 54% reduction from baseline in each group. 27 patients in the voriconazole arm had one or more exacerbations compared to 18 patients in the placebo arm. The AQLQ score improved from a mean of 4.55 at

baseline in the voriconazole group to 5.22 at the end of the treatment period (Figure 3). It then fell back within two months and was 4.85 at the end of the trial. A similar pattern was seen in the placebo group improving from 4.66 at baseline to 5.54 at the end of treatment and 5.13 at the end of the study. There were no statistically significant differences between the voriconazole and placebo groups.

Secondary outcomes

There were no significant differences between the voriconazole and placebo groups in the three other quality of life measures that we used, the ACQ6, VAS and nasal polyp questionnaire (Figure 3 and data not shown). These measures demonstrated the same pattern as the AQLQ with an improvement in both groups to the end of the treatment period followed by a rapid return towards baseline. There were no significant differences between the groups in FEV_1, blood and sputum counts or total and A. fumigatus specific IgE and IgG (see supplementary appendix)

Discussion.

As far as we are aware this is the first report of a randomised controlled study that has investigated the effects of voriconazole in asthma complicated by allergy to *A. fumigatus*. Voriconazole has a similar *in vitro* minimum inhibitory concentration against *A. fumigatus* to itraconazole and posaconazole and a good profile of tissue penetration into the lung tissue and epithelial lining fluid (23). Its use is generally restricted to invasive infections or situations where itraconazole treatment has failed, where anecdotally it appeared to have additional benefit (24). Previous clinical trials of itraconazole in ABPA and SAFS had demonstrated improved quality of life, reduced exacerbations, steroid sparing properties and reduced inflammatory and immune markers (9, 13, 16). We based our outcomes on these studies with a greater emphasis on detecting a reduction in severe exacerbations because of the link between eosinophilic inflammation (which is associated with fungal allergy), and an exacerbation phenotype

(25). It is not clear why our study found no benefit of anti-fungal treatment compared to the above studies. We recruited a similar number of patients and the treatment dose was the same although of shorter duration (12 weeks compared to 16 weeks or 32 weeks in the case of the FAST study, Our patients were similar to those recruited by the other groups in terms of the severity of asthma although the patients in the studies by Wark et al and Stevens et al had immunologically more florid disease, particularly with respect to total IgE. The patients in the study by Denning et al had a different pattern of fungal allergy as a basis for recruitment, but this may have been expected to reduce the power of their study because only a proportion had allergy to thermotolerant, potentially colonising fungi. We feel it is unlikely that a longer period of treatment would have altered the outcomes for the quality of life measures or the secondary outcomes as voriconazole should clear the fungi from the airways in weeks and we did not see any additional effect of treatment on fungal cultures after the first month. However the fungal colonisation did appear to return quite rapidly to baseline levels within a few months of cessation of treatment either as a result of re-activation of dormant spores in macrophages or re-infection so we cannot exclude the possibility of an effect of exacerbations if we had continued treatment for the full twelve months. However this would have been prohibitively expensive and resulted in increased adverse events.

We used a fixed dose of voriconazole based on the manufacturers guidance and the dose of itraconazole used in the studies quoted above. We did not attempt to adjust the dose based on voriconazole levels, not least because of the difficulty of varying the dose while maintaining a double blind design. Like Denning et al (although unlike Wark and Stevens et al), we did measure voriconazole levels to provide evidence of compliance (see supplementary appendix), but we did not rigorously measure trough levels and we have some missing data. Some patients did have levels below the recommended trough level of ~0.5υg/ml and we cannot exclude the possibility that tissue levels

were sub-optimal in those patients. There was however no association between voriconazole levels and response to treatment.

As in previous studies we found high rates of a positive sputum culture for A. fumigatus with 41% of subjects at baseline having a positive sputum and >80% of subjects having at least one positive sputum over the course of the study (see supplementary appendix). Rates in normal subjects are ~5% in our hands on a single visit, but we do not have normal values for more than two measurements. There was a trend for more positive sputum samples in the voriconazole group at baseline, but as noted above the numbers with at least one positive sputum over the course of the study were well matched between the two groups. In terms of sputum culture there were significantly more responders in the voriconazole group than the placebo group. However clearance of the sputum was not complete with five out of 24 subjects where there was sputum data having a positive sputum (albeit with only one colony in each case), at the end of the treatment period. There was also a rapid relapse with 22 out of 31 subjects in the voriconazole group with data having at least one positive sputum in the four post treatment visits. Numbers of subjects with definitive sputum data were too small to make meaningful comparison of the clinical response between sputum responders and non-responders.

There was a significant improvement in all three measures of quality of life at the end of the treatment period compared to baseline, but this was also seen in the placebo group and the between group differences were not significant. This contrasts with the study by Denning et al where the improvements in AQLQ with itraconazole were similar in magnitude to our study, but there was no placebo effect (16). Benefits with placebo are common in research studies especially with subjective symptom data due to optimisation of therapy, regression to the mean or psychological effects. Our

patients were stable when recruited and on optimal therapy. In addition the benefits of treatment in both groups were transitory. This suggests that psychological factors were the main explanation for the placebo effect in this study.

The patients were generally well matched apart from differences in gender which seems unlikely to have had a material effect on the outcome of the study. Of the 65 subjects who were consented to take part in the study and were randomised six changed their mind before taking any treatment. Five of these were in the placebo group which skewed the recruitment numbers, but again it seems unlikely this affected the outcome. Although a significant number of subjects reported side effects of the medication only one subject in the voriconazole group took less than two months treatment and only two patients were lost to follow up, both in the placebo arm.

We were powered to show a 50% reduction in severe exacerbations based on the placebo group having at least two exacerbations over the 12 month period. In the event the rate of exacerbations in the placebo group was only 1.5 which represented a ~50% reduction from the previous 12 months whereas the rate in the voriconazole group was 1.2 which similarly was a ~50% reduction from baseline. Thus although strictly speaking we were underpowered the lack of even a hint of a difference between the two groups makes it unlikely that larger numbers of subjects would have resulted in demonstration of a clinically significant reduction in severe exacerbations. The numbers in our study were very similar to those in the FAST study of Denning *et al* where they observed a significant improvement in AQLQ in the itraconazole group. We don't believe therefore that the failure to show any effect on quality of life in our study was due to a lack of power.

One possible explanation for the lack of any clinical or laboratory benefit in our study compared to those that have used itraconazole is that the benefits of itraconazole in those studies was due primarily to a pharmacokinetic effect on corticosteroid bioavailability, especially considering the high doses of inhaled and oral steroids which tend to be used in ABPA and SAFS. This is a well-recognised problem with itraconazole which confounds interpretation of the use of this drug (17, 26). This pharmacokinetic effect has not been reported with voriconazole and in the subset of subjects where we performed cortisol levels voriconazole had no discernible effect on serum cortisol (data not shown). Denning et al found that half the patients they tested who were taking itraconazole had reduced cortisol levels, but the improvement in AQLQ was no different in these patients compared to those with unchanged cortisol, although numbers were small.

In conclusion this study does not provides any evidence that patients with moderate to severe asthma, who are IgE sensitised to *A. fumigatus*, but do not fulfil all the criteria for ABPA will gain any short to medium term benefit to their asthma control from a three month course of voriconazole.

311 Table 1: Baseline Measurements

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	Voriconazole (N=32)	Placebo (N=27)	P value
Demographics			
Men	38%(12)	63%(17)	0.07
Age (mean [range], years)	59 [27-80]	59 [38-78]	0.90
Age at onset (mean [range], years)	19 [2-60]	20 [2-63]	0.50
Body mass index (mean [range])	27[18-37]	27 [17-36]	0.55
Spirometry			
FEV_1 % of predicted post-bronchodilator	72.6 ±27.7	62.7±20.3	0.13
FEV_1/FVC ratio post-bronchodilator	0.61±0.18	0.60±0.17	0.70
Leucocyte Counts and sputum analysis			
Eosinophil count in blood ($x10^{-9}$/litre) † [S.E]	0.46[0.06]	0.41[0.04]	0.49
Sputum Eosinophil counts (geometric mean [log SD]%)	2.88 [1.19]	4.68[1.04]	0.29
Sputum Neutrophil counts %	66.87 ± 22.17	60.23±23.56	0.31
Sputum cell Total cell count*10^6/mL†	6.84 (4.44-10.55)	4.05(2.41-6.82)	0.11
Sputum culture positive for Aspergillus Fumigatus Baseline (n)	50% (16)	30% (8)	0.06
Patient reported outcomes			
Asthma Quality of Life Questionnaire (Baseline [S.E])	4.55[0.25]	4.66[0.28]	0.76
Modified Juniper Asthma Control Questionnaire (ACQ 6)	2.15±0.98	2.27±1.20	0.68
Average VAS score for asthma symptoms	40.00 ± 4.58	41.45 ± 4.02	0.81
Average VAS score for nasal polyps	35.33 ± 19.4	31.41 ± 18.47	0.44
Immunoglobulins and radiology			
Total IgE†(log_{10} SD)	459(3.19)	659(3.12)	0.33

Positive atopic status to common aeroallergens% (n)*	69% (22)	70% (19)	1.00
Baseline Specific IgE to Aspergillus fumigatus; RAST †	4.79(2.38-9.65)	5.69 (2.84-13.01)	0.54
Baseline Aspergillus fumigatus IgG†	30.8(23.54-42.60)	31.7(21.70-43.80)	0.74
Bronchiectasis present or radiology report of CT scan % (n)	52% (16)	62%(16)	0.59
Smoking and steroid history			
Smoking (pack years) in ex or current smokers	14	11.9	0.68
Never Smokers,% (n)	59 % (19)	63% (17)	0.8
Rescue corticosteroid courses in previous year	2.5 (2-4)	3 (2-5)	0.19
Dose of inhaled corticosteroid-beclomethasone equivalent (median [IQR], ug/pt/day)	2000[900-2000]	2000[400-2000]	0.36
Number on maintenance prednisolone (median dose) % (n)	28% (9)	33% (9)	0.89
Median dose of maintenance Prednisolone [range]	5mg[2.5-10]	5mg[5-10]	0.89

313 ¶s.e †Geometric mean(95%C.I). *house dust mite, cat, dog, grass. **Total IgE ≥410 IU, Aspergillus
314 IgE ≥0.35, Aspergillus IgG >40mg/ml, proximal bronchiectasis.

315 P values were calculated with an independent t test for parametric values, Fishers exact test for
316 comparison of proportions and the Mann-Whitney u test for comparison of non-parametric

317

318 Figure Legends

319 *Figure 1: Recruitment of subjects*

320 Of the 184 people with asthma and IgE sensitisation approached to take part in the study about half
321 declined to be involved. A minority did not meet the inclusion criteria mainly because they had less
322 than two exacerbations in the previous twelve months. Of the 65 subjects randomised to drug or
323 placebo six (five in the placebo group), changed their mind between visit one and two. Of those who
324 took the treatment and were included in the analysis one subject in the placebo group was lost to follow
325 up between visit two and three and two subjects in the placebo group and three in the voriconazole
326 group did not complete the full course of treatment (although two of these took more than two months
327 of drug).

328 *Figure 2:Severe exacerbations.*

329 There was a linear increase in the number of severe exacerbations in both groups with no treatment
330 effect. The total number of exacerbations in the placebo group were thirty eight with thirty seven in the
331 voriconazole group which represented a 54% reduction from baseline in both groups. Five patients in
332 the voriconazole group didn't exacerbate and nine in the placebo group. This difference was not
333 significant.

334 *Figure 3: Patient reported outcomes*

335 There was a significant improvement in the quality of life scores in both groups from baseline during
336 the course of treatment with the voriconazole group increasing from a mean of 4.55 to a maximum of
337 5.3 and the placebo group from 4.66 to 5.6 ($p<0.001$). There was no significant difference between
338 groups and the improvement was not maintained after the treatment period had finished. A similar
339 pattern was observed for both the asthma control score and the mean VAS score with a significant

340 improvement from baseline in both groups during the treatment period but no significant difference
341 between groups and a return towards baseline immediately on stopping treatment.

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