1   **DP₂ antagonism reduces airway smooth muscle mass in asthma by decreasing**

2   **eosinophilia and myofibroblast recruitment**

3

4   **Authors:** Ruth Saunders[1‡], Himanshu Kaul[2‡*], Rachid Berair[1‡], Sherif Gonem[1], Amisha

5   Singapuri[1], Amanda J. Sutcliffe[1], Latifa Chachi[1], Michael S. Biddle[1], Davinder Kaur[1], Michelle

6   Bourne[1], Ian D. Pavord[3], Andrew J. Wardlaw[1], Salman H. Siddiqui[1], Richard A. Kay[4], Bindi

7   S. Brook[5], Rod H. Smallwood[6†], Christopher E. Brightling[1†*]

8   ‡These authors contributed equally to this work, †Co-senior authors, * corresponding authors

9

10   **Affiliations:**

11   [1]University of Leicester, Leicester, LE3 9QP, UK

12   [2]Himanshu Kaul was employed by University of Leicester and University of Sheffield during

13   this study, currently at University of British Columbia, Vancouver, V6T 1Z3, Canada

14   [3]University of Oxford, Oxford, OX3 7FZ, UK

15   [4]Richard Kay was an employee of Novartis Pharma AG, CH-4056, Basel, Switzerland at the

16   time that this study was conducted and completed. Currently at Medpace (UK) Ltd, Stirling,

17   FK8 1JU, UK

18   [5]Nottingham University, Nottingham, NG7 2RD, UK

19   [6]University of Sheffield, S1 4DP, UK

20   ***Corresponding authors:** ceb17@le.ac.uk, himanshu.kaul@ubc.ca

21

22   **Overline**: Asthma

23

24   **One sentence summary:** Cellular and computational models and bronchial biopsies from

25   asthma patients show that a DP₂ antagonist reduces airway smooth muscle mass in asthma.

26   **Abstract**

27   Increased airway smooth muscle mass, a feature of airway remodeling in asthma, is the

28   strongest predictor of airflow limitation and contributes to asthma-associated morbidity and

29   mortality. No current drug therapy for asthma is known to affect airway smooth muscle mass.

30   Although there is increasing evidence that prostaglandin D_2 type 2 receptor (DP_2) is expressed

31   in airway structural and inflammatory cells, few studies have addressed the expression and

32   function of DP_2 in airway smooth muscle cells. We report that the DP_2 antagonist fevipiprant

33   reduced airway smooth muscle mass in bronchial biopsies from patients with asthma who had

34   participated in a previous randomized placebo-controlled trial. We developed a computational

35   model to capture airway remodeling. Our model predicted that a reduction in airway

36   eosinophilia alone was insufficient to explain the clinically observed decrease in airway smooth

37   muscle mass without a concomitant reduction in the recruitment of airway smooth muscle cells

38   or their precursors to airway smooth muscle bundles that comprise the airway smooth muscle

39   layer. We experimentally confirmed that airway smooth muscle migration could be inhibited

40   in vitro using DP_2-specific antagonists in an airway smooth muscle cell culture model. Our

41   analyses suggest that fevipiprant, through antagonism of DP_2, reduced airway smooth muscle

42   mass in patients with asthma by decreasing airway eosinophilia in concert with reduced

43   recruitment of myofibroblasts and fibrocytes to the airway smooth muscle bundle. Fevipiprant

44   may thus represent a potential therapy to ameliorate airway remodeling in asthma**.**

Introduction

Asthma affects over 300 million people worldwide and its prevalence is increasing (1) despite currently available therapies (2). Asthma is characterised by variable airflow limitation that becomes more persistent in severe disease. Increased airway smooth muscle (ASM) mass is an important component of airway remodeling in asthma, contributing substantially to symptoms and disordered airway physiology (3-5). To date, no drug has impacted upon the increased ASM mass observed in asthma in randomized placebo-controlled trials (3, 4). However, bronchial thermoplasty has demonstrated a potential reduction in ASM mass in asthma in uncontrolled studies (6-7).

The prostaglandin (PG) D_2 type 2 receptor (DP_2, also known as chemoattractant receptor-homologous molecule expressed on T helper [Th] 2 cells [CRTh2]) is expressed by inflammatory cells critical in the immunopathogenesis of asthma, including eosinophils, Th2 lymphocytes, type 2 innate lymphoid cells, and mast cells. DP_2 activation promotes cellular release of cytokines, inflammatory cell migration, and cell survival (8-10). Its archetypal ligand PGD_2 is predominantly released by mast cells localized to the ASM-bundle (11). The DP_2 antagonist fevipiprant has been shown to improve asthma symptoms, lung function, airway eosinophilia, and epithelial integrity (4). However, the role of the PGD_2/DP_2 axis in ASM dysfunction in asthma has not been extensively studied. We hypothesized that the PGD_2/DP_2 axis may contribute to increased ASM mass in asthma, and that antagonism of DP_2 with fevipiprant might result in a decrease in ASM mass.

Here, we analysed bronchial biopsies from asthma patients treated with the DP_2 antagonist fevipiprant in a previous phase 2a randomized, placebo-controlled trial undertaken to determine the impact of drug upon airway inflammation, remodeling and asthma control (4).

70   Using an agent-based computational model representing an asthmatic airway in human patients

71   and supported by in vitro ASM cell-based observations, we propose that the reduced ASM

72   mass observed in the bronchial biopsies after fevipiprant treatment may be a consequence of

73   inhibition of eosinophilic airway inflammation together with reduced recruitment of

74   myofibroblasts and fibrocytes to the ASM bundle.

75

76   **Results**

77   **Fevipiprant reduces ASM mass in subjects with asthma in a randomized placebo-**

78   **controlled trial**

79   We obtained bronchial biopsies from moderate-to-severe asthmatics with airway eosinophilic

80   inflammation, as evidenced by increased sputum eosinophil counts. These individuals had

81   participated in a 12-week single-centre (University of Leicester), randomized, double-blind,

82   parallel-group, placebo-controlled trial of the DP_2 antagonist fevipiprant (4). We then

83   performed an a priori quantification of ASM mass (percentage of total bronchial biopsy area)

84   (11). A representative photomicrograph of a bronchial biopsy from a subject showing

85   disrupted epithelium and increased ASM mass is shown in Fig. 1A. The absolute ASM mass

86   percentage (mean ± standard error of the mean [SEM]) observed decreased significantly

87   following treatment with fevipiprant (-13 ± 5%; p=0.022, n=14) versus placebo (4 ± 5%;

88   p=0.52, n=13) (mean difference [95% confidence interval (CI)] -16.2 [-1.4 to -31.1] %;

89   p=0.034) (Fig. 1B). In view of the above data, we performed a post hoc quantification of ASM

90   mass (percentage of total biopsy area) in bronchial biopsies derived from a sub-group of

91   moderate-to-severe eosinophilic asthmatics who had participated in a 50-week single-centre

92   (University of Leicester), randomized, double-blind, parallel-group, placebo-controlled trial of

93   the anti-interleukin (IL)-5 neutralizing antibody mepolizumab (12). Although the sample size

94   was small, in contrast to fevipiprant, we observed no significant effect of mepolizumab on

ASM mass (absolute ASM mass percentage increase post-mepolizumab $2.9 \pm 4.0\%$, n=7 versus placebo $1.5 \pm 2.2\%$, n=5) (mean difference [95% CI] 1.4 [-9.9 to 12.7] %; p=0.79). For these and other data, see Data File S1.

An agent-based computational model recapitulates the features of airway remodeling observed in asthma

To interrogate the mechanisms governing the pathogenesis of asthma, we developed an agent-based computational model of airway remodeling comprising epithelial, mesenchymal, and inflammatory parameters. In agent-based modeling, a system is divided into agents (here, airway cells; table S1) capable of interacting with each other and their environment based on defined rule-sets (13-15). The initial state of the model is illustrated in Fig. S1. Our model considered interactions between epithelial (columnar and goblet cells), mesenchymal (fibroblast, myofibroblast, and ASM cells), and inflammatory (eosinophil) cell types. The various cell types, depending on their phenotype, displayed behaviours ranging from proliferation, migration, (de)differentiation, apoptosis, and synthesis of extracellular matrix (ECM) proteins and cytokines (table S1). These virtual cells were simulated within a Strahler Order 3 virtual airway with a lumen diameter of 1.21 mm and wall area of 1.79 mm^2. The number of each cell type within the model was based on geometrical constraints and published data. The rule-set governing agent behaviours and interactions was derived from existing in vitro, animal, and clinical studies (table 1). The underpinning agent interactions and rule-sets attributed to the agents are summarized in table 1 and represented schematically in Fig. S2.

In the model, we initially damaged the epithelium to cause 50% epithelial denudation. We then simulated the consequent normal injury repair and pathological airway remodeling over 180 days by introducing alterations to model parameters (tables S2 – S4). The following

pathological markers and value ranges were considered necessary in the model to reflect the key hallmarks of severe asthma (1, 2): eosinophilic inflammation (eosinophils/mm^2 sub-mucosa) >10; epithelial integrity <70%; and ASM mass $\geq$10% and $\leq$50%. We conducted parametric testing by varying, both individually and collectively, parameters from all agent categories and observing which conditions best captured the above hallmarks of asthma. We defined the most parsimonious set of parameters to capture these three hallmarks (tables S2 – S4). The response to epithelial injury in this model displayed significantly increased eosinophilic inflammation (p<0.001), ASM mass (p=0.002) and persistent epithelial damage (p<0.001) compared to the model of healthy control individuals over the 180-day time course (Fig. 2A).

Computational modeling predicts that a reduction in eosinophil recruitment is not sufficient to decrease ASM mass

In order to predict the impact of reducing eosinophil number in our model of airway remodeling, we incorporated pro-apoptotic or anti-recruitment elements into the model. These variables were chosen to represent the major respective effects of neutralizing IL-5 (12, 26), an obligate cytokine for eosinophil survival and maturation, and of blocking activation of DP$_2$, which promotes eosinophil recruitment (4). We tested an increasing range of intervention doses and found they resulted in a progressive reduction in airway eosinophilia and ASM mass in our model of airway remodeling (Fig. 2B, C).

We then used our computational model of a remodeled asthmatic airway to determine the predicted percentage increase in eosinophil apoptosis and percentage reduction in eosinophil recruitment required to reduce the number of bronchial wall eosinophils to that seen in vivo in clinical trials of mepolizumab (27) and fevipiprant (4), which reduced the eosinophil count in

patients by 55% and 80%, respectively compared to placebo control. To attain the reduction in airway eosinophil number clinically observed with mepolizumab, the model predicted that 15% of the eosinophil population must be induced to undergo apoptosis (reduction in airway eosinophilia versus control of 54.1 ± 4.1 %; Fig. 2B, chequered bar). To attain the reduction in airway eosinophil number clinically observed for fevipiprant, the model predicted that a 40% reduction in eosinophil recruitment was required (reduction in airway eosinophilia versus control of 81 ± 0.6 %; Fig. 2B, hatched bar).

We subsequently used the pro-apoptotic (15%) and anti-recruitment (40%) models resulting in a reduction in eosinophil number equivalent to that seen in the mepolizumab and fevipiprant clinical trials to predict the impact of each intervention on ASM mass. When assuming an increase in eosinophil apoptosis of 15%, the pro-apoptosis model predicted a small mean $\pm$ SEM decrease in ASM mass (absolute reduction 4.0 ± 0.6%; relative reduction 12 ± 2% versus control; Fig. 2C, chequered bar). This is consistent with the mepolizumab clinical trial (12) in which no significant change in ASM mass was observed. When assuming a decrease in eosinophil recruitment of 40%, the anti-recruitment model predicted a modest reduction in ASM mass (absolute reduction 8.1 ± 0.5%; relative reduction 25 ± 1% versus control; Fig. 2C, hatched bar), which was not sufficient to result in the observed response to fevipiprant (13% absolute and 44% relative reduction in ASM mass). The model therefore suggested the existence of additional mechanisms that, along with a reduction in airway eosinophilia, mediated the reduction in ASM mass following treatment with fevipiprant.

The ASM PGD$_2$/DP$_2$ axis mediates ASM migration

To explore the mechanism by which DP$_2$ antagonism resulted in a decrease in ASM mass, we assessed the expression and function of DP$_2$ in ASM. We found that DP$_2$ was expressed in the

ASM-bundle in bronchial biopsies from patients recruited for research bronchoscopies (Fig. 3A), in line with the previous finding that PGD_2 primes migration of ASM cells towards platelet-derived growth factor via DP_2 (28). However, DP_2 expression was not significantly different between subjects with severe asthma (60 ± 1; n=8) and healthy controls (57 ± 5; n=11) (mean difference [95% CI] 2.6 [-9.9 to 15.0]; p=0.67). We also confirmed DP_2 expression in primary human ASM cells at the mRNA (Fig. 3B) and protein levels (Fig. 3 C, D and Fig. S3).

PGD_2 can activate PGD_2 type 1 (DP_1), DP_2 and thromboxane (TP) receptors (9). Therefore, we investigated the effect of the selective DP_2 agonist 13,14-Dihydro-15-keto-PGD_2 (DK-PGD_2) and selective DP_2 antagonists (fevipiprant, CAY10471 and OC000459) on DP_2 receptor activation, phenotype and behaviour of primary human ASM cells. DK-PGD_2 (10-100nM) stimulated a small but significant increase in filamentous actin (F-actin) polymerisation and intracellular calcium elevation (Fig. 3 E-F, area under curve of the dose response [AUC DR] p=0.01 and p=0.002, respectively). Although this did not translate to an effect of DK-PGD_2 on ASM cell migration in vitro (Fig. 4A), blocking activation of DP_2 by endogenous PGD_2 with the DP_2 antagonist fevipiprant significantly inhibited ASM cell migration in vitro at the highest drug concentrations (percentage reduction in cells migrating into the wound after 24h vs vehicle control: 10 nM, 8.8 ± 7.8, p=0.294; 50 nM, 7.7 ± 7.4, p=0.332; 100 nM, 12.8 ± 4.9, p=0.034, 500 nM, 17.4 ± 6.6, p=0.034; Fig. 4A). We confirmed this effect using other DP_2 antagonists including CAY10471 (percentage reduction in cells migrating into the wound after 24h vs vehicle control: 10 nM, 11.6 ± 2.2, p=0.010; 50 nM, 9.3 ± 3.7, p=0.038; 100 nM, 13.6 ± 4.4, p=0.027; Fig. 4A), and OC000459 (percentage reduction in cells migrating into the wound after 24h vs vehicle control: 10 nM, 8.4 ± 3.3, p=0.043; 50 nM, 6.7 ± 4.2, p=0.157; 100 nM, 8.8 ± 1.9, p=0.003, Fig. 4A). Representative photomicrographs of primary ASM monolayer cultures wounded by scratching followed by incubation with different treatments

195 for 24h are shown in Fig. 4B. Thus, we hypothesized that PGD_2 was released into the

196 extracellular milieu by ASM cells to affect ASM behaviour in an autocrine manner. Indeed,

197 genes involved in PGD_2 biosynthesis and metabolism, including PGD_2 synthase, were

198 expressed by ASM cells from subjects with and without asthma (tables S5 and S6). Consistent

199 with previous reports (29), PGD_2 was released by ASM, albeit at a low concentration compared

200 with mast cells (30-31), and this PGD_2 release increased following wounding (129 ± 18 versus

201 176 ± 22 pg $PGD_2/ml/10^5$ ASM, mean difference [95% CI] 52.0 [4.8 to 99.2]; p=0.02, Fig.

202 4C). In addition to myofibroblasts, we demonstrated that the ASM progenitor fibrocytes

203 expressed DP_2 (Fig. 4D). The correlation between the change in ASM percentage observed in

204 those treated with fevipiprant or placebo and the change in lamina propria myofibroblast or

205 fibrocyte number supported the view that the effects of fevipiprant on ASM mass and lamina

206 propria mesenchymal cells may have occurred in parallel (Fig. 4E, F). These findings suggested

207 that anti-DP_2 might, in part, reduce ASM mass via a direct and concomitant effect upon ASM

208 and myofibroblast or fibrocyte recruitment to the ASM bundle.

209

210 The effects on ASM cell migration were not due to cytotoxic effects on the ASM cells as there

211 was no effect on cell number following treatment for 24h with DK-PGD_2 (100 nM), fevipiprant

212 (500 nM), CAY10471 (100 nM) or OC000459 (100 nM) (Fig. S4A). DK-PGD_2 (100 nM),

213 fevipiprant (500 nM) and CAY10471 (100 nM) had no effect on apoptosis or necrosis (Fig.

214 S4B). This was supported by a lack of effect of DK-PGD_2 (100 nM), fevipiprant (500 nM) or

215 CAY10471 (100 nM) on cell size or granularity, which are known to change during

216 apoptosis/necrosis (Fig. S4C-D). Furthermore, DK-PGD_2 (10 - 100 nM, Fig. S5A), CAY10471

217 (10 - 100 nM, Fig. S5B) and fevipiprant (10 - 500 nM, Fig. S5C) neither induced proliferation

218 of ASM in the presence of serum-free media nor inhibited the ASM cell proliferation induced

219 by fetal bovine serum (FBS) over 3 days, as assessed by the 3-(4,5-dimethylthiazol-2-yl)-5-(3-

220 carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H tetrazolium inner salt (MTS) assay and

221 carboxyfluorescein succinimidyl ester (CFSE) fluorescence (Fig. S5D, E). In the fevipiprant

222 clinical trial, there was no change in proliferating cell nuclear antigen (PCNA) staining in

223 bronchial biopsies pre- versus post- fevipiprant treatment or placebo after 12 weeks of

224 treatment. Additionally, DK-PGD$_2$ (10 – 100 nM), fevipiprant (10-500 nM) or CAY10471 (10-

225 100nM) had no effect on ASM α-smooth muscle actin (SMA) expression (Fig. S6A). This was

226 supported by a lack of effect of DK-PGD$_2$ (100 nM), fevipiprant (500 nM) or CAY10471 (100

227 nM) on basal or bradykinin (BK)-stimulated ASM contraction (Fig. S6B-C).

228

229 **Modeling predicts that reductions in myofibroblast and eosinophil recruitment are**

230 **required for fevipiprant to decrease ASM mass**

231 To support our in vitro findings, we reduced the myofibroblast recruitment (0-50%) in the

232 computational model together with the 40% reduction in eosinophil recruitment required to

233 reflect the observed reduction in bronchial biopsy eosinophils after fevipiprant treatment in

234 patients with asthma as described above. The resulting model predicted that a 50% reduction

235 in myofibroblast recruitment in concert with reduced eosinophil recruitment would result in a

236 decrease in ASM mass equivalent to that seen after fevipiprant treatment (cross hatched bars,

237 Fig. 4G). In contrast, a reduction in myofibroblast recruitment alone was predicted to result in

238 minimal effects on ASM mass (1.8 ± 1.2% relative reduction). A comparison of the

239 computational model and fevipiprant trial findings is summarised in Fig. S7.

240

241 **Discussion**

242 We report that a drug intervention in asthma, namely fevipiprant (a DP$_2$ antagonist), reduced

243 ASM mass in bronchial biopsies from asthma patients who had participated in a previous

244 randomized placebo-controlled trial (4). This is in contrast to the lack of an effect on ASM

245   mass in response to mepolizumab that we report here, corticosteroids or the anti-IL-13

246   monoconal antibody tralokinumab (3, 32, 33). Our computational model and in vitro work

247   supported the view that the reduction in ASM mass in response to fevipiprant was a

248   consequence of inhibiting eosinophilic inflammation in concert with a direct reduction in the

249   recruitment of myofibroblasts to the ASM-bundle. Thus, fevipiprant may be a potential therapy

250   to target airway remodeling in asthma and its clinical benefits observed previously could be in

251   part due to its effects upon ASM.

252

253   One limitation of our study is that the number of paired biopsies collected in the fevipiprant

254   trial included modest numbers of subjects despite the trial being one of the largest biopsy

255   studies undertaken in subjects with asthma. Therefore, it is important to extend and confirm

256   these findings in future studies. Likewise, it is possible that the lack of effect observed with

257   other anti-inflammatory interventions is due to lack of statistical power conferred by the small

258   sample sizes. However, our computational model data suggest that these anti-inflammatory

259   approaches are unlikely to be effective unless they have additional direct effects upon ASM.

260   Indeed, a small reduction in ASM mass was previously reported following treatment with the

261   calcium channel blocker gallopamil, which had been proposed to have direct effects upon ASM

262   activation, but the reduction in ASM mass was no different from placebo (34).

263

264   Another limitation of our study is that we cannot completely exclude the possibility that the

265   effect of fevipiprant upon ASM mass both in vivo and in vitro is an off-target effect. However,

266   we used 3 selective and specific DP_2 antagonists, including fevipiprant, for the in vitro

267   experiments, and therefore we consider it unlikely that the findings we report on ASM

268   activation and migration are due to off-target effects. Our in vitro findings also imply that the

269   major effect of DP_2 antagonism upon ASM function was the inhibition of migration of ASM

270 progenitors to the airway, either from the blood or via attenuation of epithelial-mesenchymal

271 transition, rather than through effects on proliferation or apoptosis. This is consistent with the

272 concept that mesenchymal cells exhibit plasticity in phenotype (35). In keeping with our in

273 vitro observations, we did not identify any changes in PCNA staining in the ASM bundle in

274 vivo, suggesting there was no active proliferation of ASM. Taken together, these results

275 suggest that neither proliferation nor apoptosis contribute to the effects of DP_2 antagonism

276 upon ASM mass, although we cannot completely exclude some contribution from these

277 processes.

278

279 A strength of our study is the integration of findings from in vivo clinical trials and in vitro and

280 computational models. We developed a comprehensive agent-based model of airway

281 remodeling during asthma. Previous computational approaches have been applied to uncover

282 mechanisms driving unresolved allergic inflammation and airway hyper-responsiveness in

283 asthma (15, 36), but not airway remodeling. Our agent-based model was created to represent

284 the airway in 3-dimensions. A possible limitation is that our model was utilized for one layer

285 of agents and simulated a distal airway to balance model resolution and computational

286 complexity. However, we believe our model is representative as it captures the features of the

287 normal and pathological workings of the entire airway. Specifically, our computational model

288 displayed features consistent with moderate-to-severe asthma including damaged bronchial

289 epithelium, eosinophilic inflammation and increased ASM mass. This model responded to

290 perturbations reflective of changes in eosinophil survival and trafficking and provided new

291 insights into possible mechanisms of action of DP_2 antagonists versus anti-IL5 upon airway

292 remodeling. This model has also given us insights into the effects of DP_2 antagonism, which

293 would not be possible in vitro due to the limitations of studying multiple cell-cell interactions

294 within a complex airway structure. Although our computational model is not patient-specific

295 it represents an average patient with asthma and airway remodeling. This 'virtual patient'

296 represents a step towards patient-specific modeling in respiratory medicine. We anticipate our

297 integrated approach combining agent-based modeling with in vivo clinical data and in vitro

298 findings will provide further insights into asthma in future studies.

Materials and methods

Study design

The objective of the study was to use an integrated strategy encompassing samples from a randomized placebo-controlled trial in asthma patients evaluating fevipiprant (DP_2 antagonist) and mepolizumab (anti-interleukin-5 antibody), in vitro experiments, and predictive computational agent-based models simulating asthma pathogenesis to investigate the impact of DP_2 antagonism upon ASM mass and determine the mechanisms driving this effect.

Subjects with persistent moderate-to-severe asthma and an elevated sputum eosinophil count (n=61) participated in a single-centre (University of Leicester) randomized placebo-controlled trial of the DP_2 antagonist fevipiprant (225mg twice per day orally) in addition to standard of care (4). In an independent study, subjects (n=61) who had refractory eosinophilic asthma participated in a single-centre (University of Leicester), randomized placebo-controlled trial of an anti-interleukin-5 neutralising antibody mepolizumab (750 mg intravenous infusions every 4 weeks over 50 weeks) in addition to standard of care (12). A subgroup of subjects underwent bronchoscopy and bronchial biopsy in each independent study before and after administration of drug or placebo. The studies were approved by the Leicester and Northamptonshire ethics committee (05/Q2502/98 and 11/EM/0402, respectively) and registered with ClinicalTrials.gov (ISRCTN75169762, NCT01545726 and with EudraCT, number 2011-004966-13). The studies were carried out in accordance with CONSORT guidelines (4, 12).

The sample size for the fevipiprant and mepolizumab randomized controlled trials were determined based on change in the sputum eosinophil count as the primary outcome as described in Gonem et al (4) and on the number of exacerbations of asthma per subject as the primary outcome as described in Haldar et al (12). Assessing change in airway smooth muscle

324   mass was in the pre-specified exploratory analysis plan for the fevipiprant clinical trial, and

325   was performed post-hoc for mepolizumab. The inclusion and exclusion criteria for the

326   fevipiprant and mepolizumab trials, randomisation and blinding procedures are described in

327   Gonem et al (4) and Haldar et al (12), respectively. For the in vitro experiments the observers

328   analysed the experiment blinded to conditions.

329

330   Additional asthmatic subjects and healthy controls were recruited from a single-centre

331   (University of Leicester) for research bronchoscopies from which tissue sections and primary

332   ASM cells could be derived. Those with asthma gave an appropriate history and had objective

333   evidence of variable airflow obstruction or airway hyper-responsiveness, as described

334   previously (37). Healthy controls had no history of asthma and possessed normal lung function.

335   The study was approved by the Leicestershire and Northamptonshire Ethics Committee

336   (08/H0406/189).

337

338   Immunohistochemistry

339   To determine DP_2 expression by ASM, bronchial biopsies from healthy controls (n=11 donors)

340   and asthmatic subjects (n=8 donors) were embedded in glycomethacrylate (GMA) (11). For

341   each subject, sequential 2 µm sections were cut and stained using polyclonal anti-DP_2 antibody

342   (Thermo-Fisher Scientific) or rabbit immunoglobulin (Ig) G isotype control (Immunostep), and

343   an α-SMA antibody (clone 1A4, Dako) or mouse IgG2a isotype control (clone DAK-GO5,

344   Dako). Antibody binding was detected using the EnVision FLEX kit (Dako). For determining

345   ASM mass pre- and post-treatment with fevipiprant (n=14) or placebo (n=13), and pre- and

346   post-treatment with mepolizumab (n=7) or placebo (n=5) bronchial biopsies from asthmatic

347   subjects were embedded in GMA and stained for α-SMA as above. ASM mass was determined

348   as the percentage of the total assessable biopsy area as previously described by a single

349 observer (RB). Repeatability of ASM mass assessment was tested and was excellent within

350 and between observers with intraclass correlations of 0.95 and 0.96 respectively.

351 Myofibroblasts were identified as α-SMA positive stained cells in the lamina propria that were

352 neither located as part of the ASM-bundle nor as vascular smooth muscle cells adjacent to

353 vessels per mm^2 of submucosa. To identify fibrocytes in bronchial biopsies pre- and post-

354 fevipiprant (n=12) or placebo (n=13), for each subject sequential 2 μm sections were cut and

355 stained using an mouse monoclonal anti-cluster of differentiation (CD) 34 antibody (Dako) or

356 mouse IgG1 isotype control (Dako) and α-SMA as above. Fibrocytes were identified as the

357 subset of α-SMA positive cells/mm2 lamina propria that also stained positive for CD34 in

358 sequential sections. The intensity of DP_2 stain was quantified as reciprocal intensity (38) on a

359 scale out of 250, assessed by a single observer. Assessors were blind to clinical characteristics,

360 treatment allocation and order of bronchial biopsy in the clinical trial.

361

362 **Cell culture**

363 ASM bundles were isolated from bronchial biopsies (n=27 asthmatic, 2 non-asthmatic) and

364 lung resection material (n=4, non-asthmatic). The clinical characteristics of subjects that

365 underwent bronchoscopy to provide primary ASM cultures are as shown (table S5). Primary

366 ASM cells were cultured in DMEM with Glutamax-1 supplemented with 10% FBS, 100U/mL

367 penicillin, 100μg/mL streptomycin, 0.25 μg/mL amphotericin, 100 μM non-essential amino-

368 acids, and 1 mM sodium pyruvate (Gibco). Cells were characterized for α-SMA expression

369 using a mouse monoclonal anti-α-SMA antibody (clone 1A4, Dako) or mouse IgG2a isotype

370 control (clone DAK-GO5, Dako) by flow cytometry and used between passage 2-6.

371

372   Fibrocytes (n=6) were isolated from peripheral blood mononuclear cells as described

373   previously (39). PBMCs were washed twice with HBSS and cultured in tissue culture flasks

374   coated with 40 µg/ml fibronectin for 5 -10 days prior to experimentation.

375

376   Prior to experimentation ASM cells from each individual donor were incubated in media in the

377   presence of a selective DP_2 agonist (DK-PGD$_2$, Cayman Chemical Company (40)) or selective

378   DP_2 antagonists (CAY10471, OC000459 and fevipiprant, Cayman Chemical Company and

379   Novartis (41-43) vs appropriate vehicle controls (Dimethyl sulfoxide (DMSO) for DK-PGD$_2$,

380   CAY10471 and OC000459, and 10% dH20 in DMSO for fevipiprant).

381

382   **Wound healing assay**

383   ASM cells from individual donors were seeded onto 6 well plates coated with 10 µg/ml

384   fibronectin at a density of $2×10^5$ cells and allowed to adhere and reach 90-100% confluence.

385   Cells were then serum deprived for 24h. Cells were wounded by scratching using a sterile 200

386   µl pipette tip in a predetermined grid pattern (44). Following wounding ASM cells were

387   washed x4 with serum free media prior to addition of serum free media with DK-PGD$_2$ (10-

388   100 nM), fevipiprant (10-500 nM), CAY10471 (10-100 nM) or OC000459 (10-100 nM) or

389   vehicle control for 24 h. Photographs of 4 different wounded areas per condition were then

390   photographed at baseline and after 24h using an EVOS xl core cell imaging system (Thermo

391   Fisher Scientific), and the outline of the wound at time zero transposed onto the corresponding

392   24h photograph. The number of cells that had moved into the wounds were analysed by a

393   blinded observer.

394

Computational model approach and framework

The computational model capturing airway remodeling was developed via the agent-oriented approach (13), which charts the spatiotemporal evolution of a system as a result of flexible, high-level interactions between agents as well as agents and their environment (14). The *Flexible Large-scale Agent-based Modelling Environment* (www.flame.ac.uk), an agent-based platform employing *stream communicating X-machines* (45) as agents, was utilized to develop the model.

The baseline model and computational iterations

Based on the agents, set of rules, and initial conditions (table 1, table S1, Fig. S1, Fig. S2, supplementary materials) a baseline model of airway remodeling, comprising epithelial, mesenchymal, and inflammatory parameters, was developed that captures trends observed during normal airway remodeling, and additionally following introduction of abnormal levels of variation within model parameters leads to the emergence of patterns observed during pathological remodeling and, as such, the key hallmarks of asthma – this approach is referred to as *pattern-oriented modelling* (13). After review of clinical literature, the following clinical markers were considered to appropriately reflect the key hallmarks of asthma: i) eosinophilic inflammation (defined as eosinophils/mm^2 submucosal area) >10 (5, 11, 46), ii) epithelial integrity <70% (4-5, 47-50) and iii) airway muscle mass/wall area ≥10% and ≤50% (4-5, 47, 49).

The model starts by assessing the epithelial integrity, the number and location of inflammatory cells (both the universal inflammatory cells and eosinophils), and the status of muscle cells. Remodeling is initiated in case of a compromised epithelium or increased inflammation within the system, resulting in a cascade of events, which, depending on the relevant boundary

420 conditions, lead to further inflammation, fibrosis, goblet cell hyperplasia, recruitment of

421 muscle, and increased collagen deposition. Furthermore, remodeling, could be exacerbated or

422 prolonged by the nature of initial or secondary conditions assigned to the computation.

423

424 More specifically, a normal or 'healthy' set of conditions triggered remodeling in the absence

425 of an intact epithelium (or a challenge that resulted in epithelial denudation) by initiating

426 fibrosis and recruiting the universal inflammatory cell. The inflammatory cell further 'released'

427 pro-inflammatory cytokines to recruit eosinophils and muscle – the latter was accounted for in

428 the model by the differentiation of fibroblasts into myofibroblasts. The eosinophils, moreover,

429 caused further damage by degranulating and releasing cytotoxic proteins, which, if close to the

430 epithelium, resulted in necrosis of the epithelial cells – thereby, prolonging remodeling. We

431 worked with the hypothesis that any set of conditions that perpetuate these interactions will

432 result in pathological remodeling, thereby capturing the hallmarks of asthma. The various

433 parameters and their quantitative values, derived from existing literature, have been, along with

434 the relevant references, listed in table 1, with a schematic interlinking the various elements of

435 the model shown in Fig. S2.

436

437 While the model does not explicitly consider cytokine activity, i.e. their release, diffusion, and

438 half-life, it implicitly accounts for it by requiring that those cells impacted by the cytokine

439 molecules share a localized region with the effector cell. For example, only ASM cells within

440 close proximity of the universal inflammatory cellswill undergo hypertrophy or contraction,

441 refer to supplementary material for more details.

442

443 Finally, the simulations progress in a number of time steps, with each time step matching 1

444 hour of real time. Time intervals of 30 minutes and 2 hours were also tested on the baseline

445 case (Case I) and yielded results indistinguishable from simulations conducted with 1-hour

446 time steps. Thus, we opted for the 1-hour interval to strike a balance between computational

447 costs and ensuring adequate resolution regarding activities we wished to capture via the model.

448 The total simulated time for all simulations, including remodeling and intervention, was ~6

449 months (4350 iterations). This time period allowed investigation of both the short- and long-

450 term response following either a challenge or intervention. Each model was simulated five

451 times (n=5) to assess the sensitivity of the model to inherent stochastic elements (such as cell

452 cycle, new coordinates of the daughter cells and migration of the universal inflammatory cells).

453 The internal random elements accounted for intra- and inter-cellular biological stochasticity.

454 Testing model for insensitivity to these random elements also served to provide an indicator

455 for model precision.

456

457 A detailed description of the model, its development, and its validation is provided in the

458 supplementary materials.

459

460 **Statistical analysis**

461 Statistical analysis was performed using with SAS/STAT software and GraphPad Prism. Data

462 were tested for normality using the Shapiro-Wilk test. For normally distributed data, two-tailed

463 paired t-tests, one sample t-tests, or one-way ANOVA were used as appropriate. For non-

464 parametric data, Wilcoxon matched pairs sign ranked test or Kruskal-Wallis tests were used as

465 appropriate. Correlations were performed using Spearman's correlation. Details of statistical

466 tests used are provided in figure legends. $p < 0.05$ was considered statistically significant.

467   **List of Supplementary Materials**

468   **Materials and Methods**

469   **Fig. S1: The virtual airway at baseline.**

470   **Fig. S2: Model parameters and agent interactions.**

471   **Fig. S3: Flow cytometric analysis of ASM cells.**

472   **Fig. S4: DK-PGD$_2$, fevipiprant, CAY10471, and OC000459 had no effect on ASM cell**

473   **number, apoptosis, or necrosis after 24h.**

474   **Fig. S5: DK-PGD$_2$, fevipiprant, and CAY10471 had no effect on ASM proliferation after**

475   **72h.**

476   **Fig. S6: DK-PGD$_2$, fevipiprant, and CAY10471 had no effect on basal or bradykinin-**

477   **induced ASM contraction.**

478   **Fig. S7: Conceptual summary.**

479   **Table S1. Description of agents used in computational model.**

480   **Table S2. Alterations made to epithelial parameters in the computational model.**

481   **Table S3. Alterations made to mesenchymal parameters in the computational model.**

482   **Table S4. Alterations made to inflammatory parameters in the computational model.**

483   **Table S5. Clinical characteristics of subjects that provided additional bronchial biopsies**

484   **for primary ASM cultures.**

485   **Table S6. Analysis of expression of genes involved in prostaglandin D$_2$ biosynthesis and**

486   **metabolism in ASM cells.**

487   **Table S7. Output of computations simulating pathological airway remodeling.**

488   **Data File S1. Data values for individual experiments.**

References and notes

1. A. Papi, C. E. Brightling, S. E. Pedersen, H. K. Reddel, Asthma. *Lancet.* **391**, 783-800 (2018).

2. K. F. Chung, S. E. Wenzel, J. L. Brozek, A. Bush, M. Castro, P. J. Sterk, I. M. Adcock, E. D. Bateman, E. H. Bel, E. R. Bleecker, L. P. Boulet, C. Brightling, P. Chanez, S. E. Dahlen, R. Djukanovic, U. Frey, M. Gaga, P. Gibson, Q. Hamid, N. N. Jajour, T. Mauad, R. L. Sorkness, W. G. Teague, International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur. Respir. J.* **43(2)**, 343-73 (2014).

3. R. Berair, C. E. Brightling, Asthma therapy and its effect on airway remodeling. *Drugs*. **74(12)**, 1345-69 (2014).

4. S. Gonem, R. Berair, A. Singapuri, R. Hartley, M. F. M. Laurencin, G. Bacher, B. Holzhauer, M. Bourne, V. Mistry, I. D. Pavord, A. H. Mansur, A. J. Wardlaw, S. H. Siddiqui, R. A. Kay, C. E. Brightling, Fevipiprant, a prostaglandin D2 receptor 2 antagonist, in patients with persistent eosinophilic asthma: a single-centre, randomised, double-blind, parallel-group, placebo-controlled trial. *Lancet Respir. Med.* **4(9)**, 699-707 (2016).

5. L. Benayoun, A. Druilhe, M. C. Dombret, M. Aubier, M. Pretolani, Airway structural alterations selectively associated with severe asthma. *Am. J. Respir. Crit. Care Med.* **167(10)**, 1360-1368 (2003).

6. M. Pretolani, A. Bergqvist, G. Thabut, M. C. Dombret, D. Knapp, F. Hamidi, L. Alavoine, C. Taillé, P. Chanez, J. S. Erjefält, M. Aubier, Effectiveness of bronchial thermoplasty in patients with severe refractory asthma: Clinical and histopathologic correlations. *J. Allergy Clin. Immunol*. **139(4)**, 1176-1185 (2017).

7. I.L. Chernyavsky, R.J. Russell, R.M. Saunders, G.E. Morris, R. Berair, A. Singapuri, L. Chachi, A.H. Mansur, P.H. Howarth, P. Dennison, R. Chaudhuri, S. Bicknell, F.R.A.J.

Rose, S. Siddiqui, B.S. Brook, C.E. Brightling, In vitro, in silico and in vivo study challenges the impact of bronchial thermoplasty on acute airway smooth muscle mass loss. *Eur Respir J*. **51:** 1701680 (2018).

8. S. E. Stinson, Y. Amrani, C. E. Brightling, D prostanoid receptor 2 (chemoattractant receptor-homologous molecule expressed on T(H)2 cells) protein expression in asthmatic patients and its effects on bronchial epithelial cells. *J. Allergy Clin. Immunol.* **135(2)**, 395-536 (2015).

9. R. Schuligoi, E. Sturm, P. Luschnig, V. Konya, S. Philipose, M. Sedej, M. Waldhoer, B. A. Peskar, A, Heinemann, CRTH2 and D-Type Prostanoid Receptor Antagonists as Novel Therapeutic Agents for Inflammatory Diseases. *Pharmacology*. **85(6)**, 372-82 (2010).

10. L. Xue, M. Salimi, I. Panse, J. M. Mjösberg, A. N. McKenzie, H. Spits, P. Klenerman, G. Ogg, Prostaglandin D2 activates group 2 innate lymphoid cells through chemoattractant receptor-homologous molecule expressed on TH2 cells. *J. Allergy Clin. Immunol.* **133(4)**, 1184-1194 (2014).

11. C. E. Brightling, P. Bradding, F. A. Symon, S. T. Holgate, A. J. Wardlaw, I. D. Pavord, Mast-cell infiltration of airway smooth muscle in asthma. *N. Engl. J. Med.* **346(22)**, 1699-1705 (2002).

12. P. Haldar, C. E. Brightling, B. Hargadon, S. Gupta, W. Monteiro, A. Sousa, R. P. Marshall, P. Bradding, R. H. Green, A. J. Wardlaw, I. D. Pavord, Mepolizumab and exacerbations of refractory eosinophilic asthma. *N. Engl. J. Med*. 360(10), 973-84 (2009). Erratum in: *N. Engl. J. Med*. **364(6)**, 588 (2011).

13. H. Kaul, Y. Ventikos, Investigating biocomplexity through the agent-based paradigm. *Brief Bioinform.* **16(1)**, 137-152 (2015).

14. H. Kaul, B. K. Hall, C. Newby, Y. Ventikos, Synergistic activity of polarised osteoblasts inside condensations cause their differentiation. *Sci. Rep.* **5**, 11838 (2015).

539   15. J. J. Pothen, M. E. Poynter, J. H. T. Bates, A computational model of unresolved allergic

540   inflammation in chronic asthma. *Am. J. Physiol. Lung Cell Mol. Physiol.* **308,** L384-L390

541   (2015).

542   16. D. L. Wilhelm, Regeneration of tracheal epithelium. *J. Pathol. Bacteriol.* **65(2)**, 543

543   (1953).

544   17. J. S. Erjefalt, I. Erjefalt, F. Sundler, C. G. A. Persson, In-vivo restitution of airway

545   epithelium. *Cell and Tissue Res.* 281(2), 305-316 (1995).

546   18. J. M. Zahm, H. Kaplan, A. L. Hérard, F. Doriot, D. Pierrot, P. Somelette, E. Puchelle, Cell

547   migration and proliferation during the in vitro wound repair of the respiratory epithelium.

548   *Cell Motil. Cytoskeleton* **37(1)**, 33-43 (1997).

549   19. C. L. Ordoñez, R. Khashayar, H. H. Wong, R. Ferrando, R. Wu, D. M. Hyde, J. A.

550   Hotchkiss, Y. Zhang, A. Novikov, G. Dolganov, J. V. Fahy, Mild and moderate asthma is

551   associated with airway goblet cell hyperplasia and abnormalities in mucin gene expression.

552   *Am. J. Respir. Crit. Care Med.* **163(2)**, 517-523 (2001).

553   20. J.V. Fahy, Remodeling of the airway epithelium in asthma. *Am. J. Respir. Crit. Care Med.*

554   **164(10 Pt 2)**, S46-51 (2001).

555   21. T. Aikawa, S. Shimura, H. Sasaki, M. Ebina, T. Takishima, Marked goblet cell

556   hyperplasia with mucus accumulation in the airways of patients who died of severe acute

557   asthma attack. *Chest* **101(4)**, 916-921 (1992).

558   22. B. Thomas, A. Rutman, R. A. Hirst, P. Haldar, A. J. Wardlaw, J. Bankart, C. E. Brightling,

559   C. O'Callaghan, Ciliary dysfunction and ultrastructural abnormalities are features of severe

560   asthma. *J. Allergy Clin. Immunol.* **126(4)**, 722-729.e722 (2010).

561   23. S. Siddiqui, A. Sutcliffe, A. Shikotra, L. Woodman, C. Doe, S. McKenna, A. Wardlaw, P.

562   Bradding, I. Pavord, C. Brightling, Vascular remodeling is a feature of asthma and

563   nonasthmatic eosinophilic bronchitis. *J. Allergy Clin. Immunol.* **120(4)**, 813-819 (2007).

564 24. M. A. Giembycz, M. A. Lindsay, Pharmacology of the eosinophil. *Pharmacol. Rev.* **51(2)**, 213-339 (1999).

566 25. H. Sugiura, X. Liu, F. Duan, S. Kawasaki, S. Togo, K. Kamio, X. Q. Wang, L. Mao, Y. Ahn, R. F. Ertl, T. W. Bargar, A. Berro, T. B. Casale, S. I. Rennard, Cultured lung fibroblasts from ovalbumin-challenged "Asthmatic" mice differ functionally from normal. *Am. J. Respir. Cell. Mol. Biol.* **37(4)**, 424-430 (2007).

570 26. R. Russell, C. Brightling, Mepolizumab for the reduction of exacerbations in severe eosinophilic asthma. *Expert Rev. Respir. Med*. **10(6)**, 607-17 (2016).

572 27. P. T. Flood-Page, A. N. Menzies-Gow, A. B. Kay, D.S. Robinson, Eosinophil's role remains uncertain as anti-interleukin-5 only partially depletes numbers in asthmatic airway. *Am. J. Respir. Crit. Care Med*. **167(2)**, 199-204 (2003).

575 28. K. Parameswaran, K. Radford, A. Fanat, J. Stephen, C. Bonnans, B. D. Levy, L. J. Janssen, P. G. Cox, Modulation of human airway smooth muscle migration by lipid mediators and Th-2 cytokines. *Am. J. Respir. Cell Mol. Biol.* **37(2)**, 240-247 (2007).

578 29. S. R. Johnson, A. J. Knox, Synthetic functions of airway smooth muscle in asthma. *Trends Pharmacol. Sci.* **18(8)**, 288-292 (1997).

580 30. A. Lewis, J. Wan, B. Baothman, P. N. Monk, S. K. Suvarna, P. T. Peachell, Heterogeneity in the responses of human lung mast cells to stem cell factor. *Clin. Exp. Allergy* **43**, 50-59 (2013).

583 31. M. C. Weston, N. Anderson, P. T. Peachell, Effects of phosphodiesterase inhibitors on human lung mast cell and basophil function. *Br. J. Pharmacol.* **121(2)**, 287-295 (1997).

585 32. A. R. Sousa, R. P. Marshall, L. C. Warnock, S. Bolton, A. Hastie, F. Symon, B. Hargadon, H. Marshall, M. Richardson, C. E. Brightling, P. Haldar, R. Milone, P. Chalk, R. Williamson, R. Panettieri Jr, R. Knowles, E. R. Bleecker, A. J. Wardlaw, Responsiveness

588 to oral prednisolone in severe asthma is related to the degree of eosinophilic airway
589 inflammation. *Clin Exp Allergy.* **47**, 890-899 (2017).

590 33. R. J. Russell, L. Chachi, J. M. FitzGerald, V. Backer, R. Olivenstein, I. L. Titlestad, C. S.
591 Ulrik, T. Harrison, D. Singh, R. Chaudhuri, B. Leaker, L. McGarvey, S. Siddiqui, M.
592 Wang, M. Braddock, L. H. Nordenmark, D. Cohen, H. Parikh, G. Colice, C. E. Brightling;
593 MESOS study investigators. Effect of tralokinumab, an interleukin-13 neutralising
594 monoclonal antibody, on eosinophilic airway inflammation in uncontrolled moderate-to-
595 severe asthma (MESOS): a multicentre, double-blind, randomised, placebo-controlled
596 phase 2 trial. Lancet Respir. Med. **Jul;6(7)**, 499-510 (2018).

597 34. P. O. Girodet, G. Dournes, M. Thumerel, H. Begueret, P. Dos Santos, A. Ozier, I. Dupin,
598 T. Trian, M. Montaudon, F. Laurent, R. Marthan, P. Berger, Calcium channel blocker
599 reduces airway remodeling in severe asthma. A proof-of-concept study. *Am. J. Respir.*
600 *Crit. Care Med.* **191(8)**, 876-83 (2015).

601 35. A. J. Halayko, H. Salari, X. MA, N. L. Stephens. Markers of airway smooth muscle cell
602 phenotype. *Am.J.Physiol* **270**:L1040-L1051. (1996)

603 36. A. Z. Politi, G. M. Donovan, M. H. Tawhai, M. J. Sanderson, A-M. Lauzon, J. H. T. Bates,
604 J. Sneyd, A multiscale, spatially-distributed model of asthmatic airway hyper-
605 responsiveness, *J. Theor. Biol.* **266(4)**, 614-624 (2010).

606 37. C. E. Brightling, A. J. Ammit, D. Kaur, J. L. Black, A. J. Wardlaw, J. M. Hughes, P.
607 Bradding, The CXCL10/CXCR3 axis mediates human lung mast cell migration to
608 asthmatic airway smooth muscle. *Am. J. Respir. Crit. Care Med.* **171(10)**, 1103-1108
609 (2005).

610 38. D. Nguyen, T. Zhou, J. Shu, J. Mao, Quantifying chromogen intensity in
611 immunohistochemistry via reciprocal intensity. *Cancer InCytes* **2(1)**, e (2013).

612   39. R. M. Saunders, S. Siddiqui, D. Kaur, C. Doe, A. Sutcliffe, F. Hollins, P. Bradding, A.J.

613   Wardlaw, C. E. Brightling. Fibrocyte localization to the airway smooth muscle is a feature

614   of asthma. *J Allergy Clin Immunol*. **123**:376-84 (2009).

615   40. H. Hirai, K. Tanaka, O. Yoshie, K. Ogawa, K. Kenmotsu, Y. Takamori, M. Ichimasa, K.

616   Sugamura, M. Nakamura, S. Takano, K. Nagata, Prostaglandin D2 selectively induces

617   chemotaxis in T helper type 2 cells, eosinophils, and basophils via seven-transmembrane

618   receptor CRTH2. J. Exp. Med. **193(2)**:255-61 (2001).

619   41. T. Ulven, E. Kostenis, Minor structural modifications convert the dual TP/CRTH2

620   antagonist ramatroban into a highly selective and potent CRTH2 antagonist. *J. Med. Chem.*

621   **48(4)**:897-900 (2005).

622   42. R. Pettipher, S. L. Vinall, L. Xue, G. Speight, E. R. Townsend, L. Gazi, C.J. Whelan, R.E.

623   Armer, M. A. Payton, M.G. Hunter MG. Pharmacologic profile of OC000459, a potent,

624   selective, and orally active D prostanoid receptor 2 antagonist that inhibits mast cell-

625   dependent activation of T helper 2 lymphocytes and eosinophils. J. Pharmacol. Exp. Ther.

626   **340(2)**:473-82 (2012).

627   43. V. J. Erpenbeck, E. Vets, L. Gheyle, W. Osuntokun, M. Larbig, S. Neelakantham, D.

628   Sandham, G. Dubois, W. Elbast, P. Goldsmith, M. Weiss. Pharmacokinetics, Safety, and

629   Tolerability of Fevipiprant (QAW039), a Novel CRTh2 Receptor Antagonist: Results

630   From 2 Randomized, Phase 1, Placebo-Controlled Studies in Healthy Volunteers. Clin.

631   Pharmacol. Drug Dev. **5(4)**:306-13 (2016).

632   44. D. Kaur, R. Saunders, P. Berger, S. Siddiqui, L. Woodman, A. Wardlaw, P. Bradding, C.

633   E. Brightling, Airway smooth muscle and mast cell-derived CC chemokine ligand 19

634   mediate airway smooth muscle migration in asthma. *Am. J. Respir. Crit. Care Med.*

635   **174(11)**, 1179-1188 (2006).

636 45. G. Laycock, *The Theory and Practice of Specification Based Software Testing* [PhD
637   Thesis]. Sheffield: Department of Computer Science, University of Sheffield; 1993.

638 46. M. Hoshino, Y. Nakamura, J. J. Sim, Expression of growth factors and remodelling of the
639   airway wall in bronchial asthma. *Thorax* **53(1)**, 21-27 (1998).

640 47. H. Begueret, P. Berger, J. M. Vernejoux, L. Dubuisson, R. Marthan, J. M. Tunon-de-Lara,
641   Inflammation of bronchial smooth muscle in allergic asthma. *Thorax* **62(1)**, 8-15 (2007).

642 48. S. Pegorier, N. Arouche, M. C. Dombret, M. Aubier, M. Pretolani, Augmented epithelial
643   endothelin-1 expression in refractory asthma. *J. Allergy Clin. Immunol.* **120(6)**, 1301-1307
644   (2007).

645 49. N. Carroll, J. Elliot, A. Morton, A. James, The structure of large and small airways in
646   nonfatal and fatal asthma. *Am. Rev. Respir. Dis.* **147(2)**, 405-410 (1993).

647 50. K. Amin, D. Lúdvíksdóttir, C. Janson, O. Nettelbladt, E. Björnsson, G. M. Roomans, G.
648   Boman, L. Sevéus, P. Venge, Inflammation and structural changes in the airways of
649   patients with atopic and nonatopic asthma. *Am. J. Respir. Crit. Care Med.* **162(6)**, 2295-
650   2301 (2000).

651 51. A. Sutcliffe, F. Hollins, E. Gomez, R. Saunders, C. Doe, M. Cooke, R. A. Challiss, C. E.
652   Brightling, Increased Nicotinamide Adenine Dinucleotide Phosphate Oxidase 4
653   Expression Mediates Intrinsic Airway Smooth Muscle Hypercontractility in Asthma. *Am.*
654   *J. Respir. Crit. Care Med.* **185(3)**, 267-274 (2012).

655 52. E. J. Swindle, J. E. Collins, D. E. Davies, Breakdown in epithelial barrier function in
656   patients with asthma: Identification of novel therapeutic approaches. *J. Allergy Clin.*
657   *Immunol.* **124(1)**, 23-34 (2009).

658 53. S. T. Holgate, D. E. Davies, S. Puddicombe, A. Richter, P. Lackie, J. Lordan, P. Howarth,
659   Mechanisms of airway epithelial damage: epithelial-mesenchymal interactions in the
660   pathogenesis of asthma. *Eur. Respir. J. Supplement.* **44**:24s-29s (2003).

661   54. A. Detoraki, F. Granata, S. Staibano, F. W. Rossi, G. Marone, A. Genovese, Angiogenesis

662   and lymphangiogenesis in bronchial asthma. *Allergy* **65(8)**, 946-958 (2010).

663   55. J. V. Fahy, B. F. Dickey. Airway Mucus Function and Dysfunction. *N. Engl. J. Med.*

664   **363(23)**, 2233-2247 (2010).

665   56. N. Awadh, N. L. Müller, C. S. Park, R. T. Abboud, J. M. FitzGerald, Airway wall

666   thickness in patients with near fatal asthma and control groups: assessment with high

667   resolution computed tomographic scanning. *Thorax* **53(4)**, 248-253 (1998).

668   57. A. L. James, J. G. Elliot, R. L. Jones, M. L. Carroll, T. Mauad, T. R. Bai, M. J. Abramson,

669   K. O. McKay, F. H. Green, Airway smooth muscle hypertrophy and hyperplasia in asthma.

670   *Am. J. Respir. Crit. Care Med.* **185(10)**, 1058-1064 (2012).

671   58. H. Kaul, Z. Cui, Y. Ventikos, A multi-paradigm modeling framework to simulate dynamic

672   reciprocity in a bioreactor. *PLoS One* **8(3)**, e59671 (2013).

673   59. O. Sacco, M. Silvestri, F. Sabatini, R. Sale, A. C. Defilippi, G. A. Rossi, Epithelial cells

674   and fibroblasts: structural repair and remodelling in the airways. *Paediatr. Respir. Rev.* **5**

675   **Suppl A**, S35-40 (2004).

676   60. S. Kirkham, J. K. Sheehan, D. Knight, P. S. Richardson, D. J. Thornton, Heterogeneity of

677   airways mucus: variations in the amounts and glycoforms of the major oligomeric mucins

678   MUC5AC and MUC5B. *Biochem. J.* **361**, 537-546 (2002).

679   61. N. G. Carroll, S. Mutavdzic, A. L. James, Increased mast cells and neutrophils in

680   submucosal mucous glands and mucus plugging in patients with asthma. *Thorax* **57(8)**,

681   677-682 (2002).

682   62. P. Bradding, Mast cell regulation of airway smooth muscle function in asthma. *Eur.*

683   *Respir. Journal* **29(5)**, 827-830 (2007).

684   63. S. Oddera, M. Silvestri, A. Balbo, B. O. Jovovich, R. Penna, E. Crimi, G. A. Rossi, Airway

685   eosinophilic inflammation, epithelial damage, and bronchial hyperresponsiveness in

686   patients with mild-moderate, stable asthma. *Allergy* **51(2)**, 100-107 (1996).

687   64. S.Y. Eum, S. Haile, J. Lefort, M. Huerre, B. B. Vargaftig, Eosinophil recruitment into the

688   respiratory epithelium following antigenic challenge in hyper-IgE: Mice is accompanied

689   by interleukin 5-dependent bronchial hyperresponsiveness. *Proc. Natl. Acad. Sci. U. S. A.*

690   **92(26)**, 12290-12294 (1995).

691   65. S. Adra, T. Sun, S. MacNeil, M. Holcombe, R. Smallwood, Development of a Three

692   Dimensional Multiscale Computational Model of the Human Epidermis. *PLoS One* **5(1)**,

693   e8511 (2010).

694   66. H. Kaul, Y. Ventikos, Dynamic reciprocity revisited. *J. Theor. Biol*. **370**, 205-208 (2015).

695   67. E. Shebani, S. Shahana, C. Janson, G. M. Roomans, BHR group, Attachment of columnar

696   airway epithelial cells in asthma. *Tissue & Cell* **37(2)**, 145-152 (2005).

697   68. E. M. Levina, M. A. Kharitonova, Y. A. Rovensky, J. M. Vasiliev, Cytoskeletal control of

698   fibroblast length: experiments with linear strips of substrate. *J. Cell Sci.* **114(23)**, 4335-

699   4341 (2001).

700   69. C. Twort, C. Vanbreemen, Human airway smooth-muscle in culture. *Tissue & Cell* **20(3)**,

701   339-344 (1988).

702   70. E. S. Schulman, A. Kagey-Sobotka, D. W. MacGlashan Jr., N. F. Adkinson Jr., S. P. Peters,

703   R. P. Schleimer, L. M. Lichtenstein, Heterogeneity of human mast-cells. *J. Immunol.*

704   **131(4)**, 1936-1941 (1983).

Author contributions

All authors contributed to the study concept and overall study design and read, edited and approved the final manuscript. RS designed and conducted the in vitro experiments, analysed data and wrote the draft manuscript. LC, RB, DK, AJS and MSB contributed to the design and undertaking of experiments, analysed data and contributed to figures. HK participated in computational modeling study design, developed the various agent-based models, conducted the computational modeling, analysed the data and wrote the draft manuscript. RHS, CEB, SS and BSB helped conceive the computational modeling. CEB, IDP, SS, AJW, RAK, RB, SG,

730 AS and MB contributed to the design and delivery of the clinical trials, recruitment and

731 characterisation of patients. CEB conceived the study, participated in experimental design and

732 wrote the draft manuscript.

733

734 **Conflicts of interest**

735 CEB serves on advisory boards for GlaxoSmithKline, AstraZeneca, Boehringer Ingelheim,

736 Cheisi, Roche, receives honoraria from Novartis, and receives research support from

737 GlaxoSmithKline, AstraZeneca, Chiesi, Novartis, Boehringer Ingelheim and Roche. AJW

738 serves on Advisory Boards for GSK, Astra Zeneca, Pulmocide, KNOPP Pharmaceuticals and

739 Anaxsys. In the last 5 years IDP has received speaker's honoraria for speaking at sponsored

740 meetings from Astra Zeneca, Boehringer Inglehiem, Aerocrine, Almirall, Novartis, Teva and

741 GSK and a payment for organising an educational event from AZ. IDP has received honoraria

742 for attending advisory panels with Almirall, Genentech, Regeneron, Astra Zeneca, Boehringer

743 Ingelheim, GSK, MSD, Schering-Plough, Novartis, Dey, Napp, Teva, Merck and Respivert.

744 IDP has received sponsorship to attend international scientific meetings from Boehringer

745 Ingelheim, GSK, Astra Zeneca, Teva and Napp. SG has received support to attend scientific

746 conferences from GSK and Chiesi. At the time of this study RAK was an employee of Novartis

747 Pharmaceuticals AG. SS has performed advisory services for Mundipharma, GSK, Astra

748 Zeneca, Roche, Boehringer Ingelheim & Owlstone Medical.

749

750 **Data and materials availability**

751 The agent-based model is available from figshare, doi: 10.25392/leicester.data.7610933 for

752 research purposes under the Creative Commons Attribution Non-Commercial 4.0 International

753 (CC BY-NC 4.0) license. Fevipiprant is in phase 3 trials and until licensed is not available to

754 other researchers to undertake clinical trials without permission from Novartis. Fevipiprant was

755   provided to the University of Leicester under a Material Transfer Agreement and is also

756   available from commercial suppliers. The gene array data have been deposited in the

757   ArrayExpress database at EMBL-EBI (www.ebi.ac.uk/arrayexpress) under accession number

758   E-MTAB-7346. All data are present in the main text or in the Supplementary Materials.

Category	Activity	Rules	Parameters	Baseline Value	Comments
Epithelial	Migration	Dedifferentiated epithelial cells migrate to repair the damaged epithelium, then undergo proliferation.	Migration rate	5 µm/h	Migration rate was consistent with in vitro studies (16-18).
			Migration delay	5h	Dedifferentiated cells migrate after 5h; doubled to 10 h to represent slowly recovering epithelium.
	Differentiation	Cells at the edge dedifferentiate into a flattened phenotype.	Dedifferentiation probability	50%	A 50% probability per iteration was applied to determine whether an epithelial cell dedifferentiates. This was altered to 5% per iteration for the defective epithelium.
			Delay in proliferation	2-8 h	Edge cells require 2-8h to flatten out (16); this was increased by 3X (6–24h) for slow epithelial recovery.
	Proliferation	Dedifferentiated epithelial cells proliferate following their migration to the opposite edge of a compromised epithelium.	Ciliated:**Goblet** ratio	0.7:**0.3** & 0.1:**0.9**	The number of goblet cells increase by 2–3X in severe asthma (19-22) given the inflammation status in disease. The probability of differentiating to a ciliated: goblet cell fate was either 0.7:**0.3** (less inflamed) or 0.1:**0.9** (more inflamed).
	Apoptosis	This feature was **not** included in the baseline model.	Auto-denudation	N/A	No apoptosis rate was applied to the baseline model. In 'mild' shedding every 25 h cells underwent apoptosis with 50% probability; 'moderate' apoptosis with the probability of 1% at **each** iteration; 'extreme' 10% at **each** iteration.
Inflammatory	Cell migration and activation	Inflammatory cells migrate into the airway in response to epithelial denudation, and their activation promotes eosinophil recruitment.	Frequency of recruitment	120 h	Inflammatory cells were added every 120h to the model with recruitment frequency increased to 60h and 50h in disease.
			Inflammatory cells recruited	30	Inflammatory cell number was chosen to reflect previous reports (11) and, in disease, this was increased by 1.5-2X (23).
			Eosinophil recruitment	45	Eosinophil recruitment was triggered by increased inflammatory cell number; 45, based on previous reports (11) and in disease this threshold was halved.
	Degranulation	Inflammatory cell activation promotes cell recruitment and fibroblast differentiation	Inflammatory cytokine release	25/ 75/ 105 h	The 'universal' inflammatory cell was assigned 150h life; releasing cytokines for 25h (or 75 and 105h for epithelial integrity of <55% and 30% respectively). In disease, inflammatory cell life was reduced to 65, 40, and 25 h (releasing cytokines for 85, 110, and 125h, respectively).
	Apoptosis	Inflammatory cells promote eosinophil survival	Eosinophil life in the airway	10/ 30/ 45h	Eosinophils survival is ~10h within a normal airway (24). This was increased to 30 and 45h for epithelial integrity of <55% and 30% respectively. In disease, the life of eosinophils was doubled to 20, 60, and 90 h.
Mesenchymal	Differentiation	Inflammatory cells promote increased ASM mass	ASM activation	25 cells	The threshold number of inflammatory cells above which myofibroblast-ASM differentiation is induced (11) which was reduced by 50% in disease.
	Differentiation	Inflammatory cells promote fibroblast-myofibroblast differentiation	(Myo)fibroblast differentiation probability	30%	Increased fibroblast differentiation or myofibroblast recruitment in the presence of activated inflammatory cells increased to 50% in disease.
	Proliferation	Fibroblast proliferation increased by inflammatory and epithelial cells	Fibroblast growth rate	132 iterations	Animal models indicate that lung fibroblasts divide every 5.5 days (132 iterations in model), which reduces to 2 days (48 iterations in model) in the animal model of asthma (25).
	Apoptosis	Myofibroblast survival supported by activated inflammatory cells	Myofibroblast apoptosis	5 cells	The threshold number of inflammatory cells below which myofibroblast apoptosis was induced, which was reduced to 2 in disease.

759

760 **Table 1. Agents, Rules and Model.** The computational airway model rule-set, parameters, and

761 which parameters were altered to observe airway remodeling.

Figure legends

Fig. 1. Increased ASM mass in asthma is reduced by fevipiprant. A. Representative photomicrograph of a bronchial biopsy from a participant with severe asthma in the fevipiprant (DP_2 antagonist) trial, showing increased ASM (brown stained α-SMA), disrupted epithelium and lamina propria. **B**. ASM mass, as measured by percentage α-SMA positive area, in bronchial biopsies from asthmatic subjects pre- and 12 weeks post-treatment with fevipiprant (n=14) or placebo (n=13). A two-tailed paired t-test was used for within group comparisons (p=0.022 and p=0.522), and a two-tailed unpaired t-test was used to compare the difference in ASM mass observed following treatment with fevipiprant to that seen in the placebo group (p=0.034).

Fig. 2. Computational model-based investigation of interactions between airway inflammation and ASM mass. A. The mean time course from 6 simulations of the response to epithelial injury (50% denudation at time zero) over 180 days showing increased eosinophil numbers, ASM mass and persistent epithelial damage in the model of airway remodelling in asthma versus resolution of the epithelial injury, eosinophil numbers and persistently low ASM mass in the healthy control model (p<0.01 for comparisons of each parameter over time between the patient model versus healthy control model, two-tailed unpaired t-tests). **B.** Predicted reduction in eosinophil number over 180 days following reduction in eosinophil recruitment or increase in eosinophil apoptosis in the computational model (n=5 simulations). **C.** Relative change in the ASM mass percentage at 180 days, predicted as a consequence of results in panel B (n=5 simulations).

Fig. 3. ASM cells express functional DP$_2$. A. Representative photomicrograph of DP$_2$ staining in bronchial biopsies from a subject with severe asthma (inset: isotype control). **B.** Quantitative PCR cycle threshold values for expression of ASM DP$_2$ mRNA vs the 18S ribosomal RNA housekeeping gene *RNA18S5*, mean DP2 Ct (threshold cycle) [95% CI]: 27.9 [26.1 - 29.8], n=7. **C.** Example histogram of DP$_2$ expression (black trace) in ASM cells by flow cytometry versus isotype control antibody (grey trace); fold increase in geometric mean fluorescence intensity (GMFI) of anti-DP$_2$ antibody/isotype control antibody [95% CI]: 1.3 [1.2 - 1.4], n=15 donors, p<0.001, two-tailed paired t-test against isotype control. **D.** Representative photomicrographs (x20 magnification) showing ASM α-SMA expression (green, left hand panel;isotype control antibody, inset) and ASM DP$_2$ expression (red, right hand panel; isotype control antibody, inset) by immunofluorescence staining; nuclei are stained with 4',6-diamidino-2-phenylindole (DAPI, blue). **E.** F-actin polymerisation in primary human ASM cells (n=9 donors) in response to DK-PGD$_2$ treatment or Dulbecco's Modified Eagle's Medium (DMEM) containing 50% FBS as a positive control; geometric mean AUC DK-PGD$_2$ DR [95% CI]: 46 [25 – 104] x 10^2; p=0.01, one sample t-test against a hypothetical value of zero. **F.** Intracellular calcium (Ca$^{2+}_i$) elevation in primary human ASM cells (n=6-9 donors) in response to DK-PGD$_2$ treatment or ionomycin (1.5 µg/ml) as a positive control; geometric mean AUC DK-PGD$_2$ DR [95% CI]: 130 [78 – 230] x 103; p=0.002, one sample t-test against a hypothetical value of zero. Data are plotted as mean ± sem. Two-tailed paired t-tests were performed to compare each condition with its vehicle control; * p < 0.05, except FBS where Wilcoxon matched pairs sign ranked test was used, denoted by ^ p < 0.05.

Fig. 4. A DP$_2$ antagonist reduces ASM migration and recruitment of myofibroblasts and fibrocytes. All experiments were carried out in serum-free media. **A.** Shown are data for the wound closure after 24h of ASM cells that had been grown in monolayers and then wounded

by scratching with a pipette tip followed by incubation with different treatments for 24h: DP_2 agonist $DK-PGD_2$ (n=4-5 donors), the DP_2 antagonists fevipiprant (n=8 donors), CAY10471 (n=6-8 donors) and OC000459 (n=7 donors), or DMEM culture medium containing 10% FBS as a positive control. Two-tailed paired t-tests were performed to compare each condition with its vehicle control; * $p<0.05$ vs vehicle control. Data are expressed as mean ± sem. **B.** Representative photographs of ASM monolayers wounded by scratching with a pipette tip after 24h (vehicle control for fevipiprant, 500 nM fevipiprant, DMEM containing 10% FBS control (upper panel); vehicle control for CAY10471 and OC000459, 100 nM CAY10471 and 100 nM OC000459 (lower panel), black lines represent the wound edge at 0h, scale bar = 250 µm. **C.** PGD_2 release by unwounded and wounded ASM cells after 24h; p=0.02, Wilcoxon matched pairs signed rank test, n=10 donors. Data are expressed as mean ± sem. **D.** Representative flow cytometry traces of isotype control antibodies (grey traces) versus α-SMA expression (left hand panel, black trace, mean percentage fibrocyte population positive for α-SMA expression [95% CI]; 97 [93 – 100] %, n=4 donors) and DP_2 expression (right hand panel, black trace, GMFI fold difference DP_2 antibody/isotype control antibody [95% CI]; 1.6 [1.3 – 2]; p=0.0064, two-tailed paired t-test against isotype control antibody, n=6 donors) by fibrocytes. **E.** Correlation between change in myofibroblast number in the lamina propria and absolute change in ASM mass as a percentage of the total biopsy area (fevipiprant: black circle, n=14, placebo: black triangle, n=13), Spearman r [95% CI]: r=0.347 [-0.050-0.649]; p=0.076. **F.** Correlation between change in fibrocyte number in the lamina propria and absolute change in ASM mass as a percentage of the total biopsy area (fevipiprant: black circle, n=12, placebo: black triangle, n=13), Spearman r [95% CI]: r=0.538 [0.169-0.774]; p=0.006. **G.** Predicted relative reduction in percent ASM mass at 180 days in the computational model as a consequence of reduced myofibroblast recruitment (30-50%) in combination with a 40% reduction in eosinophil recruitment in the computational model (n=5 simulations).