

RESEARCH ARTICLE

Evaluation of the PPAR- γ Agonist Pioglitazone in Mild Asthma: A Double-Blind Randomized Controlled Trial

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Abstract

Background

Peroxisome proliferator-activated receptor gamma (PPAR- γ) is a nuclear receptor that modulates inflammation in models of asthma. To determine whether pioglitazone improves measures of asthma control and airway inflammation, we performed a single-center randomized, double-blind, placebo-controlled, parallel-group trial.

Methods

Sixty-eight participants with mild asthma were randomized to 12 weeks pioglitazone (30 mg for 4 weeks, then 45 mg for 8 weeks) or placebo. The primary outcome was the adjusted mean forced expiratory volume in one second (FEV₁) at 12 weeks. The secondary outcomes were mean peak expiratory flow (PEF), scores on the Juniper Asthma Control Questionnaire (ACQ) and Asthma Quality of Life Questionnaire (AQLQ), fractional exhaled nitric oxide (FeNO), bronchial hyperresponsiveness (PD₂₀), induced sputum counts, and sputum supernatant interferon gamma-inducible protein-10 (IP-10), vascular endothelial growth factor (VEGF), monocyte chemotactic protein-1 (MCP-1), and eosinophil cationic protein (ECP) levels. Study recruitment was closed early after considering the European Medicines Agency's reports of a potential increased risk of bladder cancer with pioglitazone treatment. Fifty-five cases were included in the full analysis (FA) and 52 in the per-protocol (PP) analysis.

Results

There was no difference in the adjusted FEV₁ at 12 weeks (-0.014 L, 95% confidence interval [CI] -0.15 to 0.12, $p = 0.84$) or in any of the secondary outcomes in the FA. The PP analysis replicated the FA, with the exception of a lower evening PEF in the pioglitazone group (-21 L/min, 95% CI -39 to -4, $p = 0.02$).

Conclusions

We found no evidence that treatment with 12 weeks of pioglitazone improved asthma control or airway inflammation in mild asthma.

was approved by Nottingham Research Ethics Committee 2. There was no further input from the sponsors into the analysis, interpretation, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

Trial Registration

ClinicalTrials.gov [NCT01134835](https://clinicaltrials.gov/ct2/show/study/NCT01134835)

Introduction

Asthma is a common and chronic condition that affects 235 million people worldwide and is responsible for substantial healthcare expenditures [1]. Inhaled corticosteroids and bronchodilators are the mainstay of asthma pharmacotherapy. Although these treatments are highly effective, many patients fail to achieve optimal asthma control and are at risk of impaired quality of life, recurrent exacerbations, and even death. In some patients, these outcomes are due to poor concordance with medication, while others dislike or exhibit reduced responses to inhaled medications, creating a need to identify novel therapeutic targets if the burden of asthma is to be reduced.

The nuclear transcription factor peroxisome proliferator-activated receptor gamma (PPAR- γ) is an important regulator of cellular homeostasis and energy metabolism that also has anti-inflammatory properties [2–7]. There is evidence that PPAR- γ is involved in inflammation and airway remodeling responses in asthma [4]. In experimental models of asthma, the activation of PPAR- γ suppresses the increase in airway hyperresponsiveness, eosinophil influx, and expression of Th-2 cytokines that occur in the lung following an allergen challenge [5, 6, 8–10].

PPAR- γ is thought to exert its immune modulating effects by reducing the downstream gene transcription of targets of the pro-inflammatory transcription factors activator protein-1 (AP-1) and nuclear factor-kappaB (NF- κ B) [11–13]. In cultured human airway smooth muscle cells (HASMs), PPAR- γ activation suppresses the release of the chemokines MCP-1 (monocyte chemoattractant protein-1/CCL2) and eotaxin (CCL11) in response to the potent inflammatory stimulant tumor necrosis factor-alpha (TNF- α) [3]. This suppression of eotaxin release is secondary to reduced histone acetylation and reduced binding of NF- κ B to the eotaxin promoter, reducing gene transcription [3].

The PPAR- γ agonists pioglitazone and rosiglitazone were developed to treat the hyperglycemia and insulin resistance of type 2 diabetes mellitus, and were used widely until rosiglitazone was withdrawn due to an adverse cardiovascular safety profile [14–16]. Prior to its withdrawal, rosiglitazone had been studied in two short-term (up to 28 days) randomized controlled trials (RCTs) in asthma that found modest effects on the late asthmatic reaction and airway obstruction in smokers with asthma [17, 18]. A longer term study of pioglitazone in a subset of obese subjects with poorly controlled asthma resulted in no significant improvement in measures of asthma control [19]. The aim of the present study was to test the hypothesis that the activation of PPAR- γ with pioglitazone (which has a more favorable safety profile than that of rosiglitazone) would improve measures of asthma control and markers of airway inflammation over a 12-week period in mild asthma. Study recruitment was terminated after 68 participants had been recruited after reports emerged that pioglitazone treatment may be associated with an increased risk of developing bladder cancer [20].

Materials and Methods

Ethics statement

The study was approved by Nottingham Research Ethics Committee 2 (08/H0408/120) and the Medicines and Healthcare Products Regulatory Agency (MHRA) (08028). All participants provided written informed consent. Trial registration: NCT01134835.

Study design and participants

A single-center randomized, double-blind, placebo-controlled, parallel-group trial comparing pioglitazone and placebo over 12 weeks in adults with mild asthma was performed at City Hospital, Nottingham, UK. The study design was approved by Nottingham Research Ethics Committee 2 (08/H0408/120). All participants gave written informed consent. The full trial protocol is given in [S1 Text](#).

Potential participants aged 18–75 years with a clinical diagnosis of asthma who were taking 0–800 µg of inhaled beclomethasone dipropionate (or equivalent) and short-acting β_2 -agonists (SABAs) as required were identified from primary care practices and our research database between January 2010 and February 2012. At screening, participants were required to have a post-bronchodilator forced expiratory volume in one second (FEV_1) $\geq$ 60% of predicted with $\geq$ 12% reversibility of the pre-bronchodilator FEV_1 with 400 µg of salbutamol or $\geq$ 12% peak flow variability during the run-in period (maximum peak expiratory flow [PEF] minus minimum PEF divided by average PEF) to be eligible.

The exclusion criteria included an exacerbation of asthma within six weeks, current smoking, or a history of more than 10 pack years of smoking, or treatment with leukotriene antagonists, long-acting beta-agonists, theophylline, or oral steroids. Patients with a history of diabetes or liver or cardiovascular disease; patients who were pregnant, lactating, or not using adequate contraception; or patients with contraindications to pioglitazone were excluded for safety reasons.

Screening visit. FEV_1 was measured as the greater of two values within 100 ml using a dry bellows spirometer (Vitalograph, Buckingham, UK), prior to and 15 minutes after the inhalation of 400 µg of salbutamol via a spacer (VolumaticTM, Allen & Hanburys Ltd, Middlesex, UK). Sputum induction with hypertonic saline was performed as described previously [21, 22] using the ultrasonic nebulizer OMRON NE-U17 (OMRON Health Care Ltd, Milton Keynes, UK). A clinician reviewed and examined participants at each visit.

Run-in period. Participants entered a two-week run-in period, during which morning and evening PEF (Mini-Wright, Clement Clarke International Ltd, Harlow, UK) and asthma medication use were recorded.

Randomization. Eligible participants were randomized to 12 weeks pioglitazone (30 mg once a day for 4 weeks, then 45 mg once a day for 8 weeks) or matched placebo using an internet-based randomization schedule stratified by inhaled corticosteroid use. The allocation sequence was generated using random permuted blocks of two, four, and six and maintained on a secure server by the Nottingham Clinical Trials Unit (CTU).

The baseline clinical measurements were: the fractional exhaled nitric oxide (FeNO) in parts per billion (ppb) at 50 mL/second (NIOX[®] Flex, Aerocrine, Solna, Sweden), pre-bronchodilator FEV_1 , airway hyperresponsiveness to methacholine (PD₂₀, Ganshorn ProvoJet, Ganshorn Medizin Electronic GmbH, Niederlauer, Germany) based on the protocol of Yan, Asthma Control Questionnaire (ACQ), Asthma Quality of Life Questionnaire (AQLQ), and induced sputum [23–25]. Study visits were at same time of day $\pm$ 1.5 hours at 4, 8, 12, and 16 weeks within three days of the protocol date and the baseline clinical measurements were repeated after 12 weeks of treatment.

Study intervention. Pioglitazone tablets were over encapsulated with DBcaps[®] capsules (Capsugel Belgium NV, Bornem, Belgium), and a matched placebo backfilled with lactulose was manufactured by Catalent Pharma Solutions, Bolton, UK. An independent pharmacist issued 30 capsules of the study medication to each participant every four weeks and compliance was assessed by counting the returned capsules. All participants and clinical investigators performing the clinical assessments were blinded to allocation until all trial procedures were performed and the analysis was complete.

Outcome measures and statistical analysis. The primary outcome measure was the adjusted mean FEV₁ at 12 weeks compared between treatment groups by analysis of covariance (ANCOVA) incorporating terms for baseline value, treatment arm, inhaled corticosteroid use, age, gender, and height. The secondary outcome measures at 12 weeks included mean morning and evening PEF for 14 days prior to the study visit, ACQ and AQLQ scores, FeNO, PD₂₀, SABA use, and body mass index (BMI). We considered a PD₂₀ of 800 µg to be equivalent to a PC₂₀ of 8 mg/mL [26]. Final follow up visits were in December 2011.

In subsets of participants, induced sputum cell counts, sputum supernatant interferon gamma-inducible protein-10 (CXCL10/IP-10), vascular endothelial growth factor (VEGF), MCP-1/CCL2, and eosinophilic cationic protein (ECP) were measured. Activation of PPAR-γ was assessed by comparing levels of cytoplasmic and nuclear PPAR-γ from induced sputum cells between groups. Adverse effects (reported spontaneously at clinic visits and on diary cards) were recorded, and random glucose, full blood count (FBC), and liver function tests (LFTs) were measured pre- and post-treatment.

Where appropriate, secondary endpoints were log-transformed to approximate a normal distribution (PD₂₀, percentage eosinophil count, and FeNO) and compared between groups using ANCOVA, incorporating terms for baseline value treatment arm, inhaled corticosteroid use, and, where appropriate, age, gender, and height. Values that could not be transformed were compared using the Mann-Whitney U test. In participants with values below the lower limit of detection in laboratory assays (five participants for ECP), a value of 0.5 of the lower detection limit was used. Sputum mediators were expressed per 1 x 10⁶ cells and were reported as geometric means with 95% confidence intervals (CIs). Stata v11.0 (StataCorp LP, College Station, TX, USA) and GraphPad Prism Version 6 (GraphPad Software, La Jolla, CA, USA) were used to perform the statistical analyses.

The *a priori* calculation suggested that 88 participants would allow us to detect a difference of 150 mL between groups in the mean FEV₁ at 12 weeks, with 80% power at a 5% two-sided significance level, assuming a standard deviation (SD) of FEV₁ over eight weeks of 250 mL [27].

A Trial Steering Committee (TSC) and independent Data Monitoring and Ethics Committee (DMEC) monitored the progress and safety of the trial.

Sputum processing. Induced sputum samples were processed within two hours of induction as described previously [21, 22]. Sputum supernatant IP-10, MCP-1, and VEGF were assayed using the Bio-Plex® 200 system (Bio-Rad, Hemel Hempstead, UK) and supernatant ECP was assayed using ImmunoCAP 100 (Phadia AB, Uppsala, Sweden) according to the manufacturers' instructions. PPAR-γ was assayed in the nuclear and cytoplasmic fractions of sputum cells using a TransAM™ PPAR-γ Kit (Active Motif, Rixensart, Belgium) and a competitive enzyme-linked immunosorbent assay (ELISA) PPAR-γ kit (Biosources, Inc., San Diego, CA, USA) according to the manufacturers' instructions. The optical density at 450 nm (OD₄₅₀) was used for quantification of nuclear PPAR-γ and the reciprocal of the OD₄₅₀ was used for quantification of cytoplasmic PPAR-γ.

Results

Of the 119 individuals screened, 68 participants were randomized, 34 to pioglitazone and 34 to placebo between January 2010 and February 2012 (Fig 1). The full trial dataset is given in [S1 Data](#) and Consort checklist in [S2 Text](#).

The full analysis was performed on 55 completed cases, and after excluding those with protocol deviations, the per-protocol analysis included 52 participants. The baseline characteristics of the study population are shown in [Table 1](#). Subjects were well matched between treatment

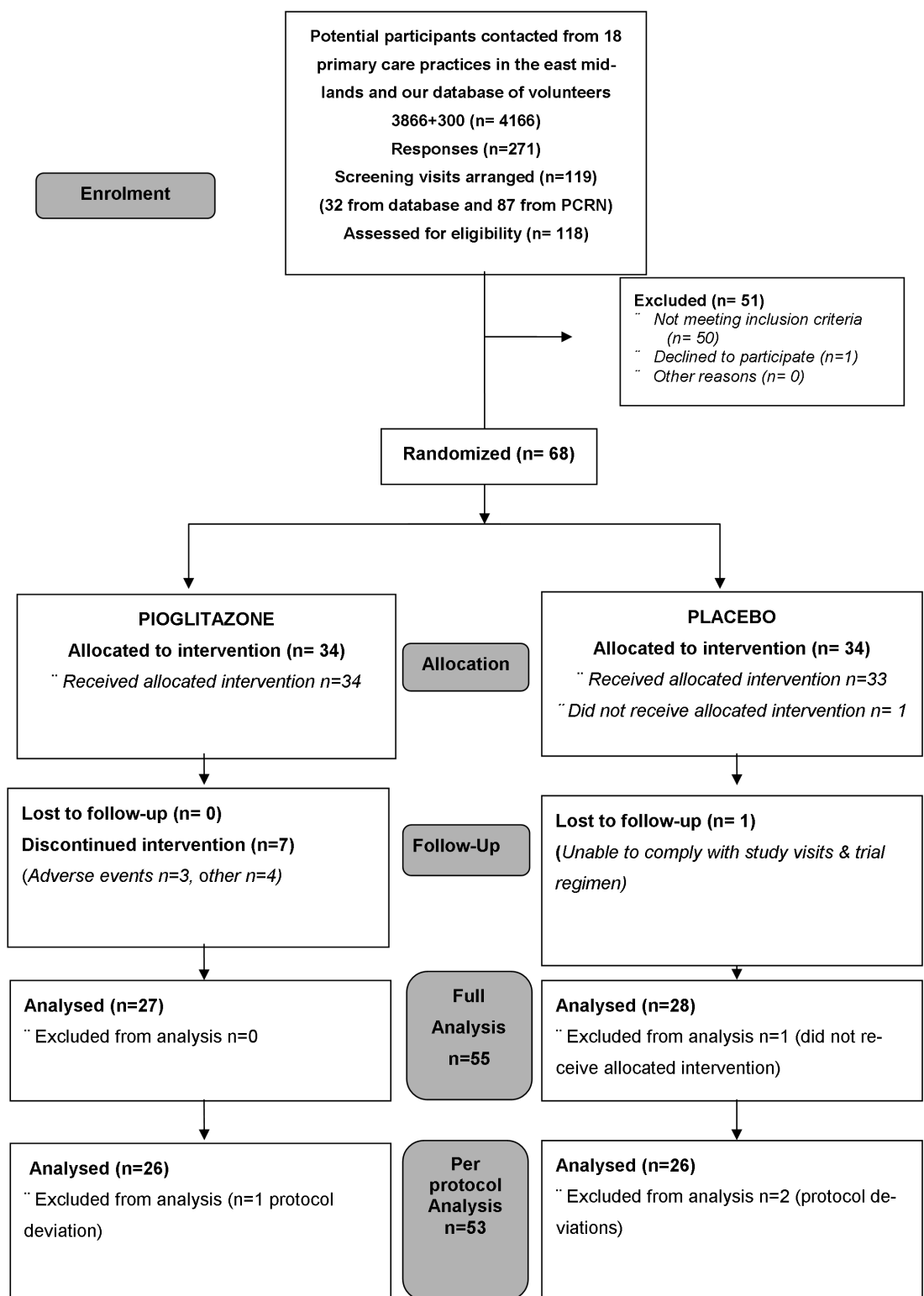


Fig 1. Consort participant flow.

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Table 1. Baseline demographics.

	All	PIOGLITAZONE	PLACEBO
Number of observations	n = 68	n = 34	n = 34
Age (years)	54.4 ± 13.6	51.6 ± 13.2	57.3 ± 13.7
• GenderMale n	• 37 (54.4%)31	• 18(52.9%)16	• 19(55.9%)
• (%)Female n (%)	(45.6%)	(47.1%)	• 15 (44.1%)
• EthnicityWhite n (%)Other (%)	• 67(98.5)1(1.5)	• 33(97.01%)1 (2.9%)	34 (100%)
Body mass index (kg/m ²) [†]	27.5 ± 3.6	28.1 ± 4.0	26.9 ± 3.0
History of smoking (Pack years) Median (range)	0 (0,10)	0.1 (0,10)	0 (0,10)
Daily inhaled steroid Yes (%)No (%)	51 (75%)17 (25%)	26 (76.5%)8 (23.5%)	25 (73.5%)9 (26.5%)
Daily inhaled corticosteroid dose (beclomethasone dipropionate equivalent [mcg/day]) Median (interquartile range)	200 (0, 400)	300 (200, 400)	200 (0, 400)
Duration of asthma (years) Median (interquartile range)	28.5 (18, 45.5)	27 (19, 41)	30.5 (13, 46)
Pre-bronchodilator FEV ₁ (liters)	2.70 ± 0.74	2.75 ± 0.73	2.65 ± 0.75
Predicted FEV ₁ (%)	90 ± 15.5	92 ± 15.5	88 ± 15.6
FVC (liters)	3.84 ± 1	3.92 ± 1	3.76 ± 1
Predicted FVC (%)	105.1 ± 15.7	107.8 ± 14.3	102.3 ± 16.8
FEV ₁ /FVC (%)	70.90 ± 9.6	71 ± 10.6	70.8 ± 8.6
FEV ₁ reversibility at screening [‡] (%) Median (interquartile range)	11.3 (7, 15) [†]	11.5 (7, 15)	11.3 (6.0, 17.0) [‡]
Exhaled nitric oxide FeNO at 50 ml flow (ppb) Geometric mean (95% CI)	24.9 (21.1, 29.4)	26.6 (20.6, 34.5)	23.20 (18.7, 28.8)
Airway hyperresponsiveness to methacholine PD ₂₀ FEV ₁ (mcg)*Geometric mean (95% CI)	97.7 (59.9, 159.5)	86.5 (39.3, 190.1) ^f	109.3 (56.7, 219.3) ^{f,f}
Juniper ACQ [#] score at baseline	0.9 ± 0.4 [~]	1.0 ± 0.7	0.9 ± 0.4 [∞]
Juniper AQLQ ⁺ score at baseline	6.2 ± 0.4 [~]	6.1 ± 0.6	6.2 ± 0.6 [∞]
Mean morning peak expiratory flow ¹	392 ± 95	391 ± 96	392 ± 95
Mean evening peak expiratory flow ²	396 ± 97	399 ± 93	396 ± 97
Peak expiratory flow variability [^] (%) Median (interquartile range)	19 (15, 24)	18 (13, 27)	18 (15, 23)
Short-acting beta ₂ -agonist use during run-in (actuations/day)	0.7 ± 1	1.5 ± 1.8	0.7 ± 1
Sputum differential eosinophil count % Geometric mean (95% CI) ^δ	0.8 (0.5, 1.4)	1.2 (0.6, 2.5)	0.5 (0.2, 1.1)

Arithmetic mean values reported as mean unless specified

± Standard deviation

Geometric means are reported with a 95% confidence Interval (CI)

* PD₂₀-The provocative dose of methacholine (in micrograms) that results in a 20% fall in the FEV₁, available in 46 participants at baseline and post-treatment. A PD₂₀ of 100 mcg is equivalent to a PC₂₀ of 1 mg/mL

‡ The percentage change in the pre-bronchodilator FEV₁ 15 minutes after 200 mcg of inhaled salbutamol

+ Juniper Asthma Control Questionnaire- A validated questionnaire providing a numerical assessment of asthma control over the preceding 7 days. The mean score ranges from 0 (fully controlled) to 6 (poorly controlled).

Juniper Asthma Quality of Life Questionnaire-The standardized 32-question AQLQ assesses the impact of asthma on 4 domains: symptoms, activity limitation, emotional function, and environmental stimuli. Mean score ranges from 1 (impaired quality of life) to 7 (no impairment of quality of life).

¹ and ² The mean peak expiratory flow calculated from the 14-day run-in period

[^]Peak expiratory flow variability calculated as the difference between the highest and lowest peak expiratory flow expressed as a percentage of the mean peak expiratory flow calculated from the 14-day run-in period

FEV₁. Forced expiratory volume in one second. FVC- Forced vital capacity. ppb- Parts per billion

[†] n = 68

[‡] n = 34

^f n = 22

^{f,f} n = 24

[∞] n = 32

[~] n = 66

^δ n = 41 (23 pioglitazone and 18 placebo)

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Table 2. Induced sputum indices at baseline.

Mean (SD)	n = 41	PIOGLITAZONE n = 23			PLACEBO n = 18		
	All	n = 23	SABA = 7	ICS = 16	n = 18	SABA = 6	ICS = 12
<i>Sputum total cell count</i> $\times 10^6/g$	1.1 $\pm$ 1.7	1.0 $\pm$ 1.2	1.3 $\pm$ 1.4 $\pm$ 1.4	0.9 $\pm$ 1.2	1.3 $\pm$ 2.6	0.6 $\pm$ 0.4	1.7 $\pm$ 2.6
<i>Sputum differential neutrophil count</i> %	49.0 $\pm$ 26.1	47.3 $\pm$ 26.3	64.6 $\pm$ 20.7	39.7 $\pm$ 25.4	51.2 $\pm$ 26.3	46.3 $\pm$ 35.4	53.6 $\pm$ 22
<i>Sputum differential eosinophil count</i> % Geometric mean (95% CI) [¶]	0.8 (0.5, 1.4)	1.2 (0.6, 2.5)	0.4 (0.08, 2.4)	1.8 (0.8, 4.2)	0.5 (0.2, 1.1)	0.4 (0.07, 2.4)	0.5 (0.2, 1.5)
<i>Sputum differential macrophage count</i> %	43.1 $\pm$ 26.0	41.8 $\pm$ 27.1	29.2 $\pm$ 20	47.4 $\pm$ 28.5	44.6 $\pm$ 25.3	47.8 $\pm$ 32.9	43.0 $\pm$ 22.0
<i>Sputum differential lymphocyte count</i> %	0.6 $\pm$ 0.6	0.7 $\pm$ 0.7	0.8 $\pm$ 1.0	0.6 $\pm$ 0.6	0.4 $\pm$ 0.4	0.4 $\pm$ 0.4	0.4 $\pm$ 0.4
<i>Sputum differential epithelial cell count</i> %	3.9 $\pm$ 4.9	5.0 $\pm$ 5.5	3.9 $\pm$ 3.8	5.5 $\pm$ 6.1	2.4 $\pm$ 3.6	4.2 $\pm$ 5.7	1.5 $\pm$ 1.7
<i>Sputum squamous contamination</i> %	4.2 $\pm$ 4.1	4.5 $\pm$ 4.8	4.0 $\pm$ 4.1	4.7 $\pm$ 5.2	3.9 $\pm$ 3.2	4.10 $\pm$ 2.0	3.8 $\pm$ 3.7

Values reported as arithmetic mean unless specified

$\pm$ Standard deviation

[¶] Geometric mean reported with a 95% confidence interval (CI)

SABA- Short-acting β_2 -agonist

ICS- Inhaled corticosteroid

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arms, 75% were taking inhaled corticosteroids with a SABA, and 25% were taking a SABA alone. Forty-one participants produced viable induced sputum samples pre- and post-treatment (Tables 1 and 2). Medication adherence assessed by tablet counting was greater than 95% in both groups over the course of the study.

Closure of recruitment

The European Medicines Agency (EMA) reported that pioglitazone may be associated with an increased risk of developing bladder cancer in July 2011 (20). Because there were no proven benefits associated with taking pioglitazone in asthma, the TSC concluded that the risk to benefit ratio had become unacceptable for participants with mild asthma and recruitment was stopped on 14 October, 2011. At that point, 68 of the minimum requirement of 88 participants had been randomized, with 52 participants having completed the study and 10 still in the treatment phase of the study. Among those still participating in the study, 70% withdrew after receiving notification of the EMA report, while three participants elected to complete the study. All participants and investigators remained blinded to allocation until the analysis was completed.

Efficacy

There was no significant difference observed in the primary outcome measure, the adjusted mean FEV₁ at 12 weeks, between the pioglitazone and placebo groups (adjusted mean difference -0.014 L, 95% CI -0.15 to 0.12, $p = 0.84$). In the FA there were no differences in any of the secondary outcomes between the groups at 12 weeks, although there was a trend toward a lower adjusted mean morning PEF (-16 L/min 95% CI -33 to 2, $p = 0.075$) and evening PEF (-17 L/min, 95% CI -35 to 1, $p = 0.068$) in the pioglitazone group. There was also a trend toward a higher use of as required SABA use in the pioglitazone group (0.6 actuations/day, 95% CI -0.05 to 1.2, $p = 0.07$) (Fig 2 and Table 3).

The per protocol analysis replicated the full analysis, except that the mean evening PEF was significantly lower in the pioglitazone group after 12 weeks (-21 L/min, 95% CI -39 to -4, $p = 0.02$) (Table 4).

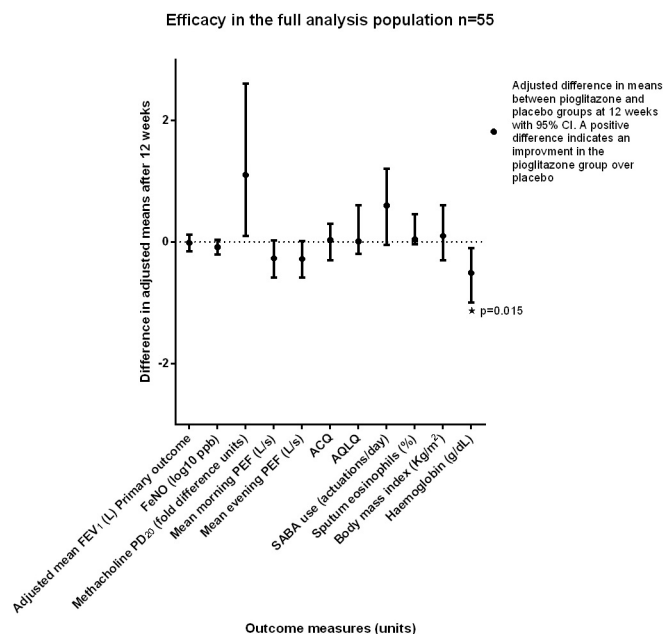


Fig 2. Efficacy analysis. The adjusted mean of the secondary clinical outcomes at 12 weeks was compared between groups using ANCOVA including terms for baseline value, treatment arm, inhaled corticosteroid use, age, gender, and height and expressed as the difference in the adjusted means with a 95% confidence interval (CI). FEV1—Forced expiratory volume in one second. FeNO—Fractional exhaled nitric oxide, ppb—Parts per billion. PD₂₀—The provocative dose of methacholine (in micrograms) that results in a 20% fall in the FEV1, available in 46 participants at baseline and post-treatment. A PD₂₀ of 100 mcg is equivalent to a PC₂₀ of 1 mg/mL. Fold difference units—antilog of the (adjusted mean difference) with 95% confidence interval. A 1-fold change is equivalent to 0 doubling doses. The mean peak flow calculated from the 14 days prior to the week 12 visit. ACQ—Juniper Asthma Control Questionnaire—A validated questionnaire providing a numerical assessment of asthma control over the preceding 7 days. The mean score ranges from 0 (fully controlled) to 6 (poorly controlled). AQLQ—Juniper Asthma Quality of Life Questionnaire—The standardized 32-question AQLQ assesses the impact of asthma on 4 domains: symptoms, activity limitation, emotional function, and environmental stimuli. Mean score ranges from 1 (impaired quality of life) to 7 (no impairment of quality of life). SABA—Short acting beta₂ agonist. Induced sputum data available for 41 participants in the full analysis.

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In subsets of participants that provided induced sputum samples pre- and post-treatment, there were no significant differences in the induced sputum differential cell counts or the induced sputum supernatant ECP, MCP-1, IP-10, or VEGF, or the nuclear and cytoplasmic levels of PPAR- γ between groups (Figs 2 and 3).

There was an increase in the reporting of expected side effects of pioglitazone in the active group, particularly ankle swelling, weight gain, insomnia, and lethargy (Table 5). There were no asthma exacerbations requiring oral corticosteroids or hospital admission in the study; however, five participants in the pioglitazone group and two in the placebo group reported mild/moderate asthma exacerbations that required an increase in SABAs.

The pioglitazone-treated group had a significantly lower adjusted mean hemoglobin (-0.51 g/dL, 95% CI -1 to -0.1, $p = 0.015$), red cell count ($-0.2 \times 10^{12}/L$, 95% CI -0.03 to -0.04, $p = 0.012$) and adjusted mean cell hemoglobin (-0.6 g/dL, 95% CI -1.1 to -0.08, $p = 0.024$) compared with the placebo-treated group at week 12 (Fig 2 and Table 6).

Discussion

In this randomized controlled trial, the administration of pioglitazone for 12 weeks in mild asthma did not result in any improvements in a range of markers of asthma control or

Table 3. Full analysis.

	PIOGLITAZONE n = 27			PLACEBO n = 28			Adjusted difference in means at 12 weeks between group(95% CI)	p-value (95% CI)
	Baseline (SD)	Week 12	Adjusted mean at 12 weeks (95% CI)	Baseline	Week 12	Adjusted mean at 12 weeks		
FEV₁ (liters)	2.71 ± 0.82.71	2.75 ± 0.82.75	2.67 (2.58, 2.76)	2.59 ± 0.82.59	2.61 ± 0.82.61	2.69 (2.60, 2.78)	-0.014 (-0.15, 0.12)-0.014	0.84
Exhaled nitric oxide FeNO (ppb)[¶] geometric mean (95% CI)	31.6 (24, 41.7)	24.0 (18.6, 30.2)	21.9 (18.2, 26.3)	23.4 (18.2, 29.5)	24.6 (19.5, 31.6)	26.6 (22.1, 31.8)	0.8 (0.6, 1.1)	0.18
Airway hyperresponsiveness to methacholine PD₂₀ FEV₁* mcg. Adjusted difference fold difference units[^] (95% CI)	86.5 (38.9, 190.6)	114.8 (55, 239.9)	129.2 (74.1, 223.9)	109.3 (56.7, 210.9)	128 (57.5, 284.8)	114.4 (67.6, 195)	1.1[^] (0.1, 2.6)	0.77
Mean morning PEF¹ (L/min)	385 ± 99	388 ± 106	388 (376, 400)	392 ± 99	404 ± 109	404 (392, 416)	-16 (-35, 2)	0.075
Mean evening PEF² (L/min)	393 ± 96	394 ± 107	390 (379, 402)	395 ± 101	404 ± 108	407 (395, 419)	-17 (-35, 1)	0.068
Juniper ACQ⁺	1.1 ± 0.7	0.7 ± 0.5	0.7 (0.5, 0.9)	0.9 ± 0.3	0.7 ± 0.5	0.7 (0.5, 0.9)	0.03 (-0.3, 0.3)	0.86
Juniper AQLQ[#]	6.0 ± 0.7	6.3 ± 0.6	6.3 (6.1, 6.5)	6.1 ± 0.6	6.3 ± 0.6	6.3 (6.1, 6.4)	0.01 (-0.2, 0.6)	0.94
Short-acting beta₂-agonist use in the 14 days preceding week 12 (actuations/day)	1.7 ± 1.8	1.1 ± 1.5	1.1 (0.7, 1.4)	0.8 ± 1	0.4 ± 0.5	0.5 (0.1, 0.9)	0.6 (-0.05, 1.2)	0.07
Body mass index at week 12 (kg/m²)	27.5 ± 3.1	27.8 ± 3.1	27.4 (27.1, 27.6)	26.8 ± 3.2	26.9 ± 3.3	27.3 (27, 27.6)	0.06 (-0.4, 0.5)	0.76

Values are reported as arithmetic mean unless specified

± Standard deviation

¶ Geometric means are reported with a 95% confidence interval (CI)

The adjusted mean of the secondary clinical outcomes at 12 weeks was compared between groups using ANCOVA including terms for baseline value, treatment arm, inhaled corticosteroid use, age, gender, and height and expressed as the difference in the adjusted means with a 95% confidence interval (CI) and p-value

*PD₂₀-The provocative dose of methacholine (in micrograms) that results in a 20% fall in the FEV₁, available in 46 participants at baseline and post-treatment.

A PD₂₀ of 100 mcg is equivalent to a PC₂₀ of 1 mg/ml

[^]Fold difference units- antilog of the (adjusted mean difference) with 95% CI. A 1 fold change is equivalent to 0 doubling doses

+ Juniper Asthma Control Questionnaire- A validated questionnaire providing a numerical assessment of asthma control over the preceding 7 days. The mean score ranges from 0 (fully controlled) to 6 (poorly controlled).

Juniper Asthma Quality of Life Questionnaire-The standardized 32-question AQLQ assesses the impact of asthma on 4 domains: symptoms, activity limitation, emotional function, and environmental stimuli. Mean score ranges from 1 (impaired quality of life) to 7 (no impairment of quality of life).

¹ and ² The mean peak flow calculated from the 14 days prior to the week 12 visit

FEV₁. Forced expiratory volume in one second

FVC- Forced vital capacity

ppb- Parts per billion

FEV₁. Forced expiratory volume in one second

ANCOVA- Analysis of covariance

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measures of airway inflammation. In fact, we observed a lower post-treatment mean evening PEF and a trend toward higher SABA reliever use in the pioglitazone group, which was not accompanied by significant deterioration in asthma control or scores on quality of life questionnaires. Although these findings most likely reflect the multiplicity of analyses, they could have been caused indirectly by side effects of pioglitazone such as insomnia and fatigue. If the reduction in PEF is a true effect, and assuming a minimum important difference (MID) for PEF of 15 to 20 L/minute, this is of a magnitude that could have been perceived by the participants [28].

Table 4. Per-protocol analysis.

	PIOGLITAZONE n = 27			PLACEBO n = 28			Adjusted difference in means at 12 weeks between group (95% CI)	p-value (95% CI)
	Baseline (SD)	Week 12	Adjusted mean at 12 weeks (95% CI)	Baseline	Week 12	Adjusted mean at 12 weeks		
FEV₁ (liters)	2.76 ± 0.8	2.80 ± 0.8	2.68 (2.59, 2.78)	2.58 ± 0.8	2.60 ± 0.8	2.71 (2.61, 2.81)	-0.03 (-0.17, 0.12)	0.71
Exhaled nitric oxide FeNO (ppb)[¶] geometric mean (95% CI)	32.7 (25, 42.8)	23.8 (18.5, 30.5)	22.2 (18.3, 27)	25.1 (19.8, 31.8)	25.9 (20.4, 33.1)	27.9 (22.8, 33.6)	0.8 (0.6, 1.1)	0.14
Airway hyperresponsiveness to methacholine PD₂₀ FEV₁* Adjusted difference in means fold difference units (95% CI)	87.8 (38, 201)	114.2 (52.5, 248.3)	123.2 (71.3, 212.9)	106.3 (53, 213.3)	133.6 (56.6, 315.5)	124.2 (72.9, 211.7)	1.0 [^] (0.4, 2.3)	0.98
Mean morning PEF¹ (L/min)	389 ± 99	393 ± 105	390 (378, 402)	393 ± 99	406 ± 110	409 (396, 421)	-19 (-38, 0.3)	0.053
Mean evening PEF² (L/min)	397 ± 95	399 ± 106	391 (379, 402)	393 ± 98	404 ± 109	412 (400, 424)	-21 (-39, 4)	0.02
Juniper ACQ⁺	1.1 ± 0.7	0.7 ± 0.6	0.7 (0.6, 0.9)	0.9 ± 0.3	0.6 ± 0.4	0.6 (0.4, 0.7)	0.2 (-0.1, 0.4)	0.23
Juniper AQLQ[#]	6.0 ± 0.7	6.3 ± 0.6	6.3 (6.2, 6.5)	6.3 ± 0.4	6.4 ± 0.5	6.4 (6.2, 6.5)	-0.1 (-0.3, 0.2)	0.69
Short-acting beta₂-agonist use in the 14 days preceding week 12 (actuations/day)	1.6 ± 1.7	1.0 ± 1.4	1.0 (0.6, 1.4)	0.8 ± 1.0	0.4 ± 0.5	0.4 (0.2, 0.80)	0.6 (-0.01, 1.2)	0.055
Body mass index at week 12 (kg/m²)	27.3 ± 2.9	27.5 ± 2.9	27.3 (27, 27.5)	26.8 ± 3.24	26.8 ± 3.3	27.1 (26.8, 27.4)	0.1 (-0.3, 0.6)	0.51

Values are reported as arithmetic mean unless specified

± Standard deviation

[¶] Geometric means are reported with a 95% confidence interval (CI)

The adjusted mean of the secondary clinical outcomes at 12 weeks was compared between groups using ANCOVA including terms for baseline value, treatment arm, inhaled corticosteroid use, age, gender, and height and expressed as the difference in the adjusted means with a 95% confidence interval (CI) and p-value.

*PD₂₀-The provocative dose of methacholine (in micrograms) that results in a 20% fall in the FEV₁, available in 43 participants at baseline and post-treatment.

A PD₂₀ of 100 mcg is equivalent to a PC₂₀ of 1 mg/mL.

[^]Fold difference units- antilog of the (adjusted mean difference) with 95% CI. A 1-fold change is equivalent to 0 doubling doses.

+ Juniper Asthma Control Questionnaire- A validated questionnaire providing a numerical assessment of asthma control over the preceding 7 days. The mean score ranges from 0 (fully controlled) to 6 (poorly controlled).

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¹ and ² The mean peak flow calculated from the 14 days prior to the week 12 visit

FEV₁- Forced expiratory volume in one second

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ANCOVA- Analysis of covariance

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Despite evidence that PPAR-γ activation has anti-inflammatory effects in experimental models of asthma, there are a number of potential reasons why pioglitazone may not have exerted an effect in the present study. Firstly, the early closure of recruitment limited the number of participants completing the study, leaving the study underpowered to detect a difference of 150 ml between groups. However, considering the narrow 95% CIs for the difference in FEV₁, the lack of change in airway hyper-responsiveness, lower PEF, and trend toward higher SABA use in the pioglitazone group, it is unlikely that we missed a clinically important effect on airway dysfunction in mild asthma.

It is possible that the observed lack of efficacy was because pioglitazone was not biologically active. Although we did not measure plasma levels of pioglitazone, more than 95% of the study

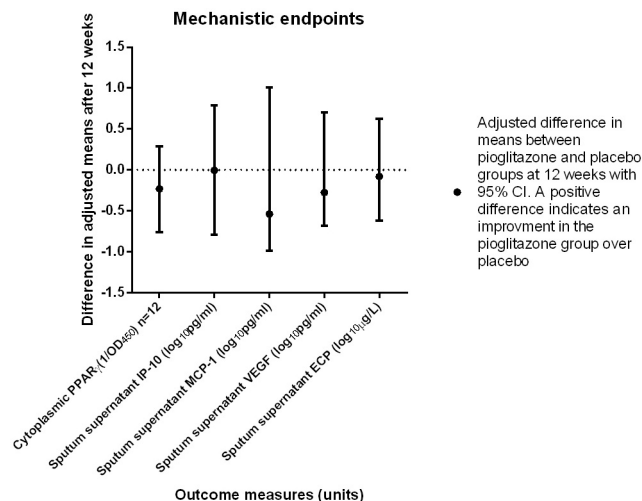


Fig 3. Mechanistic endpoints. In subsets of participants, the following assays were performed: Cytoplasmic PPAR-γ transcription factor assay. Pioglitazone n = 6, placebo n = 6. Mean of the reciprocal OD450. IP-10, MCP-1, VEGF and levels in sputum supernatant, assay (Bio-Plex) pg/mL normalized to 1×10^6 sputum cells. Pioglitazone n = 11, placebo n = 8. ECP pioglitazone n = 10 (total ECP n = 18) Phadia ImmunoCAP normalized to 1×10^6 sputum cells. PPAR-γ- Peroxisome proliferator-activated receptor gamma. OD Optical density (spectrophotometry). IP-10- Interferon gamma-inducible protein-10. VEGF- Vascular endothelial growth factor. MCP-1- Monocyte chemotactic protein-1. ECP- Eosinophil cationic protein.

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Table 5. Reported adverse events occurring in more than 1 participant by system.

	PIOGLITAZONE	PLACEBO
Respiratory system		
Upper respiratory tract infection	8	8
Exacerbation of asthma (mild/moderate)	5	2
Blood and lymphatic disorders		
Thrombocytopenia	2	0
Leukopenia or Neutropenia	1	1
Nervous system disorders		
Insomnia	5	1
Alteration of mood	3	0
Headache	1	4
Eye disorders		
Hordeolum	2	0
Ear and labyrinth disorders		
Epistaxis	2	1
Vertigo	0	2
Renal tract		
Urinary tract infection	0	2
Musculoskeletal system		
Arthralgia	1	1
General disorders		
Ankle edema (spontaneously reported)	8	2
Weight gain (spontaneously reported)	4	0
Fatigue	3	0
Investigations		
Weight increased (> 5% of baseline)	3	1

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Table 6. Safety set blood test monitoring.

	PIOGLITAZONE			PLACEBO			Difference in adjusted means between groups at 12 weeks	p-value (95% CI)
	Baseline <i>n</i> = 34 (SD)	Week 12 (<i>n</i> = 28)	Week 12 adjusted mean <i>n</i> = 27 (95% CI)	Baseline <i>n</i> = 34 (SD)	Week 12 (<i>n</i> = 29)	Week 12 adjusted mean (95% CI)		
Hemoglobin (g/dL)	13.9 ± 1.2	13.3 ± 1.3	13.5 (13.2, 13.8)	14.3 ± 1.1	14.3 ± 1.1	14.1 (13.8, 14.3)	-0.51 (-1, -0.1)	0.015*
Hematocrit	0.4 ± 0.03	0.41 ± 0.03	0.4 (0.4, 0.4)	0.4 ± 0.02	0.4 ± 0.03	0.4 (0.4, 0.4)	-0.01 (-0.03, 0.0003)	0.056
Mean cell hemoglobin concentration (g/dL)	33.1 ± 1.2	32.6 ± 1.1	32.8 (32.5, 33.1)	33.4 ± 1.1	33.6 ± 0.9	33.4 (33, 33.7)	-0.6 (-1.1, -0.08)	0.024*
White cell count × 10⁹/L	6.8 ± 1.7	6.4 ± 1.6	6.5 (6, 6.9)	6.9 ± 1.6	6.8 ± 1.8	6.7 (6.3, 7.2)	-0.3 (-1, 0.5)	0.45
Platelet count × 10⁹/L	246 ± 50	237 ± 53	233 (219, 247)	239 ± 41	244 ± 50	248 (235, 262)	-15 (-36, 5)	0.14
Mean cell volume (MCV) fL	89 ± 5	90 ± 6	90 (89, 91)	90 ± 4	90 ± 4	90 (89, 90)	0.7 (-0.5, 1.9)	0.27
Blood neutrophils × 10⁹/L	3.9 ± 1.5	3.7 ± 1.3	3.8 (3.3, 4.2)	3.9 ± 1.1	3.9 ± 1.6	3.8 (3.4, 4.3)	-0.09 (-0.7, 0.6)	0.79
Log₁₀ blood eosinophils × 10⁹/L	-0.7 ± 0.2	-0.8 ± 0.2	-0.8 (-0.9, -0.7)	-0.7 ± 0.3	-0.7 ± 0.2	-0.7 (-0.8, -0.6)	-0.09 (-0.2, 0.02)	0.11
Log₁₀ ALT U/L	1.4 ± 0.2	1.3 ± 0.3	1.3 (1.3, 1.4)	1.3 ± 0.2	1.3 ± 1.3	1.3 (1.3, 1.4)	-0.02 (-0.09, 0.04)	0.49
Log₁₀ GGT	1.41 ± 0.25	1.31 ± 0.27	1.30 (1.26, 1.33)	1.36 ± 0.16	1.36 ± 0.16	1.37 (1.33, 1.40)	-0.07 (-0.12, -0.024)	0.004*
Total bilirubin μmol/L	9.1 ± 3.6	9.7 ± 3.9	10.1 (9.1, 11.1)	10.1 ± 3.7	10.1 ± 3.7	9.84 (8.9, 10.8)	0.2 (-1.2, 1.7)	0.76
Total alkaline phosphatase U/L	69.1 ± 21.1	63.3 ± 18.9	63.8 (61.2, 66.4)	72.4 ± 15.1	70.6 ± 11	70 (67.5, 72.6)	-6.2 (-10.1, -2.4)	0.002*
Random glucose mmol/L	5.2 ± 0.8	5.0 ± 0.9	5.1 (4.7, 5.4)	5.5 ± 1.1	5.4 ± 0.8	5.4 (5.1, 5.7)	-0.3 (-0.8, 0.2)	0.21

*p value < 0.05

Values reported as arithmetic mean unless specified

± Standard deviation

The adjusted mean of the secondary outcomes at 12 weeks was compared between groups using ANCOVA including terms for baseline value, treatment arm, inhaled corticosteroid use, age, gender, and height and expressed as the difference in the adjusted means with a 95% CI and p-value.

ALT- alanine aminotransferase. GGT- gamma glutamyl transferase. ANCOVA- analysis of covariance

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capsules were taken by participants and we observed a decrease in hemoglobin, a recognized systemic side effect of pioglitazone [29–31]. However, despite evidence of systemic biological activity, we were unable to confirm that pioglitazone successfully activated PPAR-γ in the lungs to induce nuclear translocation of PPAR-γ within the induced sputum cells. As pioglitazone is highly bound to plasma proteins (>99%) and has a low volume of distribution across tissues, it may not have entered the airways in sufficient concentration to exert an effect.

An alternative explanation for the lack of efficacy is that the study participants had adequate asthma control with low levels of eosinophilic airway inflammation and preserved lung function at baseline, leaving little potential for improvement. Recruiting patients with evidence of eosinophilic inflammation would have been better but would have rendered recruitment into the study even more difficult. Therefore, we have not excluded a possible anti-inflammatory effect in patients with more severe asthma and more marked airway inflammation, and a study with longer duration in this cohort would be required to assess the effect of pioglitazone on the risk of asthma exacerbations.

To our knowledge, this is the largest and longest study of a PPAR- γ agonist in asthma performed to date [16–19]. A previously reported RCT compared rosiglitazone to inhaled corticosteroids in 46 current smokers with asthma (a group associated with poor response to inhaled corticosteroids), and reported a trend toward a higher pre-bronchodilator FEV₁ in the group treated with rosiglitazone at 28 days (183 mL, 95% CI -1.0 to 367, $p = 0.051$) (18). There was also an improvement in the forced expiratory flow between 25 and 75% of the forced vital capacity after 28 days of treatment with rosiglitazone (FEF_{25–75} 0.24 L/second, 95% CI 0.03 to 0.46, $p = 0.03$), but no statistically significant improvements in ACQ scores or sputum cell counts [18]. A second RCT compared 28 days of treatment with rosiglitazone with placebo in 32 adults with mild asthma and reported a 15% attenuation of the late asthmatic reaction, with no change in the early asthmatic reaction compared to placebo. The authors also reported no effect on airway hyper-responsiveness after allergen challenge or on a panel of inflammatory markers [17]. Dixon and colleagues reported a 12 week RCT comparing pioglitazone ($n = 23$) to placebo ($n = 19$) in obese subjects with poorly controlled moderate to severe asthma [19]. Using 30mg pioglitazone for 2 weeks increased to 45mg for 10 weeks there were no significant differences in the airway hyper-responsiveness to methacholine, FeNO or lung function between treatment and placebo. Recruitment was halted early (as in our study) due to the impact of emerging safety concerns surrounding the potential risk of bladder cancer affecting recruitment, lack of efficacy signal and significant weight gain in pioglitazone treated patients [19]. The thiazolidinediones are associated with significant adverse effects when administered systemically and these studies support our conclusion that systemic treatment with pioglitazone is not associated with beneficial effects on asthma control.

Conclusion

We found no evidence to support the hypothesis that 12 weeks of treatment with the PPAR- γ agonist pioglitazone improves asthma control or airway inflammation in individuals with mild asthma. Opportunities to further investigate the potential anti-inflammatory effects of pioglitazone in more severe asthma are currently limited by safety considerations.

Supporting Information

S1 Data. Full trial dataset.
(XLSX)

S1 Text. Protocol.
(DOC)

S2 Text. CONSORT checklist.
(DOC)

Acknowledgments

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Dr. Tim Harrison was registered as the principal investigator for this study.

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Project administration: JA KS HB DH.

Resources: AK TH.

Supervision: TH AK KM.

Validation: JA KS HB.

Writing – original draft: JA AK TH LP KM.

Writing – review & editing: JA AK TH LP KM DH DS.

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PPAR- γ : a novel therapeutic target for asthma?

Final Version 5.0
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Short title: PPAR- γ : a novel therapeutic target for asthma?

Acronym: N/A

EudraCT number: 2008-004112-13

Trial Registration: www.clinicaltrials.gov reference

ISRCTN: *as appropriate*

CTA reference: *as appropriate*

MREC reference: 08/H0408/120

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SYNOPSIS

Title	PPAR- γ : a novel therapeutic target for asthma?
Acronym	N/A
Short title	PPAR- γ : a novel therapeutic target for asthma?
Chief Investigator	Tim Harrison
Objectives	To test the hypothesis that stimulation of PPAR- γ receptors has a therapeutic role in the treatment of asthma
Trial Configuration	Randomized, double blind, placebo controlled two parallel group clinical trial
Setting	Primary care & Secondary care
Sample size estimate	Assuming the SD of differences in Forced Expiratory Volume in One Second (FEV ₁) over 12 weeks is 250 a study with 88 participants will allow us to detect a difference between treatment arms in the mean 12 week FEV ₁ of 150 ml with 80 % power at a 5% two-sided significance level. In practice we aim to recruit 100 subjects to allow for drop-outs.
Number of participants	100
Eligibility criteria	The inclusion criteria will be age 18-75 of either sex with a clinical diagnosis of asthma, FEV ₁ $\geq$ 60% predicted and an increase in FEV ₁ of greater than 12% following inhaled salbutamol 400 μ g or Peak Expiratory Flow (PEF) variability >12% during run-in. Allowed medication: 0-800 μ g inhaled beclomethasone dipropionate or equivalent and as required short acting beta agonist. The exclusion criteria will be inability to produce a sputum sample on induction current smoking, >10 pack years smoking history, treatment with leukotriene antagonists, long acting beta-agonists or theophylline, liver or cardiovascular disease, oral steroid treatment or exacerbation within 6 weeks, females who are pregnant, lactating or not using adequate contraception, any contra-indication to pioglitazone (hypersensitivity to pioglitazone, cardiac failure, history of cardiac failure, hepatic impairment, diabetic ketoacidosis), oral or insulin treatment for diabetes, treatment with gemfibrozil or rifampicin or lactose intolerance.
Description of interventions	Pioglitazone 30mg daily by mouth for 4 weeks then 45mg daily for 8 weeks and placebo 30mg daily by mouth for 4 weeks then 45mg daily for 8 weeks.
Duration of study	Planned start date 1 st January 2010 Anticipated duration 24 months Duration of involvement per participant: 18 weeks

Randomisation and blinding	Generation of randomisation sequence by a computer generated random code using random permuted blocks of randomly varying size. Sequence to be held in pharmacy in trial folder, concealed from investigators and participants. Both investigators and participants will be blinded.
Outcome measures	Primary endpoint: FEV₁ after 12 weeks treatment Secondary endpoints: Change over 12 weeks in daily asthma symptoms, mean morning and evening PEF, Juniper asthma control questionnaire and asthma quality of life questionnaire scores, exhaled nitric oxide level, bronchial hyper-responsiveness, induced sputum cell counts and analysis detailed above, adverse effects
Statistical methods	The analysis of the Primary response variable (FEV₁ at 12 weeks) will be performed using a General Linear Model incorporating terms for baseline value, treatment arm, stratum, and baseline characteristics which are thought <i>a priori</i> to strongly predict outcome. Secondary response variables will be treated similarly, after transformation to approximate Normality as required (as, for example with PD20, Percentage eosinophil count and exhaled NO concentrations). Mean morning and evening PEF and the percentage of days and nights free of symptoms and relief inhaler use will be calculated for the 14 days prior to each visit and analysed as above. All analyses will be performed using Stata v10.

ABBREVIATIONS

ADR	Adverse Drug Reaction
AE	Adverse Event
CI	Chief Investigator overall
CRF	Case Report Form
DAP	Data Analysis Plan
DMC	Data Monitoring Committee
EMA	European Agency for the Evaluation of Medicinal Products
EOT	End of Trial
GCP	Good Clinical Practice
ICF	Informed Consent Form
MHRA	Medicines and Healthcare products Regulatory Agency
NHS	National Health Service
P/GIS	Parent / Guardian Information Sheet
PI	Principal Investigator at a local centre
PIS	Participant Information Sheet
REC	Research Ethics Committee
R&D	Research and Development department
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMG	Trial Management Group
TSC	Trial Steering Committee

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TRIAL / STUDY BACKGROUND INFORMATION AND RATIONALE

Chronic asthma causes significant morbidity and mortality in Western societies. In the UK, asthma costs >£2 billion/annum in healthcare expenditure, with the 10% of patients with severe asthma accounting for 50% of this resource¹. Current approaches to treat asthma are clearly poorly effective for a sizeable proportion of patients which places a substantial social and economic burden. Additional new therapeutic approaches are therefore needed if this burden is to be reduced.

The main anti-inflammatory therapies used in asthma management are the glucocorticoids. These have widespread effects which reduce the expression of pro-inflammatory molecules by a variety of structural and inflammatory cells in the airway. Glucocorticoids act via glucocorticoid nuclear receptors (GR) which act at several sites to reduce inflammation and remodeling^{2,3}. Glucocorticoids switch on or transactivate some genes such as the β_2 -adrenoceptor whilst switching off or transrepressing others (such as chemokine genes) by complex interactions involving transcription factors and their coactivators. Post-transcriptional modification of protein production is also important⁴. The fact that activated GR can translocate to the nucleus and modify the transcription of a wide range of inflammatory genes is one of the major reasons why glucocorticoids are so effective in asthma. Glucocorticoid receptors are not the only nuclear receptors found in mammalian cells however and other families of nuclear receptor are potential targets for new therapeutic approaches in inflammatory diseases such as asthma.

Peroxisome proliferator-activated receptors (PPARs) belong to a subfamily of the nuclear receptor superfamily and are important transcription factors that modulate inflammatory responses⁵. PPARs comprise three isoforms α , δ and γ and are activated by a heterogeneous group of structurally dissimilar endogenous and synthetic agonists. The prostaglandin D₂ metabolite 15-deoxy- $\Delta^{12,14}$ PGJ₂ (15d-PGJ₂) is a direct-binding natural ligand for PPAR- γ . With the finding that PPAR- γ is important in cell metabolism a number of pharmaceutical companies have developed PPAR- γ agonists such as rosiglitazone and pioglitazone for the treatment of diabetes mellitus which act to reduce peripheral insulin resistance⁶.

PPAR- γ activation has been known to down regulate the synthesis and release of immune modulating cytokines from various cell types⁶. There is mounting evidence from studies in human cell systems and mouse asthma models to suggest that that activation of PPAR- γ has potent anti-inflammatory and immunomodulatory effects which may make this class of drugs

candidates for the treatment of asthma⁷⁻¹⁶. Inflammatory cells such as eosinophils, mast cells, T lymphocytes and neutrophils are recruited into the airways in asthma by a network of chemokines. Stimulation of the PPAR- γ ligand was found to significantly inhibit production of the Th2 type cytokines and downregulate eosinophil functions^{17,18}. Additionally treatment with synthetic PPAR- γ ligand can reduce lung inflammation and INF- γ , IL-4 and IL-2 production in experimental allergic asthma¹⁹. In subjects with asthma, PPAR- γ expression is known to be associated with airway inflammatory and remodelling responses²⁰.

We have been studying the effect of PPAR- γ agonists on release of chemokines from cultured human airway smooth muscle cells, which we and others have shown are an important source of chemokines and inflammatory mediators in asthma²¹⁻²⁹. We have shown that the endogenous PPAR- γ agonist 15d-PGJ2 and the synthetic PPAR- γ agonist troglitazone, but not the synthetic PPAR- α agonist WY-14643, inhibit TNF α -induced production of eotaxin and MCP-1, chemokines that selectively recruit eosinophils monocytes and T lymphocytes, but have no effect on the production of IL-8, the neutrophil chemoattractant. The findings suggest that both endogenous and synthetic PPAR- γ agonists act similarly to selectively modulate chemokines which are involved in allergic inflammation (Figure 1)⁹. Experiments transfecting cells with a wild type eotaxin promoter construct linked to firefly luciferase showed that this effect was on eotaxin transcription and was PPAR- γ mediated as overexpression of PPAR- γ enhanced the inhibitory effect (Figure 2). Furthermore we have also shown that PPAR- γ agonists increase release of PGE2, one of the most important endogenous bronchoprotective factors, through the induction of the enzyme cyclooxygenase-2²⁶. Collectively these studies demonstrate a potentially useful anti-inflammatory profile of PPAR- γ agonists in a key airway structural cell.

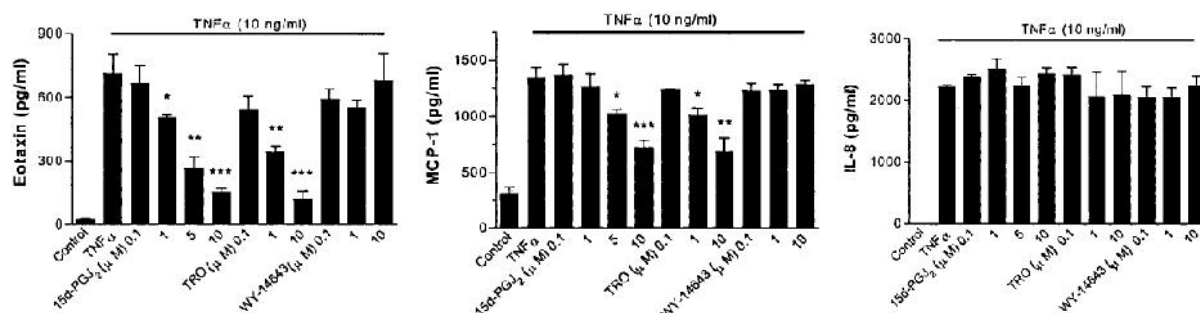


Figure 1. The endogenous PPAR- γ agonist 15d-PGJ₂ and the synthetic PPAR- γ agonist troglitazone (TRO), but not the synthetic PPAR- α agonist WY-14643, inhibit TNF α -induced production of eotaxin (left panel) and MCP-1 (middle panel), but not IL-8 (right panel), in human airway smooth muscle cells.

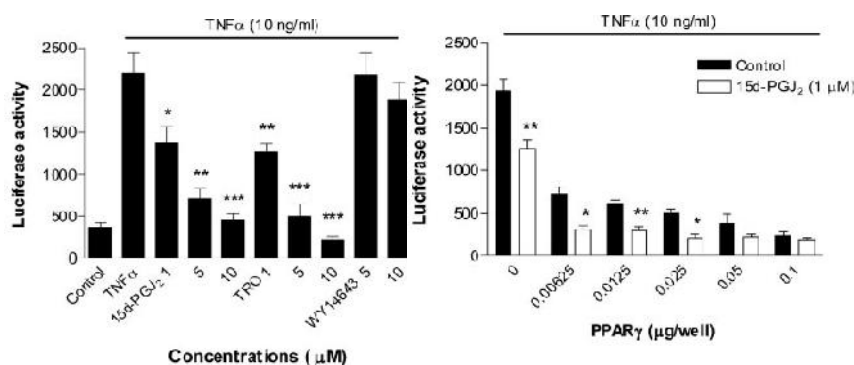


Figure 2. The endogenous PPAR- γ agonist 15d-PGJ₂ and the synthetic PPAR- γ agonist troglitazone (TRO), but not the synthetic PPAR- α agonist WY-14643, inhibit TNF α -induced eotaxin promoter activity (left panel) and PPAR- γ overexpression enhances the inhibitory effect of 15d-PGJ₂ (right panel) in human airway smooth muscle cells.

The anti-inflammatory effects of PPAR- γ agonists are not limited to airway structural cells, however. PPAR- γ agonists inhibit inflammatory cytokine release from monocytes, inhibit dendritic cell activation and induce apoptosis of T-lymphocytes³⁰⁻³². Studies from mouse models of airway inflammation and asthma also support the hypothesis that activation of PPAR- γ has potent anti-inflammatory effects. PPAR- γ agonists reduced airway hyperresponsiveness, eosinophilic inflammation, and collagen deposition after allergen challenge in mice^{10,12-15}.

A further feature of our studies is that as well as having anti-inflammatory effects on their own, PPAR- γ agonists potentiated the inhibitory effects of glucocorticoids⁹ suggesting that there may be benefits from using the drugs in combination with steroids. Figure 3 (next page) shows that the endogenous PPAR- γ agonist, 15d-PGJ₂ had additive effects when given with the glucocorticoid, fluticasone in inhibiting TNF α -induced eotaxin release from human airways

smooth muscle cells. We do not intend to study the interaction with glucocorticoids in the present study but this would provide a focus for follow on studies.

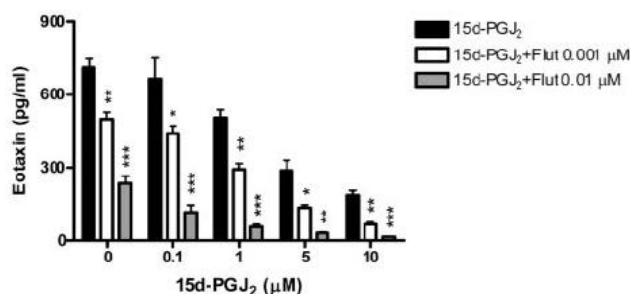


Figure 3. The endogenous PPAR-γ agonist, 15d-PGJ₂ and the glucocorticoid fluticasone additively inhibit TNFα-induced eotaxin production in human airways smooth muscle cells.

Collectively these studies provide compelling evidence to suggest that PPAR-γ agonists may have beneficial effects in airways diseases such as asthma when given on their own or in combination with glucocorticoids. We therefore propose to explore the potential therapeutic effects of stimulating the PPAR-γ receptor by using the PPAR-γ agonist pioglitazone (licensed for use in diabetes) to treat people with asthma and to explore in detail the effects of PPAR-γ receptor stimulation on FEV₁, sputum markers of airway inflammation and document any adverse effects.

DETAILS OF INVESTIGATIONAL MEDICINAL PRODUCT(S)

Description

30mg and 45mg over-encapsulated pioglitazone tablets. Pioglitazone tablets will be sourced from Takeda UK Ltd; please see separate Summary of Product Characteristics document (SmPC) that accompanies the protocol. Catalent Pharma Solutions will over-encapsulate the Pioglitazone tablets and perform dissolution testing and EU QP release.

Packaging and labelling

The drug will be packaged and labelled for individual study participants by an independent pharmacist. Standard pharmacy supplies will be used.

Storage, dispensing and return

IMP will be stored in a securely locked cupboard in the pharmacy at City Hospital, Nottingham only accessible to authorised pharmacy personnel. Supplies will be kept separately to other pharmacy supplies and labelled accordingly. When dispensed to participants, IMPs will be stored in their study packaging. Surplus or unused stock will be returned to the pharmacy for disposal.

Placebo

The placebo will consist of a lactose filled capsule (lactose monohydrate is one of the excipients in Actos) and will be labelled, distributed and stored in the same way as for the IMP. The placebo capsule will be manufactured by Catalent Pharma Solutions.

Known Side Effects

Please see separate SmPC that accompanies this protocol.

TRIAL / STUDY OBJECTIVES AND PURPOSE

PURPOSE

The key objective is to determine whether stimulation of PPAR γ receptors (using Pioglitazone) is effective in patients with mild-to-moderate asthma. The study design will allow us to estimate the size of the effect, record potential adverse effects and explore the presumed mechanism of action in-vivo by measuring sputum markers of airway inflammation. Definite evidence of clinical efficacy and biological activity in patients with asthma would justify exploring PPAR- γ stimulation as a novel therapeutic modality.

PRIMARY OBJECTIVE

To determine the effect of 12 weeks' treatment with pioglitazone on FEV₁ (measured using a dry bellows spirometer, *Vitalograph Buckingham UK* as the larger of two values within 100mls)

SECONDARY OBJECTIVES

To determine the effect of 12 weeks' treatment with pioglitazone on change over 12 weeks in daily asthma symptoms, mean morning and evening PEF, Juniper asthma control questionnaire³³ and asthma quality of life questionnaire scores³⁴, exhaled nitric oxide level (NIOXX Flex, Aerocrine), bronchial hyper-responsiveness (PD₂₀) to methacholine (Provojet, Ganshorn Medzin Elecronic GMBH), induced sputum cell counts and analysis, body mass index, random glucose, eosinophil count and adverse effects.

Detailed sputum analysis will be carried out as follows:

Our previous studies have suggested that the mechanism of action of PPAR- γ agonists in vitro involves effects on gene transcription. Gene transcription is a complex process involving binding of transcription factors to their recognition sequences on gene promoters. Access to these recognition sequences requires DNA to unravel and this process is controlled by covalent modification of core histone molecules around which DNA is wrapped. The most important covalent modification is acetylation of histone H4 which is regulated by two competing groups of enzymes, histone acetyl transferases (HATs) and histone deacetylases (HDACs). HATs increase transcription whereas HDACs reduce it. In our studies of eotaxin we found that acetylation of histone H4 was reduced by PPAR γ agonists but we have not determined whether this is due to a reduced HAT or increased HDAC activity. To examine things further we will perform studies in cells obtained from induced sputum in subgroups of

the participants to analyse 1) HDAC and HAT activities by HDAC Fluorescent Activity Assay Kit (BIOMOL) and HAT Assay Kit (Upstate Biotechnology). 2) PPAR- γ activation (nuclear translocation) by western blotting as described previously⁹. This will be conducted on a subset of samples from each group to confirm the effect of pioglitazone. We will also perform studies in supernatant obtained from induced sputum to analyse 3) the concentration of chemokines (eotaxin, MCP-1, IP-10) and growth factors (VEGF) by Luminex, (Luminex corporation, Austin TX); 4) effector mediators (cyst-leukotriens, histamine by ELISA as described previously⁹ and eosinophilic cationic protein (Immunocap, Phadia UK). These analyses will be conducted with samples obtained from participants at baseline and week 12 (the end of each treatment). The results will be normalised against total cell numbers to allow comparison.

TRIAL / STUDY DESIGN

TRIAL / STUDY CONFIGURATION

Single centre randomised two parallel group, double blind placebo controlled trial. Please see separate schematic diagram of study design.

Primary endpoint

FEV₁ after 12 weeks treatment

Secondary endpoint

Change over 12 weeks in daily asthma symptoms, mean morning and evening PEF, Juniper asthma control questionnaire and asthma quality of life questionnaire scores, exhaled nitric oxide level, bronchial hyper-responsiveness, induced sputum cell counts and analysis detailed above, adverse effects.

Safety endpoints

Adverse effects (spontaneously reported, reported at clinic visits, reported on diary cards)

Stopping rules and discontinuation

Since this is a pilot study we do not anticipate obtaining data before completion of the study that would lead to its discontinuation. For individual participants, discontinuation will be decided on an individual basis.

RANDOMIZATION AND BLINDING

The treatment to which a patient is assigned will be determined by a computer generated pseudo-random code using random permuted blocks of randomly varying size, created by the Nottingham Clinical Trials Unit (CTU) in accordance with their standard operating procedure (SOP) and held on a secure server, employing the Stata add-in ralloc.

Participants will be allocated with equal probability to the two treatment arms, stratified according to whether they use a regular inhaled corticosteroid or short acting β_2 agonist alone.

Access to the sequence will be confined to the CTU Data Manager and will be concealed until interventions have been assigned and recruitment, data collection, and all other trial-related assessments are complete.

On behalf of investigators an independent senior research pharmacist will access the treatment code for each participant by means of a password-protected remote, internet-based system developed and maintained by the Nottingham CTU.

Participants, those administering the interventions and those assessing the outcomes will be blinded to group assignment.

Participants will be enrolled by Dr Tim Harrison, Dr Kevin Mortimer, Dr Dominick Shaw, Professor Alan Knox or Dr John Anderson from asthma clinics in Nottingham. We will work with the Primary care research network (PCRN East Midlands and South Yorkshire) to coordinate recruitment from GP practices within Nottinghamshire, Bassetlaw, Derbyshire and Lincolnshire. Treatment will be provided by pharmacy according to the randomisation code.

Treatment allocation will be concealed until interventions are all assigned and recruitment, data collection and laboratory analyses are complete.

Maintenance of randomisation codes and procedures for breaking code

Study medications (active and placebo) will be produced to MHRA standards by Catalent Pharma Solutions.

Investigators may identify the treatment for each participant in the event of a medical emergency when treatment is dependent on knowledge of the actual drug received. This will be by means of opening a sealed envelope containing the details of drug allocation to each participant.

If the treatment code for a participant is broken, an urgent meeting of the study personnel (Independent trials pharmacist, Dr T Harrison and Dr K Mortimer, Dr John Anderson or Professor Alan Knox). The date and reason(s) for breaking the code will be recorded. The participant will continue follow up unless consent is withdrawn.

When the un-blinding is part of managing an SAE, unblinding will be reported with the SAE, however, in cases where un-blinding was not associated with an SAE, such actions will be reported in a timely manner. The default will be to use the same timeline requirements for investigator reporting of SAEs (notification of Sponsor immediately as practicable by phone or fax, followed by a written narrative of the event within 48 hours).

At the end of the study, randomisation and code break envelopes will be returned to pharmacy for disposal.

Treatment allocations will be identified by revealing the randomisation code at the end of the study.

TRIAL MANAGEMENT

This small pilot study will be managed by the investigators who will meet on a monthly basis to assess progress. The Trial management group (TMG) will include Dr T Harrison, Dr J Anderson, C Minter, A Samuels, Dr K Mortimer, Dr D Hodgson, Kiara Parker and Denise Barber.

DURATION OF THE TRIAL / STUDY AND PARTICIPANT INVOLVEMENT

Planned start date 1st January 2010

Anticipated duration 24 months

Duration of involvement per participant: 18 weeks (2 weeks run-in, 12 weeks intervention, observation visit at 16 weeks) Enrolment will begin in January 2010 and is anticipated to close in January 2011.

End of the Trial

The end of the trial will be the last visit for treatment of the last recruited participant.

SELECTION AND WITHDRAWAL OF PARTICIPANTS

Recruitment

Participants will be recruited from a single centre, Nottingham University Hospitals Trust through respiratory out-patient clinics in Nottingham and from our register of volunteers with asthma (who have agreed to be contacted about research projects like this one) as well as from a number of primary care practices within the East Midlands.

The initial approach in secondary care will be from a member of the patient's usual care team (which may include the investigator), and information about the trial will be on display in the relevant clinical areas. The investigator or their nominee, e.g. from the research team or a member of the participant's usual care team, will inform the participant or their nominated representative (other individual or other body with appropriate jurisdiction), of all aspects pertaining to participation in the study. If needed, the usual hospital interpreter and translator services will be available to assist with discussion of the trial, the participant information sheets, and consent forms but the consent forms and information sheets will not be available printed in other languages. It will be explained to the potential participant that that entry into the trial is entirely voluntary and that their treatment and care will not be affected by their decision. It will also be explained that they can withdraw at any time but attempts will be made to avoid this occurrence. In the event of their withdrawal it will be explained that their data collected so far cannot be erased and we will seek consent to use the data in the final analyses where appropriate.

As we plan to recruit patients with mild-to-moderate asthma many of whom will be followed-up in primary care we also plan to recruit from a number of GP practices within the East Midlands & South Yorkshire Primary Care Research Network area (PCRN). The Primary Care Trusts we have identified include; Nottingham City PCT, Nottinghamshire County PCT, Bassetlaw PCT, Derbyshire County PCT and Lincolnshire Teaching PCT. We will work with the PCRN to identify a number of suitable GP surgeries, who will search their databases to identify potential participants with mild-to-moderate asthma. Potential participants will be sent an invitation letter by post from their GP on practice headed note paper informing them of the study aims and level of participation required. It will be made clear in the invitation letter that involvement in the trial is entirely voluntary and their usual medical care will not be affected

should they decide not to partake. The letter will provide a contact telephone number for interested patients' or their nominated representative (other individual or other body with appropriate jurisdiction) to contact a member of the study team for more information. We will then be able to provide patient information leaflet and arrange a discussion of all aspects pertaining to participation in the study prior to screening visit in the same way as patients recruited from secondary care clinics.

We will also invite potential participants to find out information about the trial with an A4 sized Poster. This poster will be displayed on official notice boards within Nottingham University Hospitals Trust and in a reduced size format as a Newspaper advertisement in a variety of local Newspapers.

Inclusion criteria

Aged 18-75 of either sex with a clinical diagnosis of asthma, $FEV_1 \geq 60\%$ predicted and an increase in FEV_1 of greater than 12% following inhaled salbutamol 400µg or PEF variability >12% during run-in. Allowed medication: 0-800µg inhaled beclomethasone dipropionate or equivalent and as required short acting beta agonist.

Exclusion criteria

Inability to produce sputum on induction, current smoking, >10 pack years smoking history, treatment with long acting beta-agonists, leukotriene antagonists or theophylline, liver or cardiovascular disease, oral steroid treatment or exacerbation within 6 weeks, females who are pregnant, lactating or not using adequate contraception, any contra-indication to pioglitazone (hypersensitivity to pioglitazone, cardiac failure, history of cardiac failure, hepatic impairment, diabetic ketoacidosis), oral or insulin treatment for diabetes, treatment with gemfibrozil or rifampicin or lactose intolerance.

Expected duration of participant participation

Study participants will be participating in the study for 18 weeks.

Removal of participants from therapy or assessments

Subjects may be withdrawn from the trial either at their own request or at the discretion of the Investigator. The subjects will be made aware that this will not affect their future care.

Subjects will be made aware (via the information sheet and consent form) that should they

withdraw the data collected to date cannot be erased and may still be used in the final analysis.

Data will be collected on reasons for and timing of discontinuation/withdrawal.

Enrolled participants who are not yet randomised will be replaced but participants who withdraw after randomisation will not be replaced.

Abrupt termination of study treatment is not anticipated to affect participant safety.

Informed consent

All subjects will provide written informed consent. The Informed Consent Form will be signed and dated by the subject before they enter the trial. The Investigator will explain the details of the trial and provide a Participant Information Sheet, ensuring that the subject has sufficient time to consider participating or not. The Investigator will answer any questions that the subject has concerning study participation.

Informed consent will be collected from each subject before they undergo any interventions (including physical examination and history taking) related to the study. One copy of this will be kept by the subject, one will be kept by the Investigator, and a third will be retained in the patient's hospital records.

Should there be any subsequent amendment to the final protocol, which might affect a subject's participation in the trial, continuing consent will be obtained using an amended Consent form which will be signed by the subject.

TRIAL / STUDY TREATMENT AND REGIMEN

Pre-study consent visit

Detailed verbal and written information will be provided in clinic or in the clinical sciences building and interested subjects will be asked to sign the consent form and return for a screening visit. Subjects will be asked to withhold inhaled β -agonists for 6 hours before each visit and caffeine-containing food and drink from the previous midnight.

Screening visit

At the screening visit, the inclusion and exclusion criteria will be checked as part of the medical history followed by clinical examination and measurement of body mass index. Venesection will be performed for full blood count, liver function test, random glucose and if the patient agrees a single additional EDTA sample will be taken (approximately 10mls) at the same time to prepare plasma to store in the laboratory for future testing in respiratory

research studies. We will seek prior patient consent at the pre-study clinic visit as an 'opt-in' item on the patient consent form, for permission to save a sample of their plasma in the laboratory to permit future testing of novel biomarkers. This will be fully explained in the patient information leaflet and in person and collection would occur at the same time as venepuncture for safety monitoring. The blood sample will be taken by an experienced doctor or research nurse and cells from the blood will be discarded and no genetic testing will be performed on the stored plasma. Subsequently spirometry (Vitalograph; Buckingham, UK) with measurement of the FEV₁ reversibility twenty minutes following administration of 400µcg of inhaled salbutamol via a spacer device (Volumatic™, Allen and Hanburys UK) followed by sputum induction will be performed.

Run-in period

Subjects will enter a two week run in period to complete a diary of their morning and evening peak flow, asthma medication use, nocturnal awakenings and other asthma symptoms.

Randomisation

Subjects fulfilling all inclusion criteria with no exclusion criteria will enter the study and allocation to treatment order will be by a computer generated randomization list prepared by the Clinical Trials Unit, Nottingham University, with treatment allocated by an independent pharmacist.

Treatment period

Subjects will start treatment with 30mg pioglitazone or placebo daily, increasing to 45mg daily after 4 weeks for the remaining 8 weeks of treatment. Participants will attend for data collection at the same time of day +/-1.5 hours at 0, 4, 8, 12 and 16 weeks within 3 days of the protocol date.

Data collection

Baseline

The inclusion criteria will be checked again, 30 mg of trial medication or placebo dispensed as per randomisation code. Diary cards will be checked (symptoms and PEF), use of medication confirmed and adverse effects recorded. The Juniper asthma control and asthma quality of life questionnaires completed in the validated paper format followed by measurement of body mass index and clinical examination. Subsequently exhaled nitric oxide, spirometry, methacholine challenge for PD₂₀ and induced sputum will be measured.

Week 1

Patients will be contacted by the research team by telephone one week following randomisation to ensure there are no early problems with trial medication or diary card monitoring.

Weeks 4 and 8

Diary cards (symptoms and PEF) will be checked, use of medication confirmed, adverse effects recorded then 45mg of trial medication or placebo will be dispensed according to randomisation code at week 4 and week 8. Subsequently measurement of body mass index, clinical examination and spirometry performed. Venesection for liver function tests and random glucose will be performed at week 4.

Week 12

At week 12 the treatment phase of the study has ended. Diary cards (symptoms and PEF) will be checked, use of medication confirmed, adverse effects recorded, Juniper asthma control questionnaire and asthma quality of life questionnaire completed. Subsequently clinical examination, body mass index, measurement of exhaled nitric oxide, spirometry, methacholine challenge, induced sputum and venesection for full blood count, liver function test and glucose in that order.

Week 16 (Observation)

Diary cards (symptoms and PEF) will be checked, adverse effects recorded. Subsequently the Juniper asthma control questionnaire, asthma quality of life questionnaire completed and spirometry performed.

Compliance

Compliance will be assessed by review of diary cards and counting of returned medication.

Accountability for drugs & placebos

Dispensing will occur from pharmacy and dates and quantities recorded in each participant's CRF. Dates and quantities of returned medication will also be recorded and confirmation of destruction or return of partially used/unused supplies recorded in pharmacy and CRF.

Management of study drug overdose

Specific antidotes are not available. General appropriate supportive measures should be used with discussion with the local poisons advice centre if appropriate.

Criteria for terminating trial

Since this is a pilot study we do not anticipate obtaining data before completion of the study that would lead to its discontinuation. For individual participants, discontinuation will be decided on an individual basis.

STATISTICS

Methods

The findings will be evaluated by Dr Harrison, Dr Mortimer, Dr Anderson, Dr Pang, Professor Knox and Dr Silcocks.

The analysis of the Primary response variable (FEV₁ at 12 weeks) will be performed using a General Linear Model incorporating terms for baseline value, treatment arm, stratum, and baseline characteristics which are thought *a priori* to strongly predict outcome.

Secondary response variables will be treated similarly, after transformation to approximate Normality as required (as, for example with PD20, Percentage eosinophil count and exhaled NO concentrations). Mean morning and evening PEF and the percentage of days and nights free of symptoms and relief inhaler use will be calculated for the 14 days prior to each visit and analysed as above. All analyses will be performed using Stata v10.

No interim or sub-group analyses are planned.

Full details will be given in a separate Statistical Analysis Plan to be approved before data lock.

Sample size and justification

Assuming the SD of differences in FEV₁ over 8 weeks is 250³⁵ a study with 88 participants will allow us to detect a difference between treatment arms in the mean 12 week FEV₁ of 150 ml with 80 % power at a 5% two-sided significance level. (Stata v10). In practice we aim to recruit 100 subjects to allow for drop-outs.

Assessment of efficacy

Primary and secondary efficacy endpoints detailed above.

Assessment of safety

Safety endpoints detailed above.

Procedures for missing, unused and spurious data

Missing, unused and spurious data will be reported as such in the ensuing publication.

Definition of populations analysed

Safety set: All randomised participants who receive at least one dose of the study drug.

Full Analysis set: All randomised participants, who take at least one dose of study medication and for whom at least one post-baseline assessment of the primary endpoint is available.

Per protocol set: All participants in the Full Analysis set who are deemed to have no major protocol violations that could interfere with the objectives of the study.

Efficacy will be assessed on both the full analysis set and the per protocol set. Safety summaries will be performed on the safety set.

ADVERSE EVENTS

Definitions

An adverse event is any unfavourable and unintended sign, symptom, syndrome or illness that develops or worsens during the period of observation in the study.

An AE does include a / an:

1. exacerbation of a pre-existing illness.
2. increase in frequency or intensity of a pre-existing episodic event or condition.
3. condition detected or diagnosed after medicinal product administration even though it may have been present prior to the start of the study.
4. continuous persistent disease or symptoms present at baseline that worsen following the start of the study.

An AE does not include a / an:

1. medical or surgical procedure (e.g., surgery, endoscopy, tooth extraction, transfusion); but the condition that lead to the procedure is an AE.
2. pre-existing disease or conditions present or detected at the start of the study that did not worsen.
3. situations where an untoward medical occurrence has not occurred (e.g., hospitalisations for cosmetic elective surgery, social and / or convenience admissions).
4. disease or disorder being studied or sign or symptom associated with the disease or disorder unless more severe than expected for the participant's condition.
5. overdose of concurrent medication without any signs or symptoms.

A Serious Adverse Event (SAE) is any adverse event occurring following study mandated procedures, having received the IMP or placebo that results in any of the following outcomes:

1. Death
2. A life-threatening adverse event
3. Inpatient hospitalisation or prolongation of existing hospitalisation
4. A disability / incapacity
5. A congenital anomaly in the offspring of a participant

Important medical events that may not result in death, be life-threatening, or require hospitalisation may be considered a serious adverse event when, based upon appropriate

medical judgment, they may jeopardize the patient or participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

All adverse events will be assessed for seriousness, expectedness and causality:

A distinction is drawn between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined using the criteria above. Hence, a severe AE need not necessarily be serious.

Causality

Not related or improbable: a clinical event including laboratory test abnormality with temporal relationship to trial treatment administration which makes a causal relationship incompatible or for which other drugs, chemicals or disease provide a plausible explanation. This will be counted as “unrelated” for notification purposes.

Possible: a clinical event, including laboratory test abnormality, with temporal relationship to trial treatment administration which makes a causal relationship a reasonable possibility, but which could also be explained by other drugs, chemicals or concurrent disease. This will be counted as “related” for notification purposes.

Probable: a clinical event, including laboratory test abnormality, with temporal relationship to trial treatment administration which makes a causal relationship a reasonable possibility, and is unlikely to be due to other drugs, chemicals or concurrent disease. This will be counted as “related” for notification purposes.

Definite: a clinical event, including laboratory test abnormality, with temporal relationship to trial treatment administration which makes a causal relationship a reasonable possibility, and which can definitely not be attributed to other causes. This will be counted as “related” for notification purposes.

An AE whose causal relationship to the study IMP is assessed by the Chief Investigator as “possible”, “probable”, or “definite” is an Adverse Drug Reaction.

With regard to the criteria above, medical and scientific judgment shall be used in deciding whether prompt reporting is appropriate in that situation.

Reporting of adverse events

Participants will be asked to contact the study site immediately in the event of any serious adverse event. All adverse events will be recorded and closely monitored until resolution, stabilisation, or until it has been shown that the study medication or treatment is not the cause. The Chief Investigator shall be informed immediately of any serious adverse events and shall determine seriousness and causality in conjunction with any treating medical practitioners.

In the event of a pregnancy occurring in a trial participant or the partner of a trial participant monitoring shall occur during the pregnancy and after delivery to ascertain any trial related adverse events in the mother or the offspring. Where it is the partner of a trial participant consent will be obtained for this observation from both the partner and her medical practitioner.

All serious adverse events will be recorded and reported to the MHRA and REC as part of the annual reports. SUSARs will be reported within the statutory timeframes to the MHRA and REC as stated below. The Chief Investigator shall be responsible for all adverse event reporting.

SUSARs

A serious adverse event that is either sudden in its onset, unexpected in its severity and seriousness or not a known side effect of the IMP *and* related or suspected to be related to the IMP is classed as Suspected Unexpected Serious Adverse Reaction and requires expedited reporting as per the clinical trials regulations.

All serious adverse events that fall or are suspected to fall within these criteria shall be treated as a SUSAR until deemed otherwise.

The event shall be reported immediately of knowledge of its occurrence to the Chief Investigator.

The Chief Investigator will:

- Assess the event for seriousness, expectedness and relatedness to the study IMP
- Take appropriate medical action, which may include halting the trial and inform the Sponsor of such action
- If the event is deemed a SUSAR, shall, within seven days, complete the CIOMS form and send to the MHRA.
- Shall inform the REC using the reporting form found on the NRES web page within 7 days of knowledge of the event

- Shall, within a further eight days send any follow-up information and reports to the MHRA and REC.
- Make any amendments as required to the study protocol and inform the ethics and regulatory authorities as required

Participant removal from the study due to adverse events

Any participant who experiences an adverse event may be withdrawn from the study at the discretion of the Investigator.

ETHICAL AND REGULATORY ASPECTS

ETHICS COMMITTEE AND REGULATORY APPROVALS

The trial will not be initiated before the protocol, informed consent forms and participant and GP information sheets have received approval / favourable opinion from the Medicines and Healthcare products Regulatory Agency (MHRA), Research Ethics Committee (REC), and the respective National Health Service (NHS) Research & Development (R&D) department. Should a protocol amendment be made that requires REC approval, the changes in the protocol will not be instituted until the amendment and revised informed consent forms and participant and GP information sheets (if appropriate) have been reviewed and received approval / favourable opinion from the REC and R&D departments. A protocol amendment intended to eliminate an apparent immediate hazard to participants may be implemented immediately providing that the MHRA, R&D and REC are notified as soon as possible and an approval is requested. Minor protocol amendments only for logistical or administrative changes may be implemented immediately; and the REC will be informed.

The trial will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, 1996; the principles of Good Clinical Practice, in accordance with the Medicines for Human Use Regulations, Statutory Instrument 2004, 1031 and its subsequent amendments and the Department of Health Research Governance Framework for Health and Social care, 2005.

INFORMED CONSENT AND PARTICIPANT INFORMATION

The process for obtaining participant informed consent or assent and parent / guardian informed consent will be in accordance with the REC guidance, and Good Clinical Practice (GCP) and any other regulatory requirements that might be introduced. The investigator or

their nominee and the participant or other legally authorised representative shall both sign and date the Informed Consent Form before the person can participate in the study.

The participant will receive a copy of the signed and dated forms and the original will be retained in the Trial Master File. A second copy will be filed in the participant's medical notes and a signed and dated note made in the notes that informed consent was obtained for the trial.

The decision regarding participation in the study is entirely voluntary. The investigator or their nominee shall emphasize to them that consent regarding study participation may be withdrawn at any time without penalty or affecting the quality or quantity of their future medical care, or loss of benefits to which the participant is otherwise entitled. No trial-specific interventions will be done before informed consent has been obtained.

The investigator will inform the participant of any relevant information that becomes available during the course of the study, and will discuss with them, whether they wish to continue with the study. If applicable they will be asked to sign revised consent forms.

If the Informed Consent Form is amended during the study, the investigator shall follow all applicable regulatory requirements pertaining to approval of the amended Informed Consent Form by the REC and use of the amended form (including for ongoing participants).

RECORDS

Drug accountability

Drug supplies will be kept in a secure, limited access storage area under the storage conditions specified by Pharmacy.

The investigator and the local site pharmacist shall maintain records of the study drug's delivery to the pharmacy, an inventory at the site, the distribution to each participant, and the return to the pharmacy or alternative disposition of unused study drugs. These records will include dates, quantities received, batch / serial numbers, expiration dates, and the unique code numbers (patient trial number) assigned to the trial participant. Investigators and /or the local site pharmacists will maintain records that document adequately that the participants were provided with the correct study medication. These records will be part of each patient's Case Report Form (CRF). All study medication packs and bottles received by the pharmacy shall be accounted for.

Case Report Forms

Each participant will be assigned a trial identity code number, allocated at randomisation, for use on CRFs other trial documents and the electronic database. The documents and

database will also use their initials (of first and last names separated by a hyphen or a middle name initial when available) and date of birth (dd/mm/yy). Data entry will occur directly into a web-based electronic CRF (eCRF) set-up and maintained by Nottingham Clinical Trials Unit. The eCRF includes data encryption for personal information as well as an audit trail to log all online data entry and any changes in the data fields.

CRFs will be treated as confidential documents and held securely in accordance with regulations. The investigator will make a separate confidential record of the participant's name, date of birth, local hospital number or NHS number, and Participant Trial Number (the Trial Recruitment Log), to permit identification of all participants enrolled in the trial, in case additional follow-up is required.

CRFs shall be restricted to those personnel approved by the Chief or local Principal Investigator and recorded on the 'Trial Delegation Log.'

All paper forms shall be filled in using black ballpoint pen. Errors shall be lined out but not obliterated by using correction fluid and the correction inserted, initialled and dated.

The Chief or local Principal Investigator shall sign a declaration ensuring accuracy of data recorded in the CRF.

Source documents

Source documents shall be filed at the investigator's site and may include but are not limited to, consent forms, current medical records, laboratory results and pharmacy records. A CRF may also completely serve as its own source data. Only trial staff as listed on the Delegation Log shall have access to trial documentation other than the regulatory requirements listed below.

Direct access to source data / documents

The CRF and all source documents, including progress notes and copies of laboratory and medical test results shall be made available at all times for review by the Chief Investigator, Sponsor's designee and inspection by relevant regulatory authorities (e.g., MHRA, EMEA).

DATA PROTECTION

All trial staff and investigators will endeavour to protect the rights of the trial's participants to privacy and informed consent, and will adhere to the Data Protection Act, 1998. The CRF will only collect the minimum required information for the purposes of the trial. CRFs will be held securely, in a locked room, or locked cupboard or cabinet. Access to the information will be limited to the trial staff and investigators and relevant regulatory authorities (see above).

Computer held data including the trial database will be held securely and password protected. All data will be stored on a secure dedicated web server. Access will be restricted by user identifiers and passwords (encrypted using a one way encryption method).

Information about the trial in the participant's medical records / hospital notes will be treated confidentially in the same way as all other confidential medical information.

Electronic data will be backed up every 24 hours to both local and remote media in encrypted format.

QUALITY ASSURANCE & AUDIT

INSURANCE AND INDEMNITY

Insurance and indemnity for trial participants and trial staff is covered within the NHS Indemnity Arrangements for clinical negligence claims in the NHS, issued under cover of HSG (96)48. There are no special compensation arrangements, but trial participants may have recourse through the NHS complaints procedures.

The University of Nottingham has taken out an insurance policy to provide indemnity in the event of a successful litigious claim for proven non-negligent harm.

TRIAL CONDUCT

Trial conduct will be subject to systems audit of the Trial Master File for inclusion of essential documents; permissions to conduct the trial; Trial Delegation Log; CVs of trial staff and training received; local document control procedures; consent procedures and recruitment logs; adherence to procedures defined in the protocol (e.g. inclusion / exclusion criteria, correct randomisation, timeliness of visits); adverse event recording and reporting; drug accountability, pharmacy records and equipment calibration logs.

The Trial Coordinator, or where required, a nominated designee of the Sponsor, shall carry out a site systems audit at least yearly and an audit report shall be made to the Trial Steering Committee.

TRIAL DATA

Monitoring of trial data shall include confirmation of informed consent; source data verification; data storage and data transfer procedures; local quality control checks and procedures, back-up and disaster recovery of any local databases and validation of data manipulation. The Trial Coordinator, or where required, a nominated designee of the Sponsor, shall carry out monitoring of trial data as an ongoing activity.

Entries on CRFs will be verified by inspection against the source data. A sample of CRFs (10%) will be checked on a regular basis for verification of all entries made. In addition the subsequent capture of the data on the trial database will be checked. Where corrections are required these will carry a full audit trail and justification.

A Trial Steering committee will also convene to include, Professor A Knox, Dr T Harrison, Dr J Anderson, Dr K Mortimer, an independent chair (Professor Anne Tattersfield) and two other independent members.

An independent Data Monitoring and Ethics (DMEC) will be established which will report to the TSC. The DMEC will be chaired by Dr Dave Singh. The DMEC will have access to all available study data with the option to unblind the data if considered necessary for the safety of the participants.

Trial data and evidence of monitoring and systems audits will be made available for inspection by the regulatory authority as required.

RECORD RETENTION AND ARCHIVING

In compliance with the ICH/GCP guidelines, regulations and in accordance with the University of Nottingham Research Code of Conduct, the Chief or local Principal Investigator will maintain all records and documents regarding the conduct of the study. These will be retained for at least 7 years or for longer if required. If the responsible investigator is no longer able to maintain the study records, a second person will be nominated to take over this responsibility.

The Trial Master File and trial documents held by the Chief Investigator on behalf of the Sponsor shall be finally archived at secure archive facilities at the University of Nottingham. This archive shall include all trial databases and associated meta-data encryption codes.

DISCONTINUATION OF THE TRIAL BY THE SPONSOR

The Sponsor reserves the right to discontinue this trial at any time for failure to meet expected enrolment goals, for safety or any other administrative reasons. The Sponsor shall take advice from the Trial Steering Committee and Data Monitoring Committee as appropriate in making this decision.

STATEMENT OF CONFIDENTIALITY

Individual participant medical information obtained as a result of this study are considered confidential and disclosure to third parties is prohibited with the exceptions noted above.

Participant confidentiality will be further ensured by utilising identification code numbers to correspond to treatment data in the computer files.

Such medical information may be given to the participant's medical team and all appropriate medical personnel responsible for the participant's welfare.

Data generated as a result of this trial will be available for inspection on request by the participating physicians, the University of Nottingham representatives, the REC, local R&D Departments and the regulatory authorities.

PUBLICATION AND DISSEMINATION POLICY

We intend to submit the study results for publication. Participants will not be identified in any publications.

USER AND PUBLIC INVOLVEMENT

We hold regular meetings for service users which are an opportunity to present the findings of recently completed research, discuss ongoing work and invite participation of service users in development of future work such as the current study. The participant information sheet for current study was prepared with input from one of our service users. In addition one service user will be appointed to as one of the two independent members of the Trial Steering Committee (TSC).

STUDY FINANCES

Funding source

This study is funded by the Nottingham University Biomedical Research Unit and a successful application has been submitted to the Efficacy and Mechanism Evaluation programme for funding.

Participant stipends and payments

Participants will be given an inconvenience allowance of £25. Travel expenses will be offered for any hospital visits in excess of usual care.

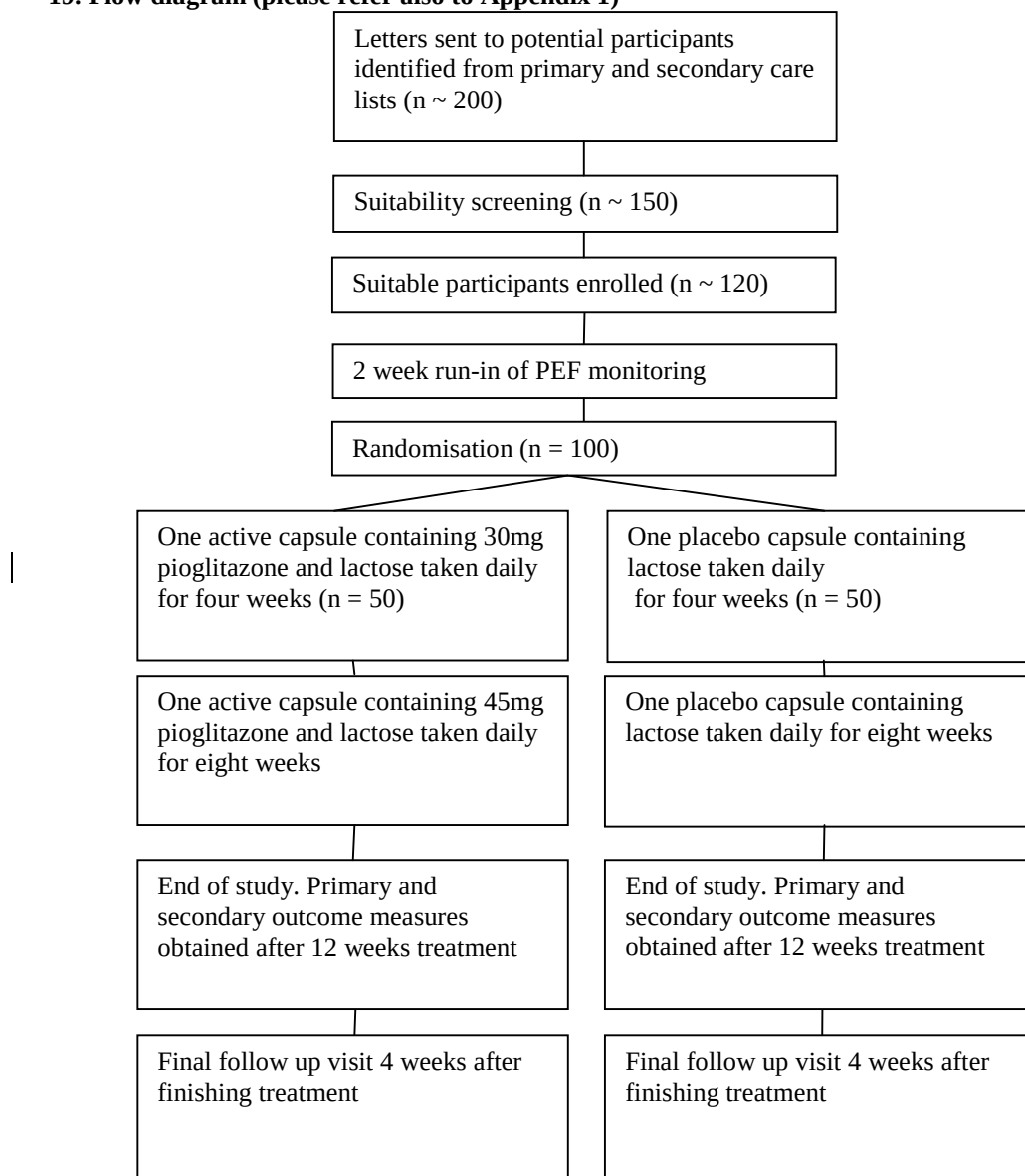
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19. Flow diagram (please refer also to Appendix 1)



SIGNATURE PAGES

Signatories to Protocol:

Chief Investigator: (name) _____

Signature: _____

Date: _____

Co- investigator: (name) _____

Signature: _____

Date: _____

Trial Statistician: (name) _____

Signature: _____

Date: _____

Trial Pharmacist: (name) _____

Signature: _____

Date: _____



CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4
	2b	Specific objectives or hypotheses	5
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	5
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	14
Participants	4a	Eligibility criteria for participants	5
	4b	Settings and locations where the data were collected	5
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	7
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	7
	6b	Any changes to trial outcomes after the trial commenced, with reasons	
Sample size	7a	How sample size was determined	8
	7b	When applicable, explanation of any interim analyses and stopping guidelines	
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	6
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	7
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	7
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	7
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	7

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	8
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	10
	13b	For each group, losses and exclusions after randomisation, together with reasons	10
Recruitment	14a	Dates defining the periods of recruitment and follow-up	10
	14b	Why the trial ended or was stopped	14
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	11
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	10
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	14
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	14
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	17
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	21
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	24
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	24
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	25
Other information			
Registration	23	Registration number and name of trial registry	1
Protocol	24	Where the full trial protocol can be accessed, if available	Submitted
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	Submitted

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming; for those and for up to date references relevant to this checklist, see www.consort-statement.org.