The Wessex Severe Asthma Cohort Study

The Wessex Severe Asthma Cohort (WSAC) is an observational cross-sectional study providing detailed characterisation of severe asthma patients recruited from specialist severe asthma clinics at Portsmouth and Southampton Hospitals between April 2009 and January 2014. In addition to the severe asthma group two comparator groups including a healthy control group and a group with stable mild/moderate asthma were recruited and underwent the same detailed characterisation.

1.1 Study Participants

The eligibility criteria were designed to create an inclusive cohort representative of the patients seen in the specialist severe asthma clinics at the two sites. Participants were aged between 18 and 80 years with no restrictions according to gender, race or smoking status.

Eligibility for the severe asthma group required:

- A diagnosis of asthma confirmed by an asthma specialist in accordance with the BTS/SIGN guidelines 2009 with alternative causes for symptoms excluded and treatment for co-morbidities optimised and had been under follow up with a specialist for at least 6 months at the time of enrolment as recommended in the ERS severe asthma guidelines 1999.
- Features of poor disease control with persistent symptoms requiring regular short acting beta-agonist rescue medication and at least one severe exacerbation in the preceding year despite high-intensity maintenance asthma treatment.

Severe exacerbations defined as a worsening of asthma requiring systemic corticosteroids, or an increase in maintenance dose of systemic corticosteroids, for at least three days[1].
High-intensity maintenance asthma treatment included patients taking ≥1000µg/day BDP equivalent inhaled corticosteroid (or maintenance systemic corticosteroids) and a long acting beta-2 agonist or alternative controller medications (steps 4 and 5 of the BTS/SIGN Asthma Guideline treatment algorithm 2009[2]).

Participants eligible for the mild-moderate asthma group were taking ≤800 µg/day BDP equivalent inhaled corticosteroid (steps 1-3 of the BTS/SIGN Asthma Guideline treatment algorithm 2009) and had well controlled disease with no disease exacerbations requiring systemic corticosteroids over the preceding year. Participants eligible for the healthy control group had no current or historical symptoms suggestive of asthma and normal lung function. Patients with significant co-morbid disease other than asthma and those unable to comply with investigational procedures were excluded.

Potential participants with severe asthma who fulfilled the trial eligibility criteria were identified in the specialist severe asthma clinics and provided with a Patient Information Sheet. Those wishing to take part returned for a specific study appointment. Adverts and existing databases were used to identify participants for the healthy and milder asthma groups.

1.2 Data Collection

All participants underwent a detailed characterisation protocol (see Table 5) including a detailed asthma and medical history; disease control, quality of life, and comorbidity questionnaires; pulmonary physiology; allergy testing; exhaled nitric oxide testing; HRCT imaging of the chest; sputum induction, nasal lavage, blood, and urine samples for biological measures of inflammation and genomic testing. The characterisation procedures used are all standard, and established guidelines were followed. Study-specific Standard Operating Procedures (SOPs) were created for sputum induction, nasal lavage and sample processing to ensure consistency between the two recruiting sites.

All data was entered onto a paper Case Report Form (CRF) at the time of review and uploaded onto the secure WSAC database after completion of all study procedures.

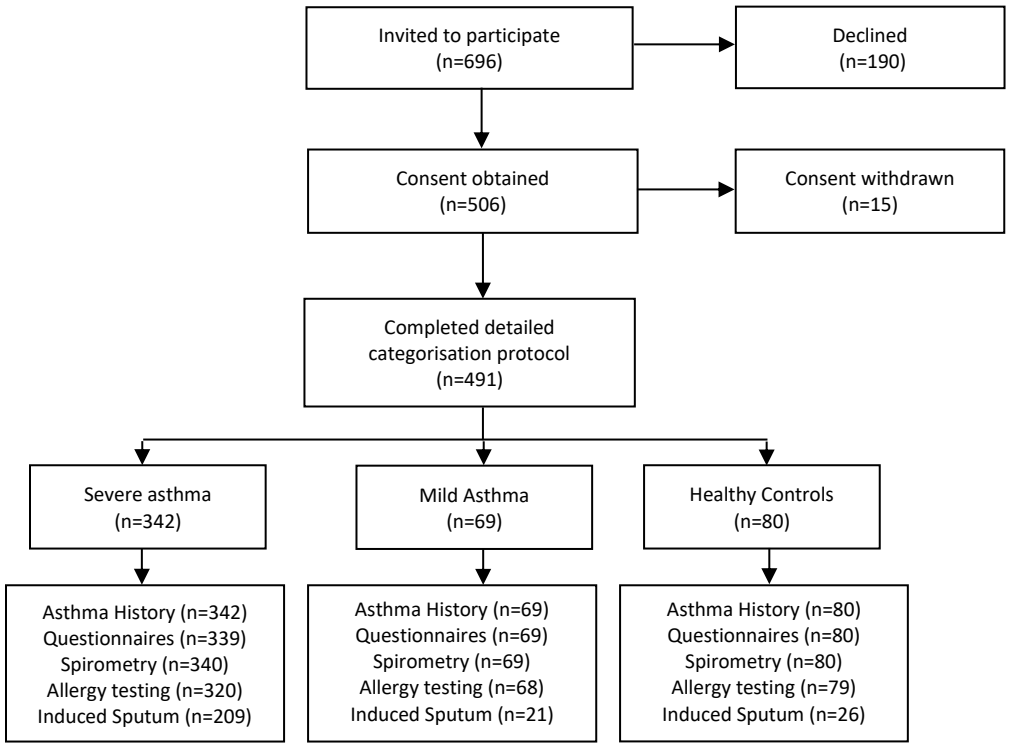
1.3 Confidentiality and Ethics

The study was conducted in accordance with the International Conference on Harmonisation Guidance for Good Clinical Practice and the clinical principles outline in the Declaration of Helsinki. Independent ethics committee approval was obtained (MREC No. 09/H0502/37), and all participants provided written informed consent. Unique identification codes were used to identify participants within the trial database as well as their biological samples. All participant data has been stored securely and is only accessible to study staff and authorised personnel. The study is funded by the UK Medical Research Council Patient Research Cohorts Initiative. The study was sponsored by University Hospital Southampton NHS Foundation Trust and was adopted onto the UKCRN Portfolio (http://public.ukcrn.org.uk/Search/StudyDetail.aspx?StudyID=7547).

2. Recruitment

491 participants were recruited and underwent the detailed characterisation protocol between April 2009 and January 2014. Details of recruitment are shown in Figure 1.

Figure 1: Wessex Severe Asthma Cohort Study Recruitment



Within the severe asthma group induced sputum was obtained from 61.1% of participants but in the milder and control cohorts this was lower at 30.4% and 32.5% respectively. This reflected participants being either unable to produce sputum (particularly in the healthy control population) or not being willing to have this procedure performed. However, sputum samples were obtained in a similar number of participants when compared to other published severe asthma cohorts (see Table 1).

Table 1: Sputum Induction in Severe Asthma Cohorts/Registries

	WSAC	**SARP**[3]	**UBIOPRED**[4]	**BSAR**[5]	**BIOAIR**[6]
Cohort size (n)	342	204	421	350	93
Successful sputum induction (%)	61.1	60.7	43.0	32.2	24.6

3. Study Procedures

3.1 Spirometry

Spirometry was performed using a portable spirometer (Vitalograph Alpha Touch®, Vitalograph Ltd, Buckingham, UK) in accordance with ATS/ERS guidelines[7] . The best forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) values, from three acceptable manoeuvres, were recorded and analysed according to the European Coal and Steel Community (ECSC) 1993 predicted values. Bronchodilator reversibility testing was performed 15 minutes after the administration of 2.5mg nebulised salbutamol with significant reversibility defined as a 200ml and 12% increase in FEV_1.

3.2 Single Breath Gas Transfer

Single Breath Gas Transfer measures were performed in accordance with ATS/ERS guidelines. Mean results of the transfer factor for carbon monoxide (TL_{CO}) and the transfer coefficient of the lung for carbon monoxide K_{CO} (not corrected for haemoglobin) were reported as a percent predicted using equations recommended by the ATS/ERS taskforce[8].

3.3 Impulse Oscillometry (IOS)

Impulse oscillometry (CareFusion MasterScreen™ IOS) was measured on a sub population of the cohort. A minimum of three tests of 30 seconds tidal breathing at Functional Residual capacity (FRC) were performed as per ERS taskforce recommendations[9]. Impulse oscillometry was performed before and after 2.5mg nebulised salbutamol. Mean oscillometric parameters were recorded and included but not limited to Z5, R5, R20, X5, R5-R20.

3.4 Exhaled Nitric Oxide (FeNO)

The fraction of exhaled nitric oxide (FeNO) was measured at the standard flow rate of 50ml/sec (NIOX MINO®, Aerocrine AB®, Solna, Sweden) in accordance with ATS/ERS guidelines[10]. FeNO was measured prior to other lung function tests and at least 2-hours after eating or drinking with the mean of at least two reproducible values recorded as parts per billion (ppb). In a sub population, FeNO was measured at higher flow rates of 100 and 200ml/sec (NIOX Flex®, Aerocrine AB®, Solna, Sweden) to calculate alveolar NO (CANO) and bronchial NO flux (JawNO) using the linear NO model.

3.5 Nasal Nitric Oxide (nNO)

Nasal nitric oxide measures (NIOX Flex®, Aerocrine AB®, Solna, Sweden) were performed in a sub population. A minimum of two reproducible values (ppb) were obtained from each nostril using the breath hold manoeuvre and the mean value from each nostril was reported.

4. Sample collection

All biological samples were obtained under consistent conditions with standardised processing procedures followed by the cohort characterisation team.

4.1 Sputum Induction

Sputum was induced using a DeVilbiss® Ultraneb (DeVilbiss, NY, USA) following a standardised protocol based on the methods described by ten Brinke et al[11]. Patients were bronchodilated with short acting beta-agonist (SABA) medication prior to sputum induction and lung function (FEV_1) was measured after each 5 minute nebulisation (4.5% saline) to check if a 20% drop from post bronchodilator FEV_1 had been reached at which point the induction would be stopped. For severe asthmatics at risk of bronchoconstriction, clinical judgement determined if nebulisation protocol should begin with 0.9% saline followed by 3% and finally 4.5% if tolerated. Lung function (FEV_1) was measured after each 5 minute nebulisation and after 2 minutes of nebulisation if the subject's FEV_1<1.5L. After a maximum of 20 minutes total nebulisation time for stable subjects, 15 minutes for at risk subjects or when an adequate sample was obtained, the procedure was stopped. Samples were stored on ice

during collection and transport to the laboratory for processing. Sputum samples were processed as soon as possible and within 2 hours of expectoration.

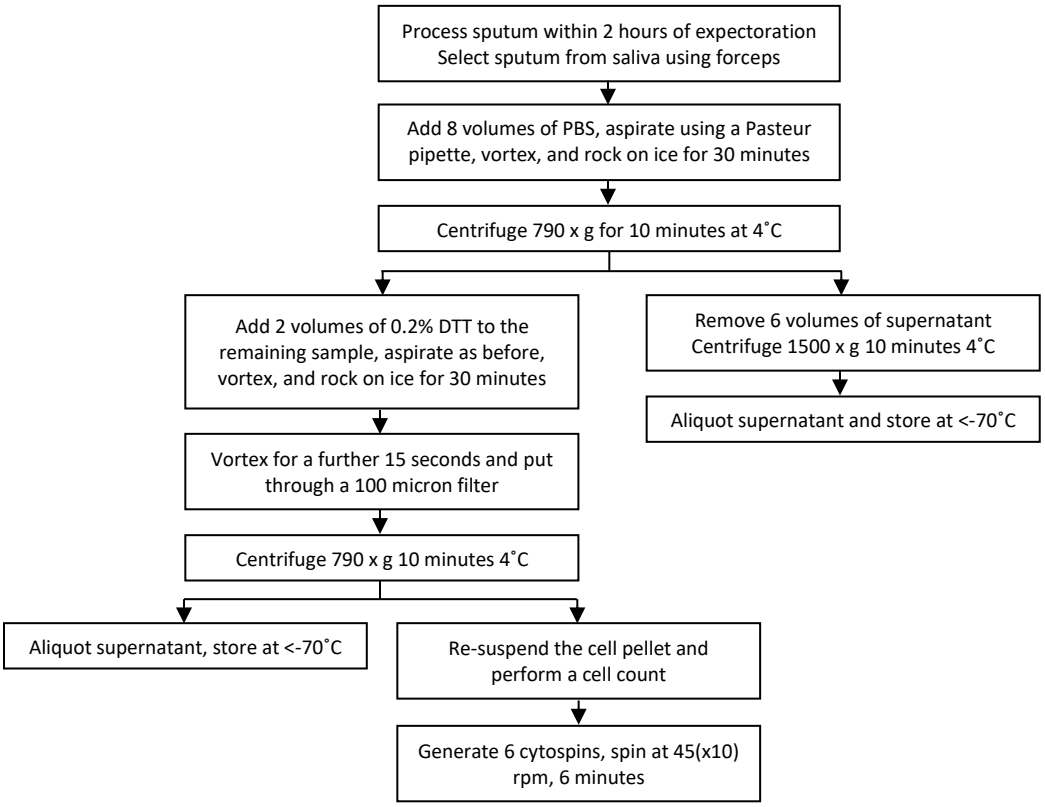
4.2 Nasal Lavage

Subjects were seated in a forward-flexed position and for each nostril, 2.5ml of 0.9% saline (warmed to 37%) was passed slowly into the nasal cavity using a 10ml syringe and then left to dwell for 10 seconds. The saline was withdrawn into the syringe and collected in a sterile tube kept cool on ice. This process was repeated twice. The sample was then passed through a 100µm nylon mesh filter before centrifuging at 4◦C for 10 minutes at 790g. The supernatant was stored at <-70°C.

5. Sample Processing

- Serum samples were coagulated for 30-60 minutes, centrifuged for 15 minutes at 1500g at 4°C, the serum layer removed and stored at <-70°C until analysis.
- Whole blood was stored at <-70°C and a simple salting procedure was performed for extracting DNA[12].
- The concurrent method of sputum processing was performed providing PBS and DTE supernatant for analysis[13]. Sputum samples were processed as soon as possible and within 2 hours of expectoration with 8x volume of phosphate buffered saline (PBS) and a proportion of supernatant was then removed and the sample was further incubated with 0.2% dithioerythritol (DTE) giving a final concentration of 0.1% DTE using the process described in Figure 2. Cytospins were stained using by rapid Romanowski staining (Fisher Scientific, Loughborough, UK). The proportion of inflammatory cells were assessed by counting 400 respiratory cells + squamous to give a mean percentage of respiratory cells and an independent mean percentage of squamous cells counted to indicate the level of salivary contamination.

Figure 2: Sputum Processing



- Nasal lavage was stored on ice and processed as soon as possible and within 30 minutes of collection. A small portion was removed for bacteriology analysis and the remaining sample was filtered through a 100µm cell strainer. Samples were then centrifuged at 790 x G and stored at <-70°C until further analysis.
- Urine was aliquotted and stored as soon as possible at <-70°C until further analysis. A small portion was analysed immediately for urinary cotinine.
- Inflammatory mediators were measured by:
    - Enzyme-linked immunosorbent assays (ELISA) – myeloperoxidase, elastase (Hycult® Biotech, Uden, The Netherlands), eosinophil cationic protein (MBL® International, MA, USA), interleukin (IL)-5 (Abnova®, Taiwan), IL-6, high-sensitivity-CRP (Biomerica®, CA, USA), ENA-78, eotaxin, FGF, osteopontin, ST2/IL-1 R4, VEGF,YKL-40 (Quidel Corporation, CA, USA), periostin (K Izuhara), tryptase, and α2-macroglobulin (A Walls)
    - Fluoroenzyme immunoassay – total IgE (ImmunoCAP, Phadia®, Uppsala, Sweden)
    - Cytokine bead array (Luminex®, R&D Systems, Oxford, UK): matrix metalloproteases (MMPs), metallopeptidase inhibitor 1 (TIMP-1), G-CSF, GM-CSF, Gro-α, CCL1, ICAM-1, IFN-γ, IL-1 α, IL-1 β, IL-1RA, IL-2, IL-4, IL-8, IL-10, IL-12, IL-13, IL-17, MCP-1, MIP-1 α, MIP-1 β, TNF-α (R&D Systems, Abingdon, Oxford, UK)
- Urinary cotinine was measured by lateral flow chromatographic immunoassay NicoScreen® One Step Cotinine Test Device (Modern Health Systems Ltd, Shipley, UK).

Table 2: Characteristics of the Wessex Severe Asthma Cohort

		Severe Asthma	Mild-Mod Asthma	Healthy Controls
Number (n)		342	69	80
Patient Demographics:				
Age (y)		48.2±13.8	38.9±12.6	37.2±12.8
Female (%)		67.5%	58%	63.8%
BMI (Kg/m^2); BMI>30 (%)		29.7 (25.6-35.6); 48.2%	27.8 (25.2-33.3); 43.5%	23.7 (21.9-26.9); 13.8%
Smoking status:				
Never smoker (%)		55%	68%	66.3%
Ex-smoker (%); Pack-years (y)		39.2%; 10 (4.4-22.5)	29%; 2.5 (1.5 -8)	25%; 2 (1-6)
Current smoker (%); Pack-years (y)		5.8%; 20 (8.4 -35)	3%; 1 (1-1)	8.8%; 5 (2.5 -7.2)
Asthma Characteristics:				
Age at asthma onset (y); onset ≤12 years (%)		22±19; 40.4%	18±14; 50.7%	-
Asthma duration (y)		26±17	21±14	-
Family history of asthma or allergy (%)		43.9%	73.9%	50%
Asthma Treatment:				
ICS dose (BDP equivalent µg/day)		2369±1149	278±302	-
LABA/LTRA/LAMA/Theophylline (%)		95.8%/67.6%/31.3%/26.2%	29%/1.4%/0%/0%	-
Maintenance OCS (%); Prednisolone equivalent dose (mg)		34.2%; 15.9±12	0%; 0	0%; 0
BTS Step 1/2/3[2] (%)		0%/0%/0%	46.4%/26.1%/27.5%	-
BTS Step 4/5 (%)		65.1%/34.9%	0%/0%	-
Omalizumab/Long-term macrolide/Antifungal		2.9%/11%/3%	0%/1.4%/0%	0%/1.3%/0%
Asthma Control:				
Rescue OCS courses in previous year		2 (1-4)	0 (0-0)	-
≥1 Hospital admission in previous year		50.3%	0%	-
ACQ 6; ACQ6 >1.5 (%)		2.74±1.24; 82.5%	0.85±0.73; 14.5%	0.01± 0.04; 0%
Previous intensive care admission for asthma (%)		18.1%	0%	-
Co-morbidities:				
Gastro-oesophageal reflux disease (%); PPI use (%)		48.5%; 45.5%	8.7%; 5.8%	10%; 6.3%
Rhinosinusitis (%); Rhinosinusitis treatment[∞] (%)		72.5%; 72.8%	31.9%; 4.3%	5%; 3.8%
Nasal polyps (%)		14%	1.4%	2.5%
Aspirin sensitivity (%)		27.7%	5.9%	1.3%
SNOT-20 score		35.5±19.4	15.5±11.3	7.2±8.2
HADS anxiety/depression		7.3±5.2/8.6±4.3	3.4±3.8/3.1±3.5	2.7±2.6/2.7±2.5
Antidepressant use (%)		20.2%	7.2%	2.5%
Diabetes/OSA/Osteoporosis (%)		7.6%/2.9%/9.6%	2.9%/1.4%/0%	2.5%/0%/0%
Asthma and Generic Quality of Life Questionnaires:				
AQLQ		4.09 ± 1.26	6.15 ± 0.76	6.96 ± 0.1
Short Form-36 Health Survey		47.9 ± 20.4	81.0 ± 13.5	86.2 ± 9.7
Physiological Measures:				
FEV_1 pre-BD (% predicted)		69.6±24.9	94.3±18.4	104.6±11.7
FEV_1/FVC ratio pre-BD (%)		66.9 (55-76)	76.1 (71.1 – 81.4)	82.1 (77.1-86.5)
FEV_1 pre-BD <80% predicted and FEV_1/FVC <0.7 (%)		66.5%	20.3%	1.3%
FEV_1 reversibility (% from baseline); ≥12% reversibility[§]		13.3 (5.2-26.4); 43.3%	12.9 (9.4-32.9); 26.5%	8.6 (7.1-10.2); 0%
K_{CO} (% predicted)		99.2±17.5	98.6±14.1	92.3±14.2
Atopic status:				
Atopic* (%)		72.4%	82.4%	45.6%
Serum total IgE (IU/ml)		91.7 (23-331)	104 (30-200)	33 (15.7-106)
Measures of inflammation:				
$FeNO_{50}$ measured (%)		94.7%	100%	100%
$FeNO_{50}$ (ppb); $FeNO_{50}$ ≥50ppb		20.7(12.7-41); 20.7%	22 (15-41); 18.8%	15.1 (11-24); 8.8%
Sputum induction successful (%)		61.1%	30.4%	32.5%
Sputum Inflammatory Phenotype (%):	Eosinophilic (≥3%)	41.1%	28.6%	11.5%
	Neutrophilic (≥61%)	35.9%	0%	15.4%
	Mixed granulocytic	10.5%	0%	3.8%
	Paucigranulocytic	32.1%	71.4%	76.9%
Blood Eosinophil Count (x10^9/L); ≥0.3 (%)		0.2 (0.1-0.5); 49%	0.2 (0.1-0.4); 38.2%	0.1 (0.1-0.2); 18.4%
Serum Periostin (ng/ml); ≥50 (%)		67 (56-83); 85.9%	66.5 (54-75.5); 85.3%	73.5 (58-92); 92.3%
Blood Neutrophil Count (x10^9/L)		5.6 (4.2-7.8)	3.7 (3.3-4.4)	3.6 (2.9-4.5)

Type2-high phenotype[†]	56.2%	37.7%	25%
ATS/ERS Severe Asthma Criteria 2014[14]			
GINA Step 4/5 treatment	100%	0%	-
ACQ(6)>1.5, ≥2 OCS bursts in previous year, ≥1 hospital admission in previous year, persistent airflow limitation or deterioration in asthma control on tapering steroid dose	100%	29%	-

Data presented as mean±SD, median (IQR), n or %

[∝]Rhinosinusits treatment includes the use of nasal corticosteroids, oral corticosteroids and oral antihistamines

[§] Reversibility testing performed 15-mintues after 2.5mg nebulised Salbutamol

* Atopic defined as ≥1 positive skin prick test

[†] TH2-high phenotype identified by either FeNO≥50ppb, ≥3% Sputum Eosinophils, Blood Eosinophil count >$0.3x10^9$/L

BMI (Body Mass Index), ICS (Inhaled Corticosteroids), BDP (Beclometasone Diproprionate), LABA (Long Acting Beta-2 Agonist), LTRA (Leukotriene Receptor Antagonist), LAMA (Long Acting Muscarinic Antagonist), OCS (Oral Corticosteroids), BTS (British Thoracic Society), ACQ (Asthma Control Questionnaire), PPI (Proton Pump Inhibitor), SNOT-20 (20 Question Sino-Nasal Outcome Test Score), HADS (Hospital Anxiety and Depression Score), OSA (Obstructive Sleep Apnoea), AQLQ (Asthma Quality of Life Questionnaire), Pre-BD (Pre-Bronchodilator), $FeNO_{50}$ (Exhaled Nitric Oxide level at 50ml/sec flow rate).

Table 3: Comparison of WSAC with Existing Severe Asthma Cohorts/Registries

	WSAC	**ENFUMOSA**[15]	**SARP**[3]	**BTS**[16]	**BSAR**[5]	**BIOAIR**[6]
Cohort size (n)	342	163	204	382	350	93
Patient Demographics:						
Age (y)	49.4±13.6	42.4±12.1	41±13	NA	55±14	50.0±12.5
Female (%)	67.5%	81.6%	64.0%	63.1%	55%	58.0%
BMI (Kg/m^2)	29.7 (25.6-35.6)	27±5	NA	28 (24-32)	26 (16-43)	28.5±5.8
Smoking status:						
Never smoker (%)	55%	NA	NA	61%	57%	NA
Ex-smoker (%); Pack-years (y)	39.2%; 10 (4.4-22.5)	NA	NA	29.8%	31%; 15 (11-24)	NA
Current smoker (%); Pack-years (y)	5.8%; 20 (8.4 -35)	NA	NA	5.8%	12%; 11 (10-15)	NA
Clinical Characteristics:						
Age at asthma onset (y)	22 ± 19	NA	16±16	17 (3-35)	NA	NA
Asthma onset ≤12 years (%)	40.4%	NA	NA	NA	32%	NA
Asthma duration (y)	26±17	20.8±2.5	25±14	NA	NA	NA
Asthma medications:						
ICS dose (BDP equivalent µg/day)	2000 (1600-3000)	1676±667	NA	2000 (1000-2000)	2000 (190-6000)	2064±939
Maintenance OCS (%)	34.2%	32.5%	32%	41.7%	24%	NA
Co-morbidities:						
Aspirin sensitivity (%)	27.7%	NA	NA	9.5%	8%	NA
Rhinosinusitis (%)	72.5%	NA	54%	36.6%	49%	NA
GORD (%)	48.5%	NA	41%	41.4%	36%	NA
Quality of Life:						
AQLQ	4.09 ± 1.26	NA	NA	NA	4.14 (1.2-7)	NA
Disease Control:						
Rescue OCS courses in previous year	2 (1 - 4)	NA	NA	4 (2-6)	2.03 (0-7)	NA
Hospital admissions in previous year	0 (0 – 1)	NA	NA	0 (0-2)	0.95 (0-7)	NA
ACQ6	2.74±1.24	NA	NA	NA	2.57±1.31	2.03±0.96
ACQ6 >1.5 (%)	82.5%	NA	NA	NA	77%	NA
Physiological Measures:						
FEV1 Pre-BD (% predicted)	69.6±24.9	71.8±23.1	62±22	65.9±23.6	68±21	70.4±20.3
FEV1 <80% predicted (%)	66.5%	NA	78%	NA	60%	NA
FEV1/FVC ratio Pre-BD (%)	66.9 (55-76)	79.9±16.6	65±13	63.1±15.2	63±12	67±9.6
FEV1 reversibility (% from baseline)	13.3 (5.2-26.4)	NA	20±24	NA	11±13	9.4±7.7
≥12% reversibility (%)	43.3%	NA	61%	NA	36%	NA
K_{CO} (% predicted)	99.2±17.5	90.6±19	NA	101.5±17	97±20	NA
Atopic status:						
Atopic (%)	72.4%	58%	71%	NA	70%	43%
SPT Positive HDM (%)	55.3%	NA	NA	71.0%	NA	NA
SPT Positive Cat (%)	34.6%	NA	NA	65.4%	NA	NA
Serum total IgE (IU/ml)	91.7 (23-331)	109 (85-139)	NA	130 (53.5-292)	207 (2-10000)	NA
Measures of inflammation:						
FeNO measured (% of cohort)	94.7%	NA	66.2%	34.8%	77.4%	NA
$FeNO_{50}$ (ppb)	20.7(12.7-41)	NA	40±38	34.5(16-65)	26 (4-250)	46.3±59.7
Sputum induction successful (%)	61.1%	NA	60.7%	NA	32.2%	24.6%
Sputum Eosinophil Count (%)	1.5 (0.3 - 8.5)	11±2	NA	3 (0.3-11.3)	7 (0-92)	16.7±33.7
Sputum Neutrophil Count (%)	49.6 (26.3 – 67.3)	37±3	NA	NA	51 (0-99)	42.2±35.7
Sputum Inflammatory Phenotype (%):						
Eosinophilic (≥3%)	41.1%	NA	NA	NA	60.5%	NA
Neutrophilic (≥61%)	35.9%	NA	NA	NA	27.9%	NA
Mixed granulocytic	10.5%	NA	NA	NA	5.8%	NA
Paucigranulocytic	32.1%	NA	NA	NA	17.4%	NA
Blood Eosinophil Count ($x10^9/L$)	0.2 (0.1-0.5)	NA	NA	0.3 (0.2-11)	0.24 (0-3.1)	NA

Data presented as mean±SD, median (IQR), n or %; NA= data not available

BMI (Body Mass Index), ICS (Inhaled Corticosteroids), BDP (Beclometasone Diproprionate), OCS (Oral Corticosteroids), GORD (Gastro-oesophageal Reflux Disease), AQLQ (Asthma Quality of Life Questionnaire), ACQ (Asthma Control Questionnaire), Pre-BD (Pre-Bronchodilator), SPT (Skin Prick Test), $FeNO_{50}$ (Exhaled Nitric Oxide level at 50ml/sec flow rate).

Table 4: Comparison of WSAC with UBIOPRED (Severe Asthmatics)

	Non-smokers*		Smokers and Ex-smokers*	
	WSAC	**UBIOPRED**	**WSAC**	**UBIOPRED**
Cohort size (n)	215	311	115	110
Patient Demographics:				
Age (y)	47.4±14.1	51.01±0.8	51.0±12.1	54.51±1.08
Female (%)	68.9%	66%	31.1%	51%
BMI (Kg/m^2); BMI>30 (%)	31.0±8.1; 47.7%	29.11±6.34; 38.6%	31.5±7.0; 51.8%	29.59±6.29; 40%
Smoking status:				
Never smoker (%)	83.3%	84.9%	0%	0%
Ex-smoker (%); Pack-years (y)	16.7%; 1.5 (0.9-3.0)	2 (1-4)	83.5%;15.2(8.8-30)	17.38(10-26)
Current smoker (%); Pack-years (y)	0%		16.5%; 20(8.4-35)	
Clinical Characteristics:				
Age at diagnosis (y)	17 (4-32)	20 (7-38)	32 (9-43)	38 (20-48)
Asthma onset ≤12 years (%)	43.3%	NA	30.4%	NA
Asthma duration (y)	27±16	NA	23±18	NA
Asthma medications:				
ICS dose (BDP equivalent µg/day)	2000 (1600-3000)	NA	2000 (1600-2800)	NA
Maintenance OCS (%)	36.3%	45.8%	29.6	44.7%
Co-morbidities:				
Rhinosinusitis (%)	75.3%	74.0%	66.1%	60.4%
GORD (%)	47.9%	46.7%	48.7%	63.6%
Quality of Life:				
AQLQ	4.1±1.3	4.48±1.16	4.0±1.3	4.44±1.25
Disease Control:				
Rescue OCS courses in previous year	3.2±2.7	2.48±2.29	2.8±2.2	2.55±2.73
ACQ7	2.75±1.10	2.67±1.33	2.90±1.23	2.62±1.18
Physiological Measures:				
FEV1 Pre-BD (% predicted)	71.5±25.7	67.5±22.1	65.7±22.1	67.2±19.3
FEV1/FVC ratio Pre-BD (%)	0.67±0.14	0.64±0.18	0.63±0.13	0.61±0.10
Atopic status:				
Atopic (%)	74.4%	78.3%	67.3%	71.3%
Serum total IgE (IU/ml)	78 (20-304)	119.5(45-342)	110 (36.7-353)	126(63-328)
Measures of inflammation:				
FeNO measured (% of cohort)	95.3%	93.2%	94.8%	94.5%
$FeNO_{50}$ (ppb)	22.4 (13.9-47.0)	26.5 (16-47)	18.0 (11.0-36.0)	23.5 (12-42)
Sputum induction successful (%)	59.1%	41.1%	67.0%	48.2%
Sputum Eosinophil Count (%)	2.0 (0.3-11.3)	2.75 (0-19)	1.0 (0.3-6.5)	4.13 (1-14)
Sputum Neutrophil Count (%)	52.0 (26.5-67.5)	53.69 (34-75)	46.5 (25.0-64.0)	55.15 (35-65)
Sputum Eosinophils >1.9%	50.4%	57.81%	37.7%	60.38%
Blood Eosinophil Count ($x10^9/L$)	0.3 (0.1-0.5)	0.2±0.3	0.2 (0.1-0.4)	0.22±0.29

Data presented as mean±SD or median (IQR), n or %; NA= data not available

*Non-smokers defined as having not smoked for 12-months with <5-pack year smoking history

BMI (Body Mass Index), ICS (Inhaled Corticosteroids), BDP (Beclometasone Diproprionate), OCS (Oral Corticosteroids), GORD (Gastro-oesophageal Reflux Disease), AQLQ (Asthma Quality of Life Questionnaire), ACQ (Asthma Control Questionnaire), Pre-BD (Pre-Bronchodilator), $FeNO_{50}$ (Exhaled Nitric Oxide level at 50ml/sec flow rate).

Table 5 – Wessex Severe Asthma Cohort Detailed Characterisation Protocol

Demographics	• Age (yrs) • Gender • Predominant Race (Caucasian/Asian/Black/Other) • Smoking history (pack year history if ever smoked) • Height • Weight • BMI
Clinical Characteristics	**Asthma History:** • Age at diagnosis (yrs) • Predominant symptoms (Wheeze/Chest tightness/Breathlessness/Cough/Other) • Triggers (Cold air/Exercise/Climate/ Air pollution/Fumes/Allergens/Medications including aspirin sensitivity/Emotion/Hormonal/Foods/Workplace/Alcohol/Viral RTI/Other • Family history of asthma • Asthma treatments (BTS Stage/ICS Dose/Controller Medications/Anti-IgE) • Exacerbation history **Medical History:** • Medical& surgical co-morbidity including rhino-sinusitis / gastro-oesophageal reflux / depression • Current treatments
Questionnaires	**Disease Control:** • Asthma Control Questionnaire 7 • Asthma Control Diary **General Quality of Life:** • Short Form 36 Health Survey **Asthma Specific Quality of Life:** • Asthma Quality of Life Questionnaire (Symptoms / Activity / Emotional / Environmental / Total) **Rhino-sinusitis Assessment:** • Sino-nasal Outcome Test (20) **Anxiety and Depression Assessment:** • Hospital Anxiety and Depression Score (Anxiety / Depression / Total)
Physiological Measures	• Random oscillometry pre and post-bronchodilator (R4-16 / R0 / I / FN) • Impulse oscillometry pre and post-bronchodilator (Z at 5Hz / R at 20 Hz / R at 5 Hz / X at 5Hz / AX / Resonant Frequency) • Spirometry pre and post-bronchodilator (FEV_1 / FVC / FEV_1/FVC Ratio / MEF 75 / MEF 50 / MEF25 / PEF) with % reversibility derived from this. • Carbon monoxide transfer factor (Vinsp / V Asb / TLco / Kco) • 2-week PEF diary
Measures of Atopic Status	• Allergy history (Perennial or seasonal allergy / Food allergy / Drug allergy / Antihistamine use) • Skin prick testing(Aspergillus fumigatus / Alternaria tenuis / Grass pollen / Birch / Rape / Dermatophagoides pteronyssinus / Dermatophagoides farinae / Dog / Cat • Serum total IgE
Biological Measures	• Exhaled Nitric Oxide (FeNO) at 50ml/sec low rate (ppb) • Nasal Nitric Oxide Measurements **Nasal Lavage:** • Tryptase (ng/ml) • Eosinophilic Cationic Protein (ng/ml) • Myeloperoxidase (ng/ml) • α-2 macroglobulin (ng/ml) **Serum:** • Periostin level • Staphylococcus aureus enterotoxin IgE • Peripheral blood eosinophil & neutrophil count ($x10^9$/L) • High-sensitivity C-Reactive Protein (mg/L) • Eosinophilic Cationic Protein (ng/ml) • IL-6 (pg/ml) • ST2/IL-1R4(pg/ml) • YKL-40(ng/ml)

	Induced Sputum: • Inflammatory Cell Counts (Macrophages / Neutrophils / Eosinophils / Lymphocytes / Epithelial Cells / Squamous Cells) • IL-10(pg/ml) • IL-5(pg/ml) • IL-4 (pg/ml) • IL-1receptor antagonist(pg/ml) • IL-1α (pg/ml) • IL-1 β (pg/ml) • Granulocyte Macrophage Colony-Stimulating Factor (pg/ml) • Vascular Endothelial Growth Factor (pg/ml) • IL-17 (pg/ml) • Monocyte Chemotactic Protein 1/CCL2 (pg/ml) • Tumour Necrosis Factor-α (pg/ml) • Macrophage Inflammatory Protein 1β/CCL4 (pg/ml) • Fibroblast Growth Factor (pg/ml) • Granulocyte Colony-Stimulating Factor (pg/ml) • Interferon-γ (pg/ml) • IL-2 (pg/ml) • Macrophage Inflammatory Protein 1α/CCL3(pg/ml) • Eotaxin (pg/ml) • TIMP MetallopeptidaseInhibitor 1(ng/ml) • Matrix metalloproteinase-1 (pg/ml) • Matrix metalloproteinase-2 (pg/ml) • Matrix metalloproteinase-3 (pg/ml) • Matrix metalloproteinase-7 (pg/ml) • Matrix metalloproteinase-8 (pg/ml) • Matrix metalloproteinase-9 (pg/ml) • Matrix metalloproteinase-12 (pg/ml) • Matrix metalloproteinase-13 (pg/ml) • IL-6 (pg/ml) (PBS & DTE) • IL-6 Soluble Receptor (pg/ml) • Tryptase(ng/ml) (DTE) • Eosinophilic Cationic Protein (ng/ml) (DTE) • Myeloperoxidase (ng/ml) (DTE) • YKL-40 (ng/ml) (DTE) • Osteopontin(pg/ml) • CXCL5/ENA-78 (pg/ml) • IL-8 (pg/ml) (DTE) • CXCL1/Gro-α (pg/ml) (DTE) • α-2 macroglobulin (ng/ml) (DTE) • Elastase (ng/ml) • IL-13 (pg/ml) **Urine:** • Urinary eosinophil-derived neurotoxin/protein X (mg/ml) • Urinary Cotinine
Additional Measures:	• University of Pennsylvania Smell Identification Test • HRCT Chest

Table 6: Summary of Trial Eligibility Criteria (Phase IIb/III RCTs of Novel Therapies in Severe Asthma since 2000)

Target	Drug	Authors	Year	Phase	Trial Number	N	Eligibility Criteria								
							Steroid Dose		Airflow Obstruction	Reversibility	Exacerbation Frequency	Asthma Control	BMI or Weight	Smoking	Biomarker criterion
							ICS	OCS							
IgE	Omalizumab	Holgate et al[17]	2004	III	x	246	•		•	•				•	•
		Humbert et al[18]	2005	II	x	419	•	•	•	•	•			•	•
		Hanania et al[19]	2011	III	NCT00314575	850	•		•	§	•		•	•	•
IL-5	Mepolizumab	Haldar et al[20]	2009	II	ISRCTN75169762	61	•		•	§	•			•	•
		Nair et al[21]	2009	II	NCT00292877	20	•	•	•					§	•
		Pavord et al[22]	2012	III	NCT01000506	621	•		•	§	•		•	•	§
		Bel et al[23]	2014	III	NCT01691508	135	•	•	•	§			•	•	•
		Ortega et al[24]	2014	III	NCT01691521	576	•		•	§	•		•	•	•
		Chupp et al[25]	2017	III	NCT02281318	556	•		•	§	•			•	•
	Reslizumab	Castro et al[26]	2011	II	x	106	•	•	•	§		•		•	•
		Castro et al[27]	2015	III	NCT01287039	489	•	•		•	•	•		•	•
		Castro et al[27]	2015	III	NCT01285323	464	•	•		•	•	•		•	•
		Bjermer et al[28]	2016	III	NCT01270464	315	•			•		•		•	•
		Corren et al[29]	2016	III	NCT01508936		•	•		•		•		•	
	Benralizumab	Bleecker et al[30]	2016	III	NCT01928771	1205	•		•	•	•	•	•	•	
		FitzGerald et al[31]	2016	III	NCT01914757	1306	•		•	•	•	•	•	•	
IL-13	Tralokinumab	Piper et al[32]	2013	II	NCT00873860	194		•	•	§	•	•	•	•	
		Brightling et al[33]	2015	II	NCT01402986	452	•		§	§	•	§	•	•	
	Lebrikizumab	Corren et al[34]	2011	II	NCT00930163	219	•	•	•	•		•	•	•	
		Hanania et al[35]	2016	III	NCT01867125	1081	•	•	•	•		•		•	
		Hanania et al[35]	2016	III	NCT01868061	1068	•	•	•	•		•		•	
	GSK679586	De Boever et al[36]	2014	II	NCT00843193	198	•		•	•		•		•	
IL-4Rα	AMG317	Corren et al[37]	2010	II	NCT00436670	294	•	•	•	•		•		•	•
	Dupilumab	Wenzel et al[38]	2013	II	NCT01312961	104	•	•	•	•	•	•		•	§
		Wenzel et al[39]	2016	II	NCT01854047	769	•	•	•	•	•	•		•	
	Pitrakinra	Slager et al[40]	2012	II	NCT00801853	534	•	•	•	§	•	•		•	
IL-4/5	Suplatast	Tamaoki et al[41]	2000	II	x	85	•	•	•	•				•	
DP2 receptor	Fevipiprant	Gonem et al[42]	2016	II	NCT01545726	61		•		§	•	•		•	•
IL-2Rα	Daclizumab	Busse et al[43]	2008	II	NCT00028288	115	•	•	•	•				•	•
TSLP	Tezepelumab	Corren et al[44]	2017	II	NCT02054130	584	•	•	•	•	•	•	•	•	
c-kit/PDGF	Masitinib	Humbert et al[45]	2009	II	NCT00842270	44	•	•		•				•	•
CXCR2	Navarixin	Nair et al[46]	2012	II	NCT00632502	34	•		•	§				•	•
	AZD5069	O'Byrne et al[47]	2016	II	NCT01704495	640	•		•		•			•	•
IL17RA	Brodalumab	Busse et al[48]	2013	II	NCT01199289	302	•	•	•	•		•			
TNFα	Entanercept	Morjaria et al[49]	2008	II	x	39	•			§				•	
		Holgate et al[50]	2011	II	NCT00141791	132	•	•	•	•		•		•	
	Golimumab	Wenzel et al[51]	2009	II	NCT00207740	309	•			§	•	•		•	
Multiple	BT	Castro et al[52]	2010	III	NCT00231114	288	•	•	•		•			•	
	Azithromycin	Brusselle et al[53]	2013	III	NCT00760838	109	•			§	•			•	•
		Gibson et al[54]	2017	III	AZNCTR12609000197235	420	•			§				•	
	TLA	Storrar et al[55]	2017	III	ISRCTN46346208	222	•			§	•	•		•	•

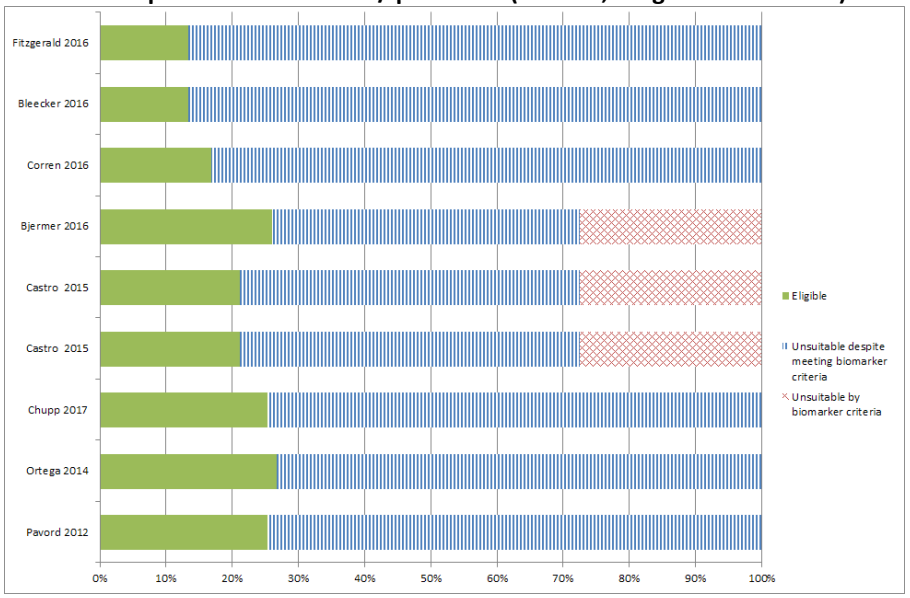
• Criteria used; § Composite criteria used

Table 7: Summary of Trial Eligibility (Phase IIb/III RCTs of Novel Therapies in Severe Asthma since 2000)

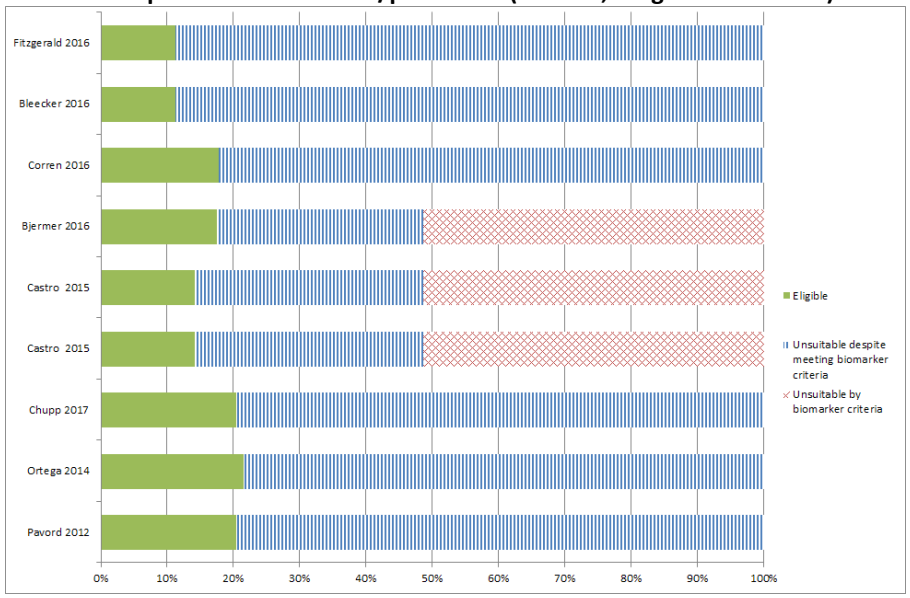
Drug	Authors	Year	Phase	Meet Eligibility Criteria (%)									Eligible (%)	
				Steroid Dose		Airflow Obstruction	Reversibility	Exacerbation Frequency	Asthma Control	BMI	Smoking	Biomarker criteria	Asthma Criteria	Overall
				ICS	OCS									
Omalizumab	Holgate et al[17]	2004	III	69.6%	100.0%	54.4%	38.9%	100.0%	100.0%	100.0%	67.3%	36.8%	12.6%	3.5%
	Humbert et al[18]	2005	II	93.9%	92.7%	54.4%	43.3%	79.5%	100.0%	100.0%	67.3%	36.8%	11.7%	4.1%
	Hanania et al[19]	2011	III	69.6%	100.0%	54.4%	81.9%	100.0%	100.0%	98.8%	67.3%	36.8%	18.4%	6.1%
Mepolizumab	Haldar et al[20]	2009	II	85.1%	100.0%	66.7%	81.6%	74.0%	100.0%	100.0%	72.8%	25.2%	23.1%	7.9%
	Nair et al[21]	2009	II	85.7%	34.2%	87.7%	100.0%	100.0%	100.0%	100.0%	82.7%	25.2%	23.1%	5.6%
	Pavord et al[22]	2012	III	72.2%	100.0%	66.7%	100.0%	74.0%	100.0%	99.7%	67.3%	57.3%	23.1%	17.5%
	Bel et al[23]	2014	III	72.2%	28.9%	66.7%	100.0%	100.0%	100.0%	99.7%	68.7%	69.6%	8.8%	4.1%
	Ortega et al[24]	2014	III	72.2%	100.0%	66.7%	100.0%	74.0%	100.0%	99.7%	68.7%	69.6%	24.0%	15.8%
	Chupp et al[25]	2017	III	72.2%	100.0%	66.7%	100.0%	74.0%	100.0%	100.0%	67.3%	69.6%	23.4%	14.9%
Reslizumab	Castro et al[26]	2011	II	72.2%	65.8%	76.3%	86.3%	100.0%	86.5%	100.0%	83.3%	25.2%	22.8%	4.7%
	Castro et al[27]	2015	III	93.9%	84.2%	100.0%	43.3%	100.0%	86.0%	100.0%	83.3%	45.3%	23.7%	8.8%
	Castro et al[27]	2015	III	93.9%	84.2%	100.0%	43.3%	100.0%	86.0%	100.0%	83.3%	45.3%	23.7%	8.8%
	Bjermer et al[28]	2016	III	93.9%	100.0%	100.0%	43.3%	100.0%	86.0%	100.0%	83.3%	45.3%	29.2%	10.8%
	Corren et al[29]	2016	III	93.9%	65.8%	100.0%	43.3%	100.0%	86.5%	100.0%	83.3%	100%	15.5%	15.5%
Benralizumab	Bleecker et al[30]	2016	III	93.9%	100.0%	66.7%	38.9%	74.0%	86.5%	99.7%	67.3%	100%	11.1%	11.1%
	FitzGerald et al[31]	2016	III	93.9%	100.0%	66.7%	38.9%	74.0%	86.5%	99.7%	67.3%	100%	11.1%	11.1%
Tralokinumab	Piper et al[32]	2013	II	100.0%	84.2%	87.7%	81.9%	100.0%	86.5%	86.3%	67.3%	100%	26.9%	26.9%
	Brightling et al[33]	2015	II	93.9%	100.0%	54.4%	81.9%	63.2%	86.5%	86.8%	68.7%	100%	11.4%	11.4%
Lebrikizumab	Corren et al[34]	2011	II	59.6%	65.8%	54.4%	43.3%	100.0%	86.5%	98.5%	68.7%	100%	6.1%	6.1%
	Hanania et al[35]	2016	III	89.5%	65.8%	54.4%	43.3%	100.0%	86.5%	100.0%	68.7%	100%	7.6%	7.6%
	Hanania et al[35]	2016	III	89.5%	65.8%	54.4%	43.3%	100.0%	86.5%	100.0%	68.7%	100%	7.6%	7.6%
GSK679586	De Boever et al[36]	2014	II	93.9%	100.0%	58.2%	43.3%	100.0%	86.0%	100.0%	70.5%	100%	18.4%	18.4%
AMG317	Corren et al[37]	2010	II	59.6%	65.8%	43.0%	43.3%	100.0%	86.5%	100.0%	67.3%	73.7%	3.8%	3.5%
Dupilumab	Wenzel et al[38]	2013	II	93.9%	65.8%	43.0%	38.9%	100.0%	47.7%	100.0%	68.7%	36.6%	2.9%	2.1%
	Wenzel et al[39]	2016	II	93.9%	84.2%	54.4%	38.9%	100.0%	86.5%	100.0%	68.7%	100%	10.5%	10.5%
Pitrakinra	Slager et al[40]	2012	II	93.9%	65.8%	61.4%	81.9%	100.0%	100.0%	100.0%	67.3%	100%	23.4%	23.4%
Suplatast	Tamaoki et al[41]	2000	II	83.6%	65.8%	62.6%	34.5%	100.0%	100.0%	100.0%	83.3%	100%	6.4%	6.4%
Fevipiprant	Gonem et al[42]	2016	II	100.0%	84.2%	100.0%	100.0%	100.0%	86.0%	100.0%	83.3%	27.2%	59.7%	16.7%
Daclizumab	Busse et al[43]	2008	II	93.9%	65.8%	43.0%	43.3%	100.0%	100.0%	100.0%	67.3%	73.7%	6.7%	5.3%
Tezepelumab	Corren et al[44]	2017	II	99.4%	84.2%	54.4%	38.9%	79.5%	86.5%	86.3%	67.3%	100%	6.4%	6.4%
Masitinib	Humbert et al[45]	2009	II	93.9%	22.5%	100.0%	43.3%	100.0%	100.0%	100.0%	67.3%	99.1%	5.6%	5.3%
Navarixin	Nair et al[46]	2012	II	93.9%	100.0%	93.6%	81.9%	100.0%	100.0%	100.0%	74.0%	36.8%	55.6%	19.9%
AZD5069	O'Byrne et al[47]	2016	II	93.9%	100.0%	67.8%	100.0%	79.5%	100.0%	100.0%	74.0%	71.9%	37.7%	25.2%
Brodalumab	Busse et al[48]	2013	II	59.6%	65.8%	43.0%	43.3%	100.0%	86.5%	100.0%	100.0%	100%	6.7%	6.7%
Entanercept	Morjaria et al[49]	2008	II	69.6%	100.0%	100.0%	86.3%	100.0%	100.0%	100.0%	67.3%	100%	39.5%	39.5%
	Holgate et al[50]	2011	II	93.9%	84.5%	43.0%	51.8%	100.0%	74.6%	100.0%	67.3%	100%	8.2%	8.2%
Golimumab	Wenzel et al[51]	2009	II	93.9%	100.0%	100.0%	86.3%	74.0%	74.6%	100.0%	67.3%	100%	30.7%	30.7%
BT	Castro et al[52]	2010	III	93.9%	84.2%	62.6%	100.0%	68.4%	100.0%	100.0%	67.3%	100%	26.3%	26.3%
Azithromycin	Brusselle et al[53]	2013	III	69.6%	100.0%	100.0%	86.3%	74.0%	100.0%	100.0%	67.3%	74.0%	31.6%	20.8%
	Gibson et al[54]	2017	III	99.4%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	81.0%	100%	76.9%	76.9%
TLA	Storrar et al[55]	2017	III	93.9%	100.0%	100.0%	100.0%	74.0%	94.7%	100.0%	72.8%	73.7%	53.8%	41.5%

Figure 3: Trial Eligibility for Phase III IL-5 Targeted Treatments in Severe Eosinophilic Asthmatics Defined by Varying Levels of Sputum or Blood Eosinophilia

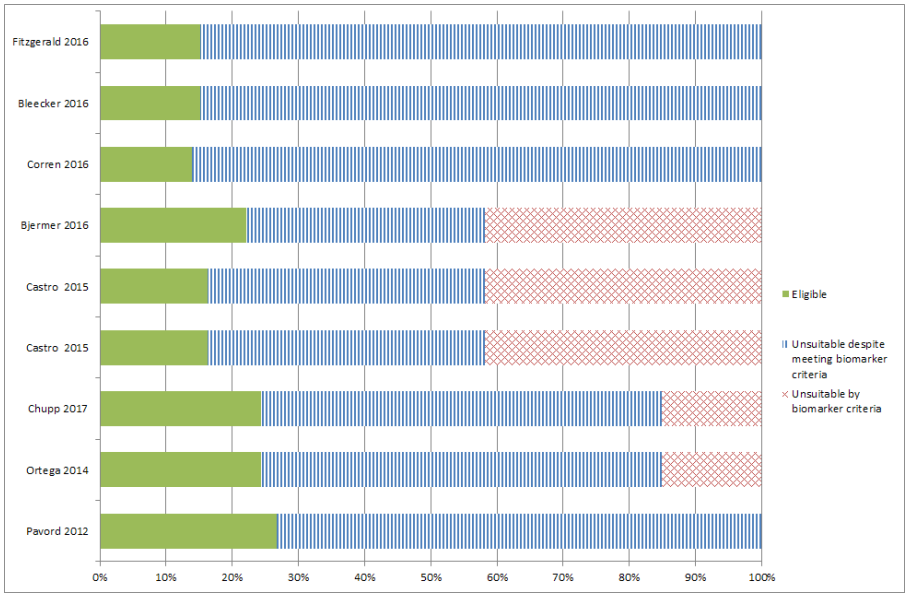
Blood Eosinophil Count ≥300 Cells/ µL - 21.1%(median; range 13.4% - 26.8%)



Blood Eosinophil Count ≥150 Cells/µL - 17.7% (median; range 11.3%-20.4%)



Sputum Eosinophil ≥3% - 16.3% (median; range 14.0% - 26.7%)



Sputum Eosinophil ≥2% - 16.1% (median; range 12.9% - 25.8%)

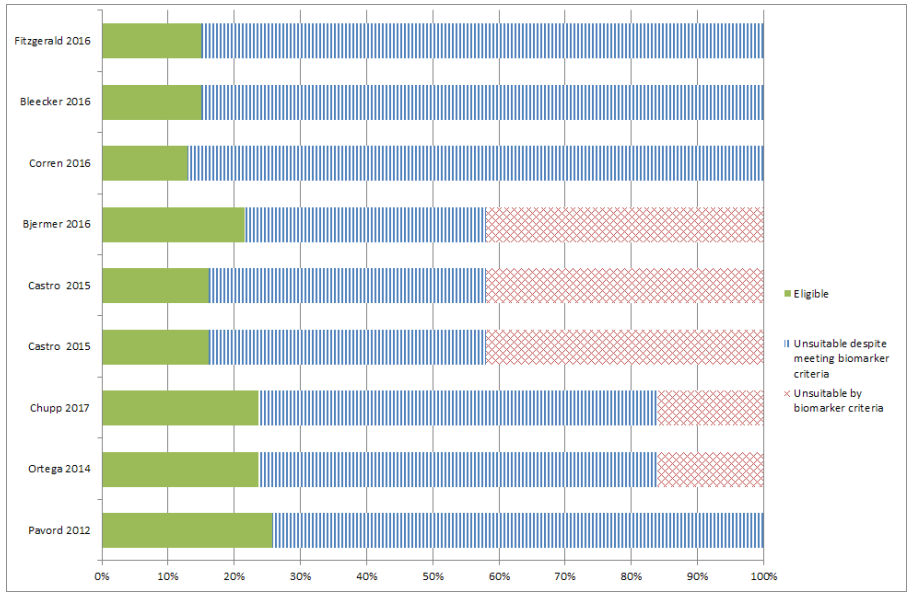


Table 8: NICE Eligibility Criteria for Biological Therapies Licensed for use in Severe Asthma

Mepolizumab (*NICE TA431; Jan 2017)*	**Reslizumab (***NICE TA479; Oct 2017)*
Severe refractory eosinophilic asthma	
Blood eosinophil count ≥300 cells/μL in last 12 months	Blood eosinophil count ≥400 cells/μL in last 12 months
≥4 severe exacerbations in last 12-months or maintenance oral corticosteroids	≥3 severe exacerbations in last 12-months
Adherence confirmed, treatment optimised, co-morbidities and asthma triggers addressed	

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