Supplementary Appendix

Kole TM, *et al. BMJ Open Respir Res* 2024; 11:e002316. doi: 10.1136/bmjresp-2024-002316

Supplementary Appendix

Inhoud

1. Eligibility Criteria

1.1 Inclusion Criteria

- Male or female patients aged ≥ 18 and ≤ 65 years, who have signed an Informed Consent form prior to initiation of any study-related procedure.
- Clinical diagnosis of asthma for at least 6 months confirmed by a chest physician according to international guidelines (GINA 2012) supported by objective evidence of any of the following at the baseline visit or in the previous 5 years:
    - Positive response to methacholine challenge test [PC20 < 8 mg/mL or PD20 < 0.7 mg for those subjects not using inhaled corticosteroids (ICS), and PC20 < 16 mg/mL or PD20 < 1.4 mg for subjects using ICS];
    - Positive response to a reversibility test, defined as ΔFEV_1 ≥ 12% and ≥ 200 mL over baseline FEV_1, within 30 minutes after administration of 400 µg of salbutamol pMDI administered with or without Spacer;
    - Peak Flow variability (i.e. highest - lowest PEF over the day/mean value of the two, × 100) > 20%, measured over a follow-up period of 7 days;
    - Documented response (defined as ΔFEV_1 ≥ 12% and ≥ 200 mL) after a cycle (e.g., 4 weeks) of regular maintenance anti-asthma treatment.
- Patients with stable asthma, on any previous regular asthma treatment ("rescue" β2-agonists alone included) at a stable dose, for at least 8 weeks prior to baseline visit.
- Current smoker, ex-smoker (since the past 12 months) or lifelong non-smoker (total lifetime smoking history < 10 packyears defined as [(number of cigarettes smoked per day)x(number of years of smoking)] / 20).

1.2 Exclusion Criteria

- Cigarette smoking > 10 packyears defined as [(number of cigarettes smoked per day) x (number of years of smoking)] / 20.
- Diagnosis of COPD confirmed by a chest physician.
- Asthma exacerbation in the 8 weeks prior to baseline visit (defined as a significant deterioration of asthma and signalled by any or more of the following: need for a systemic corticosteroid course (≥ 3 days); hospitalisation for asthma; emergency room attendance for asthma).
- Clinical or functional uncontrolled respiratory, haematological, immunologic, renal, neurologic, hepatic, endocrinal or other disease, or any condition that might, in the judgment of the investigator, compromise the results or interpretation of the study.
- Pregnant or lactating women (a urine pregnancy test will be performed).
- Participation in an interventional clinical trial with intake of the last dose of any investigational drug <12 weeks preceding baseline visit (last dose < 5 half-lives prior to baseline visit for biologics).
- Inability to comply with study procedures.
- Alcohol or drug abuse.

Kole TM, *et al. BMJ Open Respir Res* 2024; 11:e002316. doi: 10.1136/bmjresp-2024-002316

1.3 IRB or institutions that approved the protocol

The following review boards or institutions approved the protocol; Comitato Etico dell'Azienda Ospedaliera V. Cervello di Palermo (Palermo 2), Comitato Etico della Provincia di Ferrara, Comitato Etico dell'Azienda Ospedaliero-Universitaria di Parma, Comitato Etico di Area Vasta Nord-Ovest per la sperimentazione clinica, Comitato Etico per la Sperimentazione Clinica delle Province di Verona e Rovigo, Comitato Etico dell'Azienda Ospedaliera dei Colli, Comitato Etico della Fondazione Salvatore Maugeri, Comitato Etico dell'A.O.U Ospedali Riuniti di Foggia, NHS Health Research Authority NRES Committee North West - Greater Manchester South (for all UK sites), UMCG Medical Ethical Review Committee, Foreest Medical School, Jeroen Bosch Ziekenhuis, Martini Ziekenhuis, Comité Ético de Investigación Clínica, Ethikkommission bei der Ärztekammer Schleswig-Holstein, Ethik-Kommission der Medizinischen Hochschule Hannover, Ethik-Kommission bei der Sächsischen Landesärztekammer, Institutional review board for human investigation (Cleveland, OH), Western Institutional Review Board, Institutional Review Board for Baylor College of Medicine and Affiliated Hospitals, University of Arizona Institutional Review Board, Biomedical Research Ethics Board of the McGill University Health Center, Comitê de Ética em Pesquisa com Seres Humanos da Universidade Federal de Santa Catarina, Comissão de Ética para Análise de Projetos de Pesquisa – CAPPesq do HCFMUSP, Coordinator EC - Comitê de Ética em Pesquisa da Fundação ABC – FMABC, Medical Ethics Committee of the First Affiliated Hospital of Guangzhou Medical University.

2. Study Design

2.1 Measurements at each visit

	Visit 1 T=0 months		**TC 1 T=3 months**	**Visit 2 T=6 months**	**TC 2 T=9 months**	**Visit 3 T=12 months**
	1a	1b				
Written informed consent	X					
Review in-/exclusion criteria	X	X				
Medical history	X					
Concomitant medication	X		X	X	X	X
Exacerbations	X		X	X	X	X
Physical examination	X			X		X
Urine pregnancy test	X					
Blood sample	X			X		X
Phadiatop test	X					
Asthma Control Test	X		X	X	X	X
Impulse Oscillometry		X		X		X
Fraction of exhaled NO	X			X		X
Multiple Breath Nitrogen Washout		X		X		X
Body plethysmography		X		X		X
Spirometry		X		X		X
Sputum induction*		X				
Nasal brushing	X			X		
Methacholine challenge test	X					
Asthma Control Questionnaire	X		X	X	X	X
Asthma Quality of Life Questionnaire	X		X	X	X	X
Computed tomography scan*		X				

Table 2.1: Measurements at each visit. Abbreviations: NO: Nitric oxide. * Sputum induction and computed tomography were performed in a subset of patients depending on the availability of equipment in participating centers.

Table S1. Z-scores for spirometry

	Female asthma patients	Male asthma patients	P-value
N	450	323	
Prebronchodilator FEV_1 Z-score (mean (SD))	-1.36 (1.31)	-1.52 (1.37)	0.084
Postbronchodilator FEV_1 Z-score (mean (SD))	-0.73 (1.22)	-0.84 (1.28)	0.214
Postbronchodilator FVC Z-score (mean (SD))	-0.23 (1.15)	0.01 (1.13)	0.004
Prebronchodilator FEV_1/FVC Z-score (mean (SD))	-1.51 (1.25)	-1.92 (1.29)	<0.001
Postbronchodilator FEV_1/FVC Z-score (mean (SD))	-0.89 (1.27)	-1.30 (1.33)	<0.001

Table S1: Univariable analyses of spirometry Z-scores at baseline of asthma subjects in ATLANTIS, stratified for sex. FEV_1: Forced expiratory volume in 1 second, FVC: Forced vital capacity.

Table S2. Exacerbations during 1 year follow-up per GINA step by sex

	Female patients			Male patients		
Exacerbations/year	**0**	**1**	**2+**	**0**	**1**	**2+**
GINA step 1 (%)	92,8	7,2	0,0	93,5	4,8	1,6
GINA step 2 (%)	87,2	10,3	2,6	97,6	2,4	0,0
GINA step 3 (%)	87,0	9,6	3,5	83,9	11,5	4,6
GINA step 4 (%)	69,9	19,1	10,9	80,9	10,0	9,1
GINA step 5 (%)	43,8	21,9	34,4	61,5	0,0	38,5

Table S3. Medication use related to FEV_1 stratified for sex

	Quartile 1		**Quartile 2**		**Quartile 3**		**Quartile 4**		**p-value for logistic regression**
	Female	Male	Female	Male	Female	Male	Female	Male	
n	109	82	113	78	104	87	117	73	-
FEV_1 % predicted (median [IQR])	70.83 [66.07, 75.02]	70.21 [59.59, 74.18]	85.14 [82.58, 88.29]	85.22 [81.74, 88.06]	96.39 [93.72, 98.04]	95.94 [93.53, 99.14]	107.12 [104.58, 112.98]	107.68 [104.90, 111.71]	-
ICS use, n (%)	100 (91.7)	75 (91.5)	93 (82.3)	61 (78.2)	86 (82.7)	69 (79.3)	85 (72.6)	54 (74.0)	0.369
LABA use, n (%)	90 (82.6)	68 (82.9)	79 (69.9)	51 (65.4)	67 (64.4)	57 (65.5)	72 (61.5)	34 (46.6)	0.360
LAMA use, n (%)	10 (9.2)	8 (9.8)	4 (3.5)	1 (1.3)	1 (1.0)	0 (0.0)	4 (3.4)	0 (0.0)	0.087
Montelukast use, n (%)	30 (27.5)	21 (25.6)	20 (17.7)	9 (11.5)	16 (15.4)	12 (13.8)	30 (25.6)	5 (6.8)	0.007
Biological use, n (%)	8 (7.3)	7 (8.5)	5 (4.4)	3 (3.8)	2 (1.9)	1 (1.1)	5 (4.3)	0 (0.0)	0.117
Systemic corticosteroid use, n (%)	8 (7.3)	4 (4.9)	3 (2.7)	1 (1.3)	3 (2.9)	1 (1.1)	2 (1.7)	0 (0.0)	0.805

Table S2. Medication use in quartiles for (post-bronchodilator) FEV_1 % predicted stratified for sex. The presented p-values are derived from a logistic regression analysis for medication use, i.e. ICS use, LABA use etc (dependent variable), with as covariates: sex, FEV_1% predicted , FEV_1% predicted sex (interaction term). The presented p-value is that for the interaction term. FEV_1: forced expiratory volume in 1 second, GINA: Global Initiative for Asthma, ICS: inhaled corticosteroids, LABA: long-acting beta2 agonist, LAMA: long-acting muscarinic antagonists*

Table S4. Medication use in quartiles for ACQ-6 stratified for sex

	Quartile 1		**Quartile 2**		**Quartile 3**		**Quartile 4**		**p-value for logistic regression**
	Female	Male	Female	Male	Female	Male	Female	Male	
n	92	101	112	81	111	82	135	58	-
ACQ-6, median [IQR]	0.00 [0.00, 0.16]	0.00 [0.00, 0.16]	0.50 [0.33, 0.66]	0.50 [0.33, 0.66]	1.16 [1.00, 1.33]	1.13 [1.00, 1.20]	2.16 [1.83, 2.83]	2.33 [2.00, 3.27]	-
ICS use, n (%)	70 (76.1)	79 (78.2)	90 (80.4)	64 (79.0)	95 (85.6)	66 (80.5)	113 (83.7)	52 (89.7)	0.852
LABA use, n (%)	54 (58.7)	65 (64.4)	74 (66.1)	48 (59.3)	78 (70.3)	58 (70.7)	105 (77.8)	41 (70.7)	0.205
LAMA use, n (%)	1 (1.1)	1 (1.0)	2 (1.8)	3 (3.7)	8 (7.2)	1 (1.2)	9 (6.7)	4 (6.9)	0.640
Montelukast use, n (%)	12 (13.0)	11 (10.9)	19 (17.0)	11 (13.6)	22 (19.8)	15 (18.3)	44 (32.6)	10 (17.2)	0.311
Biological use, n (%)	3 (3.3)	3 (3.0)	4 (3.6)	1 (1.2)	4 (3.6)	5 (6.1)	9 (6.7)	3 (5.2)	0.651
Systemic corticosteroid use, n (%)	3 (3.3)	0 (0.0)	4 (3.6)	0 (0.0)	1 (0.9)	4 (4.9)	8 (5.9)	2 (3.4)	0.634

*Table S3. Medication use in quartiles for ACQ-6 stratified for sex. ACQ-6: Asthma Control Questionnaire, GINA: Global Initiative for Asthma, ICS: inhaled corticosteroids, LABA: long-acting beta2 agonist, LAMA: long-acting muscarinic antagonists. The presented p-values are derived from a logistic regression analysis for medication use, i.e. ICS use, LABA use etc (dependent variable), with as covariates: sex, ACQ6 score, ACQ-6 score * sex (interaction term). The presented p-value is that for the interaction term.*

Table S5. Medication use in categories of airway hyperresponsiveness stratified for sex

	Very mild - mild		**Moderate – severe**		**p-value for logistic regression**
	Female	Male	Female	Male	-
n	175	138	154	91	-
ICS use, n (%)	143 (81.7)	109 (79.0)	129 (83.8)	72 (79.1)	0.759
LABA use, n (%)	122 (69.7)	83 (60.1)	102 (66.2)	54 (59.3)	0.728
LAMA use, n (%)	5 (2.9)	2 (1.4)	5 (3.2)	2 (2.2)	0.807
Montelukast use, n (%)	33 (18.9)	15 (10.9)	26 (16.9)	11 (12.1)	0.619
Biological use, n (%)	8 (4.6)	0 (0.0)	4 (2.6)	4 (4.4)	0.990
Systemic corticosteroid use, n (%)	6 (3.4)	1 (0.7)	5 (3.2)	3 (3.3)	0.225

*Table S4. Medication use in categories of airway hyperresponsiveness stratified for sex. The p-value in the right hand column is derived from a logistic regression with medication use (i.e. ICS use, LABA use, LAMA use, etc) as a dependent variable and sex, moderate or severe airway hyperresponsiveness and sex*moderate or severe airway hyperresponsiveness (interaction term). The presented p-value is that of the interaction term. ICS: inhaled corticosteroids, LABA: long-acting beta2 agonist, LAMA: long-acting muscarinic antagonists.*

Table S6. Medication use in categories of exacerbations prior to inclusion stratified for sex

Exacerbations prior to inclusion	**0**		**1 or more**		**p-value for logistic regression**
	Female	Male	Female	Male	-
n	373	291	77	32	-
ICS use, n (%)	294 (78.8)	232 (79.7)	74 (96.1)	30 (93.8)	0.564
LABA use, n (%)	240 (64.3)	184 (63.2)	71 (92.2)	28 (87.5)	0.497
LAMA use, n (%)	12 (3.2)	6 (2.1)	8 (10.4)	3 (9.4)	0.694
Montelukast use, n (%)	70 (18.8)	38 (13.1)	27 (35.1)	9 (28.1)	0.831
Biological use, n (%)	16 (4.3)	10 (3.4)	4 (5.2)	2 (6.2)	0.664
Systemic corticosteroid use, n (%)	4 (1.1)	1 (0.3)	12 (15.6)	5 (15.6)	0.363

Table S5. Medication use in category based on exacerbations prior to inclusion stratified for sex. The p-value in the right hand column is derived from a logistic regression with medication use (i.e. ICS use, LABA use, LAMA use, etc) as a dependent variable and sex, exacerbation count ≥ 1 prior to inclusion and sex exacerbation count ≥ 1 prior to inclusion (interaction term). The presented p-value is that of the interaction term.GINA: Global Initiative for Asthma, ICS: inhaled corticosteroids, LABA: long-acting beta2 agonist, LAMA: long-acting muscarinic antagonists.*

Table S7. Medication use in categories of exacerbations during follow-up stratified for sex

Exacerbations during follow-up	**0**		**1 or more**		**p-value for logistic regression**
	Female	Male	Female	Male	-
n	340	269	98	45	-
ICS use, n (%)	271 (79.7)	214 (79.6)	90 (91.8)	41 (91.1)	0.901
LABA use, n (%)	222 (65.3)	171 (63.6)	83 (84.7)	37 (82.2)	0.838
LAMA use, n (%)	8 (2.4)	5 (1.9)	11 (11.2)	4 (8.9)	0.983
Montelukast use, n (%)	65 (19.1)	32 (11.9)	31 (31.6)	14 (31.1)	0.237
Biological use, n (%)	7 (2.1)	8 (3.0)	13 (13.3)	4 (8.9)	0.301
Systemic corticosteroid use, n (%)	7 (2.1)	2 (0.7)	9 (9.2)	3 (6.7)	0.520

Table S6. Medication use in categories of exacerbations during follow-up stratified for sex. The p-value in the right hand column is derived from a logistic regression with medication use (i.e. ICS use, LABA use, LAMA use, etc) as a dependent variable and sex, exacerbation count ≥ 1 during follow-up and sex exacerbation count ≥ 1 prior to inclusion (interaction term). The presented p-value is that of the interaction term. GINA: Global Initiative for Asthma, ICS: inhaled corticosteroids, LABA: long-acting beta2 agonist, LAMA: long-acting muscarinic antagonist*