Anti-IL5 therapy for asthma and beyond

Mukherjee et al.
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Abstract
Airway inflammation is considered to be the primary component contributing to the heterogeneity and severity of airway disorders. Therapeutic efficacies of diverse novel biologics targeting the inflammatory pathways are under investigation. One such target is IL-5, a type-1 cytokine that is central to the initiation and sustenance of eosinophilic airway inflammation. Over the past decade, anti-IL5 molecules have been documented to have mixed therapeutic benefits in asthmatics. Post hoc analyses of the trials reiterate the importance of identifying the IL-5-responsive patient endotypes. In fact, the currently available anti-IL5 treatments are being considered beyond asthma management; especially in clinical complications with an underlying eosinophilic pathobiology such as hypereosinophilic syndrome (HES) and eosinophilic granulomatosis and polyangiitis (EGPA). In addition, closer analyses of the available data indicate alternative mechanisms of tissue eosinophilia that remain uncurbed with the current dosage and delivery platform of the anti-IL5 molecules.

Keywords: Eosinophil, IL-5, Eosinophilic asthma, Hypereosinophilic syndrome (HES), Churg-strauss syndrome, Chronic bronchitis, Eosinophilic granulomatosis and polyangiitis (EGPA), Chronic obstructive pulmonary disorder (COPD), Mepolizumab, Reslizumab, Benralizumab

Introduction
The past ten years have witnessed the development and evaluation of a number of biologics that target the Th2 cytokines involved in asthma pathophysiology, particularly those that are associated with eosinophils in the airway. Eosinophils play a key role in the pathobiology of several airway disorders presenting with chronic inflammatory pathology such as asthma [1,2], chronic obstructive pulmonary disorder (COPD) [3], eosinophilic granulomatosis and polyangiitis (EGPA) [4], and hypereosinophilic syndrome (HES) [5]. Targeting Interleukin-5 (IL-5) in asthma, the central protagonist in eosinophilia (discussed in details later), was a logical derivative post promising results in animal models [6,7] and initial screenings in patients [8,9]. De-liberation arises from the mixed response of anti-IL-5 trials conducted in different asthmatic populations that document a healthy reduction in circulating eosinophils, but without much significant improvement in other clinical indices of disease severity (extensively reviewed in [10-12]). Again in a recent review, anti-IL-5 therapy has been conjectured to be effective in long-term management of HES patients [5]. The current review will critically evaluate the documented outcomes of the conducted clinical trials to date and subsequently assess the therapeutic implications of anti-IL-5 therapy in treating airway disorders with an aberrant eosinophilic pathobiology.

Eosinophil biology and the role of IL-5
A robust literature now support eosinophils to be pleiotropic, multifunctional leukocytes that facilitate the ‘innate’ response against extraneous agents in the airway, modulate the downstream ‘adaptive’ immunity cascade, maintain local immunity/inflammation and as the end-stage effector cells that can cause tissue damage via release of granule proteins, reactive oxygen species and cysteinyl leukotrienes (reviewed in [2,13]). In eosinophil biology (refer to Figure 1), IL-5 plays a central role in the production, mobilization, activation, recruitment, proliferation, survival and suppression of apoptosis in eosinophils at the site of inflammation (reviewed in,[2,6,12,13]), illustrated in a schematic diagram (Figure 1).

In 1996, a study reported that ‘IL-5 deficient’ mice failed to develop the characteristic eosinophilia and airway hyper-reactivity after ovalbumin-sensitization [7]. Soon after, 8 asthmatic patients demonstrated increase in airway eosinophil counts and methcholine PC_{20} (a provocative concentration of methcholine required to
induce 20% reduction in the forced expiratory volume in 1 second, FEV$_1$ when subjected to inhaled recombinant IL-5 [9]. Moreover, restraining sources of eosinophil recruitment and/or eosinophil-deficient animal models were observed to be healthy without any characteristic abnormalities [14]. As a logical derivative from the existing experimental and clinical evidences, several monoclonal antibodies (mAbs) were engineered to
neutralize free circulating IL-5 and/or target IL-5 receptor alpha (IL5Rα) and are now in different phases of development [10,12,15].

A comparative analysis of the Anti-IL-5 trials: asthma
As early as 1990, Bousquet et al., [8], correlated eosinophilia with asthma severity and demonstrated eosinophilic cation proteins (ECP) were associated with epithelial damage in 44 patients with asthma. A direct but modest correlation has been established between asthma severity, frequent exacerbations and the intensity of eosinophilia. Thereby, a sub-set of patients are being identified who suffer from ‘severe refractory asthma,’ consequently accounting for a high socioeconomic burden and are considered to most benefit from an eosinophil-targeted therapy [1,10].

The last 15 years have documented several clinical trials that evaluate the therapeutic relevance of anti-IL-5 biologics in asthma treatment and symptom management (refer to Table 1). As evident from the outcome summary tabulated in Table 1, Mepolizumab, a humanized mAb (IgG1) with a high affinity for binding free IL-5 (which further prevents its binding to the receptor, IL5Rα), is found to be effective in depleting eosinophil numbers in blood and the airways. On the contrary, studies in mild-moderate asthmatics documented Mepolizumab to be ineffective in improving end-point clinical symptoms (refer to Table 1, [16,19,20]), therefore raising concern over the efficacy of IL-5 as a therapeutic intervention in asthma. However, by selecting patients with persistent blood ( >0.3 × 10⁹/L) and sputum eosinophils (≥3%) coupled with frequent history of exacerbations, two independent relatively small studies in 2009 documented a significant decrease in the exacerbation frequencies (P ≤ 0.02 vs. placebo, both studies) and asthma control questionnaire (ACQ) scores (P ≤ 0.02, vs. placebo, both studies), with 750 mg infusions of Mepolizumab [21,22]; in addition to a prednisone-sparing effect [21]. Similar reduction in exacerbation frequencies with corresponding decrease in peripheral blood eosinophils was reflected in a large, double-blinded, placebo-controlled, multi-centered study conducted in 2011 [23]. Based on the dose–response observations from the DREAM study [23], 75 mg intravenous and 100 mg subcutaneous doses were investigated in a recent Phase III trial, where significant reductions in exacerbation rates by 47% and 53% respectively (P< 0.001, vs. placebo) along with depletion of blood eosinophils were recorded [28]. The 100 mg subcutaneous dose was reported in a parallel Phase III study to have a corticosteroid-sparing effect in a similar target population, with median percentage reduction of 50% in treatment group, along with 32% relative reduction in annual exacerbation rate (p= 0.04 vs placebo) [29]; a reduction though statistically significant, is less pronounced than that observed in the earlier study with higher dose and intravenous route of drug delivery [21,32]. The optimum dose, route and duration of therapy and persistence of beneficial effects for prednisone-dependent patients remain to be established.

Another anti-IL5 mAb (IgG4/κ) Reslizumab, showed similar reduction in sputum eosinophils, significant improvement in lung function (P= 0.002, vs. placebo) and a trend towards improved asthma scores (P= 0.054, vs. placebo) in patients diagnosed with severe refractory eosinophilic asthma (see Table 1). Additionally, the authors observed the improvement in ACQ scores were most pronounced in patients with nasal polyps (P= 0.012, vs. placebo), [24] which reflected the observations of Gevaert et al., in 2003 [33]. There are recent reports of Phase III trials that demonstrate significant improvement in ACQ scores and FEV₁ (p<0.05, vs. placebo) in moderate to severe asthmatics treated with 3.0 mg/kg of intravenous Reslizu- mab; charting a larger improvement in asthma control for subjects with baseline eosinophils ≥400 cells/μL [30,31].

IL5Rα expressed by both mature eosinophils and eosinophil-lineage progenitor cells [2], is targeted by Benralizumab (MEDI-563), a humanized, afucosylated mAb. Being afucosylated, this drug induces apoptosis in its target cells via enhanced antibody-mediated cellular toxicity (ADCC), and is considered to have an increased efficiency of eosinophil depletion comparative to the other anti-IL5 biologics [34]. The initial safety trial conducted by Busse et al., in 2010, documented no adverse events [25] and a further study by the same group showed 100% reduction of peripheral circulating eosinophils (Table 1) [26]. More recently, 100 mg subcutaneous Benralizumab exhibited significant improvement in annual exacerbation rates, lung function and asthma score, with greater benefits seen in patients with blood eosinophil levels ≥ 400 cells/μL [27]. Currently, there are three clinical trials registered on http://clinicaltrials.gov (NCT01914757, NCT02075255, NCT01928771, last accessed 17/09/2014), where the drug is being assessed as an adjunct therapy for ‘uncontrolled’ asthma.

Further assessment of anti-IL5 Trials: clinical insights
The mixed outcomes from anti-IL-5 clinical trials highlight the need for careful endotyping of patients, since the therapy is deemed effective on those patients whose asthma is dependent on the eosinophilic inflammatory pathway [1]. The potential ‘responders’ to IL-5 therapy are patients who present with eosinophilia (blood ≥0.3 × 10⁹/L, ≥3% sputum), are generally steroid-responsive, and suffer from frequent exacerbations. As evident from the DREAM study [23], the atopic status is inadequate for segregating ‘responders’ from the ‘non-responders,’ since approximately 50% of the patients who responded to Mepolizumab had negative radioallergosorbent test to the four most common allergens. In addition, sub-sets of patients that are aspirin-
| First author [ref] | Disease (severity) | Study design | Dosage/ delivery | Inclusion criteria: Baseline eosinophil count | Comments on eosinophilia | Outcome summary |
|-------------------|-------------------|--------------|------------------|-----------------------------------------------|--------------------------|-----------------|
| Leckie [16] 2000  | Mild atopic asthmatic n= 24 mc, db, pc | Single dose i.v., 2.5, 10 mg/kg | • Not an inclusion criteria Baseline values: | • Day 29, post-allergen 10 mg/kg dosage, blood eos 0.04 × 10^9/L compared to 0.25 × 10^9/L placebo (P< 0.0001) | • No significant effect on AHR | |
| Mepolizumab       | FEV1 ≥ 70%, predicted | | | | | |
| Böttner [17] 2003 | Mild to moderate asthmatics n= 19 mc, db, pc | Three monthly doses, i.v. No Baseline count/ median n/a | | • Decrease in blood eos (median values from 300 to 45 per mL, P< 0.05 vs. placebo) | • No asthma end-points were assessed | |
| Mepolizumab       | FEV1 > 50-80%, predicted | | | | | |
| Kips [18] 2003    | Moderate-severe asthma, FEV1 > 40-80%, predicted n= 32 db, pc, mc | Rising single dose (0.03, 0.1, 0.3, 1 mg/kg) i.v | • Not included in the inclusion criteria | • Dose dependently reduced circulating eos | | |
| Reslizumab        | | | Baseline value: blood eos (counts x 10^9/L): Placebo:0.45 ± 0.16 0.3 mg/kg: 0.28 ± 0.04 1.0 mg/kg: 0.25 ± 0.04 | • Significant dose reduction with 1 mg/kg for 30 days post dosing ( p=0.05) | • Significant increase in FEV1 post 24 hours from dose range ≥ 0.3 mg/kg (p=0.019) | |
| Flood-page [19] 2003 | Mild atopic asthma n= 24 db, pc, parallel group, | 3 i.v. doses of 750 mg per month12- wk follow up | • Not included in the inclusion criteria | • Blood eos: significant reduction in wk 4 and 10 (P<0.02, vs. placebo) | • No significant changes in other clinical indices | |
| Mepolizumab       | FEV1 ≥ 70%, predicted | Mepolizumab/ per month12- wk follow up | Baseline value: blood eos (mean x 10^9/L): Group: 0.27 Placebo: 0.4 | • Bone marrow: 70% reduction in mature eos (P= 0.017) | • Sputum eos not checked | |
| | | | | | | |
| Mukherjee et al. World Allergy Organization Journal 2014, 7:32 | | | | | | |
| Study | Drug | Comparator | No of patients | Method | Inclusion Criteria | Comparator | Results |
|-------|------|------------|----------------|--------|--------------------|------------|---------|
| Flood-page [20] 2007 | Mepolizumab | Moderate persistent asthmatics | n= 362 mc, db, pc | 3 i.v. doses of 750/250 mg Mepolizumab per month 8- wk follow up | Not included in the inclusion criteria | Blood eos: Sustained significant 80% reduction for both doses (p< 0.001 vs. placebo) | No significant change in clinical endpoints |
| Nair [21] 2009 | Mepolizumab | Severe persistent asthma with Eosinophilia | n= 20 db, pc, pilot study | 5 i.v. doses of 750 mg per month. | Yes. Inclusion criteria - Sputum eos > 3% | Blood eos; Baseline; | Significant reduction in blood eos after 1st dose (49.5/μl), last dose (64.5/μl) and follow-up (76.3/μl) (P< 0.05) vs. placebo, no significant reduction from baseline |
| Haldar [22] 2009 | Mepolizumab | Refractory eosinophilic severe asthma | n= 61 db, pc, parallel study | 12 doses of 750 mg i.v. per month | Inclusion criteria - Sputum eos > 3% | Blood eos; Baseline; | Significant reduction in blood eos after 1st dose (49.5/μl), last dose (64.5/μl) and follow-up (76.3/μl) (P< 0.05) vs. placebo, no significant reduction from baseline |
| Pavord [23] 2012 | Mepolizumab | Severe refractory asthma with ≥ 2 exacerbations in past year | n= 621 db, pc, parallel study, mc (DREAM) | 3 doses s.c., at 4 wks | Yes. Inclusion criteria - Sputum eos > 3% | Blood eos (x 10^9/L); at 52 wk, vs. placebo | Exacerbation rates at all doses were 39-52% less than those in the placebo group (P< 0.05 vs. placebo) |

Table 1 A comparative study of Anti-IL5 trials in Asthma (Continued)
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| Study | Type of Asthma | Study Design | Dose | Primary Outcome Measures |
|-------|----------------|--------------|------|--------------------------|
| Castro [24] 2011 | Poorly controlled asthma, on high dose ICS | n=106 | 3.0 mg/kg sc, at baseline and at Weeks 4, 8, and 12 | • Yes. Inclusion criteria - Sputum eos > 3% Baseline: • Blood eos, median (× 10^3/μL); Drug: 0.5 Placebo: 0.5 • Sputum eos (%): Drug: 10.7 Placebo: 8.5 | • Significant reduction in blood eosinophils (P< 0.0001, vs. placebo) • Significant decrease in sputum eos in dose-dependent fashion from baseline to 0.01 ± 0.0 × 10^3/μL, 24 hours post-dose • Significant reduction in sputum eos compared to placebo, 38.7% (p= 0.0068) • Significant improvement in ACQ score in patients with nasal polyps (p= 0.012) • Significant reduction in FEV1 in drug group (p=0.002, vs. placebo) • Significant reduction in FEV1 ≥ 80% of predicted | • Trend in reduction of asthma exacerbations in drug group (p=0.083, ns) • ACQ trend in favour of drug group (p=0.054) • Significant reduction in FEV1, in drug group (p=0.002, vs. placebo) |
| Busse [25] 2010 | Mild atopic asthma | n=44 mc, safety in open-label study | Single escalating doses (0.003-3 mg/kg, over 3 – 30 minutes) | Baseline: • Blood eos: Mean ± SD, 0.27 ± 0.2 x 10^3/μL; • ECP levels (mean) 21.4 ± 17.2 μg/L | • Significant decrease in eos in dose-dependent fashion from baseline to 0.01 ± 0.0 x 10^3/μL, 24 hours post-dose | • Acceptable safety profile |
| Benralizumab (MEDI-563) | Eosinophilic asthma | n=27 mc, db, pc | Cohort I: 1 mg/kg single dose; Cohort II: 100 mg, 200 mg, combined 3 monthly (s.c.) | Baseline: • Sputum eos of ≥ 2.5% • ECP levels were reduced from baseline to 10.3 ± 7.0 μg/L, 24 hrs post-dosing | • Significant reduction in sputum eosinophils, airway eosinophil counts and 100% reduction in bone marrow and peripheral blood | • No adverse reactions were noted. |
| Laviolette [26] 2013 | Eosinophilic asthma | n=27 mc, db, pc | Cohort I: (i.v) 1 mg/kg single dose; Cohort II: 100 mg, 200 mg, combined 3 monthly (s.c.) | Baseline: • Sputum eos (mean%) • Airway mucosal/submucosal eos: mean reduction vs. placebo: Placebo: 13.9 Drug: 6.6 Cohort I: (i.v): 61.9% (ns) Cohort II, combined (sc): 83.1% (p=0.0023) | • Additional clinical factors were not measured |
### Table 1 A comparative study of Anti-IL5 trials in Asthma (Continued)

| Study | Design | Population | Intervention | Outcomes |
|-------|--------|------------|--------------|----------|
| **Castro [27] 2014** | Uncontrolled asthma ACQ-6 score ≥ 1.5 | n= 609 | 2 mg, 20 mg, 100 mg sc for eosinophilic patients (n= 324) | • Subjects were stratified based on blood eos, Sputum eos ≥ 2%, FeNO > 50 ppb • All doses reduced blood eos < 50 cells/μl after the first dosage • In eosinophilic group, 100 mg sc improved annual exacerbation rate by 41% (p= 0.096) vs. placebo, deemed significant; ns in non-eosinophilic group, • Subgroup analysis showed greater improvement with increased baseline blood eos (100 mg sc reduced exacerbations by 70% in patients ≥ 400 cells/μl, p= 0.002) |
| **Benralizumab** | Uncontrolled asthma, Exacerbation ≥ 2/last year | Phase Ib | 2 mg, 20 mg, 100 mg sc for eosinophilic patients (n= 324) | Subjects were stratified based on blood eos, Sputum eos ≥ 2%, FeNO > 50 ppb • All doses reduced blood eos < 50 cells/μl after the first dosage • Significant improvement in FEV1 and ACQ-6 in eos subtype with all doses |
| **Ortega [28] 2014** | Severe asthma n= 576 | Cohort 1 – 75 mg i.v. (n= 191) | Blood eos 150/μl at screening or 300/μl in previous year | Reduction in eos by week 4 maintained through the entire study • Rate of exacerbations reduced by 47% and 53% in s.c and i.v. groups respectively (p< 0.001, vs. placebo) |
| **Mepolizumab** | Severe asthma n= 576 | Cohort 2 – 100 mg sc. (n= 194) | Blood eos 150/μl at screening or 300/μl in previous year | Reduction in eos by week 4 maintained through the entire study • Rate of exacerbations reduced by 47% and 53% in s.c and i.v. groups respectively (p< 0.001, vs. placebo) |
| **Bel [29] 2014** | Severe eosinophilic asthma n= 135 | 100 mg s.c. every 4 weeks for 20 weeks | Blood eos 150/μl at screening or 300/μl in previous year | Drug significantly reduced blood eos by week 4 and was maintained throughout study (p< 0.001) • Rate of exacerbations reduced by 47% and 53% in s.c and i.v. groups respectively (p< 0.001, vs. placebo) |
| **Corren [30] 2014** | Moderate-severe asthma n= 395 (drug) | 3.0 mg/kg, i.v. monthly (for 16 weeks) | Blood eos 150/μl at screening or 300/μl in previous year | Abstract does not document any reduction in blood eos • Significant reduction in ACQ score in drug arm (p= 0.04) |
| **Reslizumab** | ACQ ≥ 1.5 n= 97 (placebo) | 30 mg/kg, i.v. monthly (for 16 weeks) | Inclusion criteria does not include sputum eos | Study population stratified by baseline blood eos ≥ or ≤ 400 cells/μl • Only 20% of study population was eosinophilic (or ≤ 400 cells/μl) • FEV1 improvement for overall population by 68 ml, 270 ml for eosinophilic patients (p= 0.04 vs. placebo), ns decrease of 33 ml in non-eosinophilic patients |
| **Bjermer [31] 2014** | Eosinophilic asthma n= 311 | 0.3 – 3.0 mg/kg, i.v. monthly (for 16 weeks) | Sputum eos ≥ 400 cells/μl | Eosinophil measurement was not documented in the abstract • Overall improvement in FEV1 p ≤ 0.024, ACQ score (p ≤ 0.033) • Improvement in ACQ-5 score (p= 0.004) • Improvement in ACQ-5 score (p= 0.004) |

Index: eos= eosinophils; db= double-blind; pc= placebo-controlled; mc= multi-center; sc= single-centre; FEV1= peak expiratory flow i.v= intravenous; s.c.= sub-cutaneous; wk= week; ns= non-significant; ACQ= Asthma Control Questionnaire, ICS= inhaled corticosteroid, OCS= oral corticosteroid.
sensitive/induced asthma or present with sinusitis might also benefit from anti-IL5 therapy [35].

Using ‘sputum eosinophils’ as a biomarker to identify IL5-treatment responsive patient-groups as well as a marker for its therapeutic outcome is a topic under debate [1]. Studies that considered patients with ≥ 2.5 - 3% sputum eosinophilia in their inclusion criteria, independently recorded significant improvements with asthma scores and lung function [21,22] compared to others (see Table 1). Similarly post hoc analysis showed that patients treated with 1.0 mg/kg Reslizumab, with baseline sputum levels< 3% did not show improvement in the FEV₁, (even with depleted peripheral eosinophil levels) [18]. In fact, sputum eosinophils do not correlate with a change in circulating eosinophil numbers in the severe prednisone-dependent asthmatic patients and the former is markedly reduced before an event of exacerbations [1]. Subcutaneous doses of Mepolizumab showed neither significant reduction in sputum eosinophils (for 75 and 250 mg dosage groups) nor any relevant improvements in the symptom scores or lung function (see Table 1, [23]). The recent phase III trials documented depletion of blood eosinophils, significant improvement in asthma symptom scores and moderate reduction in exacerbation rates with lower (100 mg) subcutaneous doses (refer to Table 1), without any indication of whether the luminal eosinophila generally exhibited in the specific patient group was resolved or not [28,29]. In contrast, 750 mg intravenous infusions in the previous two studies of similar disease profiles [21,22], were able to reduce both circulating and sputum eosinophils, allow significant improvement in ACQ, FEV₁ and quality of life score along with pronounced reduction in exacerbations. This discrepancy may reflect the therapeutic significance of the drug delivery platform and dose used, a concern addressed in a recent editorial [32].

Further assessment of Anti-IL5 trials: molecular insights

The eosinophil biology is complex and outcomes from the anti-IL-5 clinical trials reiterate this. Many of the anti-IL-5 clinical trials (Table 1) document the presence of tissue eosinophilia in spite of nil/low circulating levels, post-treatment. Especially, both studies with Benralizumab showed 100% reduction of eosinophils in bone-marrow and peripheral blood, but presence of airway mucosal/sub-mucosal eosinophils [26] and detectable levels of ECP in the sputum [25], indicating an alternative mechanism to IL-5 for eosinophil initiation, recruitment, activation and survival in the tissues. Delving further, Haldar et al., [22] showed significant decrease (P< 0.002) for both circulating blood and sputum eosinophils (see Table 1) in the Mepolizumab study group, which was not reflected in the paired bronchial-biopsy specimens (obtained before and after the study). In context, antisense oligonucleotide therapy (TPI ASM8), developed to suppress the expression of surface receptors CCR3 (C-C chemokine receptor type 3, binds eotaxin) and β chain (shared receptor for IL-5, IL-3 and granulo-cyte macrophage-colony stimulating factor, GM-CSF) [36], reduced sputum eosinophil counts by 46%; while a CCR3 antagonist was recently documented to show no effect of blood or sputum eosinophilia or to have any clinically improvement in moderate to severe asthmatics [37]. Scattered evidences [2,11,38] instrument the presence of alternative pathways in situ, that can trigger, activate and maintain eosinophils in the sub-mucosal and mucosal surfaces, independent of the classical T1/2 pathway activation triggers (refer to Figure 1).

Extraneous environment-derived factors including non-allergic sources like pathogens and epithelial damage can trigger the release of epithelium-derived ‘Type-2 alarmins’ – IL-25, IL-33, and thymic stromal lymphopoietin (TSLP, see Figure 1) [15,38]. IL-25 and IL-33 can initiate mast cell (MC) response (mostly sub-mucosal localization) that leads to the release of IL-5 and CCL5. In addition, the type-2 alarmins activate the resident lineage negative, type 2, innate lymphoid cells (ILC2s) to release the classical T1/2 cytokines IL-5, IL-13, IL-9, that directly or indirectly support eosinophil recruitment and survival in the tissues (refer to Figure 1) [15,38-41]. Again, in vitro experiments demonstrated TSLP in presence of pro-inflammatory stimuli ILL1-beta/tumor necrosis factor alpha (mimicking an ongoing inflammatory state) activated MCs to release IL-5 and CCL5. IL-13 (and IL-4 in airway smooth muscle, ASM) trigger the release of eotaxins from the ASM [43] and the bronchial epithelium [44] that promote recruitment of eosinophils and eosino-phil progenitors (refer to Figure 1). Increased IL-13 and IL-4 can promote the homing of CD34⁺ haemopoietic progenitor cells into the airway parenchyma [45].

Evidence suggest that this mechanism maybe upstream of activation by epithelial cell-derived cytokines [46,47]. Understanding physiological processes that promote airway eosinophilia in severe asthma may be critical to the development of novel treatment modalities for optimal asthma control. Luminal eosinophilia in asthma arise as a result of (i) the recruitment of mature eosinophils from the periphery in response to locally elaborated chemo-attractants such as eotaxin and/or (ii) the localized maturation of eosinophil lineage-committed progenitors, termed “in situ differentiation” in the presence of locally elaborated cytokines such as IL-5 [48,49]. That haemopoietic progenitors differentiate within the tissue is inferred from findings that there is increased recruitment of eosinophil progenitor cells into the airways in asthmatics [50]. In addition CD34⁺ cells extracted from human nasal polyp
tissue and nasal explant tissue undergo IL-5 driven differentiation to form mature eosinophils [51,52]. In context, anti-IL5 mAbs reduce tissue and luminal eosinophils (see Table 1), and are most effective in severe prednisone-dependent asthmatics with eosinophilic bronchitis [21]. These findings suggest that local eosinophilopoiesis may be a more dominant mechanism for the persistence of eosinophils in the airways of patients with moderate-to-severe asthma than chemokine-dependent (for e.g. eotaxin) recruitment of mature eosinophils. Whilst this remains to be determined, it may explain our recent findings where treatment with anti-CCR3 failed to clear luminal eosinophils likely because the treatment did not attenuate local differentiative processes [37].

Controlling the development of airway eosinophilia may involve targeting multiple factors that stimulate eosinophil recruitment and modulate local differentiative processes or prolonged tissue survival. A few conducted clinical trials with mAbs targeting IL-4 and IL-13 biology, similar to anti-IL-5, has met with a mixed response in improving clinical symptoms [10]. However, a combination therapy with drugs like Dupulimab (targets the receptor complex common both IL-4 and IL-13) [53] and an anti-IL-5 mAb could synergistically curb the mechanisms of in situ eosinophilia plausibly altered in severe asthmatics, that render the airways susceptible to maintain the clinical symptoms.

Anti-IL-5 therapy for other lung eosinophilic disorders
As evident from the on-going discussion, IL-5 and IL-5 receptor alpha (IL5Ra) exhibit an undeniable eosinophil lineage-specificity. Indisputably, they have been considered as a potential therapeutic target in eosinophilic airway disorders.

Hypereosinophilic syndrome (HES)
HES is a heterogeneous rare disorder defined by the presence of >1500 eosinophils per μL of blood, persistent for ≥6 months, with eosinophil-related organ involvement or dysfunction and no identifiable secondary cause of eosinophilia [5]. Early case studies with HES patients show considerable improvement in disease symptoms, patient relief, and decrease in eosinophilia with 750 mg intravenous Mepolizumab [5]. Table 2 summarizes an open-label study with 4 patients in 2004 [54] followed by a double-blinded, multi-centered clinical trial with Mepolizumab in 2008, where 41 out of 43 patients (on the experimental drug and tapering strength of prednisone), managed to maintain a circulating blood eosinophil count <600/μL, for ≥8 weeks (p<0.0001, vs. placebo) [55]. Additionally, it could be an alternative to using high-dose OCS, otherwise prescribed to the patients diagnosed with T-lymphocyte variant of HES [56]. A number of studies are currently underway to evaluate other anti-IL5 molecules in addition to Mepolizumab as potential therapeutic interventions in HES. A study with HES patients is projected to end in early 2017, which evaluates the safety and efficacy of Benralizumab (NCT02130882) in these subjects (http://clinicaltrials.gov).

Eosinophilic Granulomatosis and Polyangitis (EGPA)
EGPA, earlier known as Churg-Strauss syndrome, is a rare form of vasculitis characterized by asthma and eosinophilia, with multi-organ involvement (lung, peripheral nerves, heart, gastrointestinal tract, skin), where systemic corticosteroid is the cornerstone of therapy [4,60]. Histology shows classic evidence of an eosinophilic inflammatory response in the airway tissue of these patients and hence Mepolizumab has been reasoned to be a potential corticosteroid-sparing therapy. A recent case study reported complete regression of asthma (discontinuation of inhaled therapy) and depletion of blood and airway eosinophils in a patient with refractory EGPA with monthly infusions of 750 mg Mepolizumab [60]. Kim et al., 2010, reported a significant (75%) reduction in circulating eosinophils with 4 monthly doses of 750 mg (i.v) Mepolizumab, in a small open label trial with 7 patients, that allowed safe reduction of OCS from a mean dose of 18.8 mg to 4.6 mg [57]. In addition, Mepolizumab at the same dosage allowed complete remission in 8 out of 9 EGPA patients in a Phase II uncontrolled trial (detailed in Table 1) [58]. Though promising, further clinical investigations are necessary to ascertain the therapeutic benefit of Mepolizumab in EGPA and a large multicenter clinical trial is ongoing.

Chronic eosinophilic pneumonia (CEP)
Chronic eosinophilic pneumonia (CEP) is an idiopathic condition that presents with peripheral eosinophilia, eosinophilic infiltrates in the lung parenchyma and may occasionally be associated with asthma. Increased levels of IL-5 and release of cytotoxic granular proteins from eosinophils constitutes an important pathomechanism in pulmonary tissue damage observed in CEP [61]. Conventionally OCS therapy is used for treatment; however, an eosinophil-targeted therapy with Mepolizumab might result in steroid-sparing therapeutic benefit in CEP patients.

Chronic bronchitis (in COPD)
Chronic bronchitis is a primary component of COPD that encompasses a high level of heterogeneity. A sputum database analysis of 2443 patients with airway diseases, revealed one-fifth of the COPD patients experience eosinophilic bronchitis (EB). Additionally, EB was also associated with the severity of airflow obstruction in non-asthmatic COPD. 18% COPD patients with frequent exacerbations were documented to have EB and associated eosinophilia. Targeting eosinophils and IL-5 levels with Mepolizumab may decrease exacerbation rates and improve lung functions for this sub-set of COPD patients [62], as documented for other eosinophil-driven airway
| First author [ref]/year | Disease (severity) | Study design | Dosage/ delivery | Baseline eosinophil count | Comments on eosinophilia | Outcome summary |
|-------------------------|-------------------|--------------|------------------|---------------------------|--------------------------|-----------------|
| Garrett [54]/2003       | HES n=4           | open label   | 3 doses 10 mg/kg or 750 mg (max) i.v. every 4 wk | Blood eos > 750/μL after an 8 wk pre-treatment run-in period | Blood eos reduced in all patients, sustained in 12 wk follow-up span | Symptoms and quality of life improved in all patients |
|                        |                   |              | 750 mg i.v. at 4 wk interval 36 wk study | Blood eos < 600/μL for 8 wks, achieved in 95% patients in drug group, 45% placebo, p<0.0001 | Primary end-point (reduction of prednisone to 10 mg or less without clinical severity) was reached 84% of patients in drug group, 43% placebo, p<0.0001 |
| Rothenberg [55]/2008    | HES n=85         | db, pc, mc, parallel group study | 750 mg i.v. at 4 wk interval 36 wk study | Blood eos ≤ 600/μL by OCS monotherapy at a daily dose of 20–60 mg. | Significant lower mean daily prednisone dose of 4.64 mg in drug dosed group, compared to 28.3 mg in placebo (P=0.014) |
|                        |                   |              |                   | Sputum eos not measured | Patients with low CCL17 levels were seen to significantly maintain blood eos ≤ 600 μl |
| Roufosse [56]/2010      | L-HES n=85       | db, pc, international study | 750 mg i.v. at 4 wk interval 36 wk study | Blood eos maintained ≤ 600/μL by L-HES (Mepolizumab group) for 8 wks and during the entire length of the study compared to placebo |
|                        |                   |              |                   | Mean eos count 3.4% | Reduction in corticosteroid 18.8 mg to 4.6 mg, P<0.001 |
|                        |                   |              |                   | Mean eos 3.8% at wk 40 | Significant improvement of ACQ during study and wash-out phase |
|                        |                   |              |                   | BVAS not does include eos as a criteria | Variations in eos levels ranged from 13 – 4282 cells/μl |
|                        |                   |              |                   | Disease extent dropped from 4 at week 0 to 0 at week 32 (p=0.009) | Eight patients achieved remission at week 32 (primary end-point), BVAS score= 0, OCS<7.5 mg/day |
|                        |                   |              |                   |     | No relapse occurred |
| Kim [57]/2010           | EGPA n=7         | open – label pilot study | 4 monthly 750 mg (i.v) 40 wk study | Mean eos count 3.4% | Reduction in eos count from 2.9% (mean) to 0.4 at wk 16 (wash-out phase) |
|                        |                   |              |                   |     | Mean eos 3.8% at wk 40 |
| Moosig [58]/2011        | Active refractory (n=3) or relapsing (n=7) active EGPA | sc, phase II, uncontrolled | 750 mg i.v. once every 4 weeks (9 infusions in total) | BVAS does not include eos as a criteria | Variations in eos levels ranged from 13 – 4282 cells/μl |
|                        |                   |              |                   |     | 6 patients (≥120 cells/μl) showed reduction in eos from their respective baseline, maintained throughout. |
|                        |                   |              |                   |     | Disease extent dropped from 4 at week 0 to 0 at week 32 (p=0.009) |
|                        |                   |              |                   |     | Eight patients achieved remission at week 32 (primary end-point), BVAS score= 0, OCS<7.5 mg/day |
|                        |                   |              |                   |     | No relapse occurred |
| Study | p | Study Design | Disease | Patient Characteristics | Treatment | Outcomes |
|-------|---|--------------|---------|-------------------------|-----------|----------|
| Brightling [59] 2014 | Moderate to severe | n= 101 | 100 mg s.c. every 4 weeks (three doses), then every 8 weeks (five doses) over 48 weeks | Inclusion criteria: Sputum eosinophils >3% at screening or past year. Sub-group analysis stratified results based on ≥150 or ≥200 or ≥300 eosinophils/μL. Increase in blood and sputum eos after final dose. | Significant reduction in both sputum and blood eosinophil levels at week 4, and maintained till week 56. Increase in blood and sputum eos after final dose. | No changes in acute exacerbation rates, lung function or symptom score between treatment and placebo arm at week 56 for overall population. Non-significant decrease in exacerbation rate compared with placebo in patients with baseline eosinophil counts of ≥150 cells/μL (p=0.84), ≥200 cells/μL (p=0.26), or 300 cells/μL (p=0.28). Changes in FEV1 at week 56 was significant in patients with blood eosinophil counts ≥150 cells/μL (p=0.031) or ≥200 cells/μL (p=0.035), and non-significant in those with counts of ≥300 cells/μL (p=0.22). |

Index: eos= eosinophils; db= double-blind; pc= placebo-controlled; mc= multi-center; sc= single-centre; FEV1= peak expiratory flow; I.V.= intravenous; s.c.= sub-cutaneous; wk= week; ns= non-significant; FIP1L1-PDGFRA: Fip1-like 1/platelet-derived growth factor receptor a fusion; ACQ= Asthma Control Questionnaire; Birmingham Vasculitis Activity score= BVAS.
disorders (discussed previously). However, as per the recent reports of Brightling and co-workers, no reduction in annual exacerbation rates was observed in moderate to severe COPD patients with eosinophilia (>3% sputum eosinophils) when treated with a monthly/bi-monthly subcutaneous dose of 100 mg Benralizumab, even though the treatment effectively depleted both airway and circulating eosinophils (refer to Table 2) [59]. Nevertheless, online database (http://clinicaltrials.gov, last accessed 16/09/2014) enlists ongoing independent studies investigating efficacy and safety of Mepolizumab as an adjunct treatment in COPD management (registration number: NCT02105961); in COPD with EB (NC T01463644); reducing exacerbations in severe COPD (NCT02105948); and Phase III trials for Benralizumab in moderate to very severe COPD (NCT02155660, NC T02138916), addressing safety and efficacy of the drug.

Competing interests
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Authors’ contributions
PN conceived the idea. MM prepared the first draft. RS contributed to the development. All authors edited and reviewed the manuscript. All authors have approved the final version of the manuscript.

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Summary
The ongoing anti-IL5 clinical trials, show promise of a safe, effective treatment option for the severe ‘eosinophilic’ asthma phenotype independent of their atopic status [63]. Beyond asthma, small pilot studies have documented their potential in treating HES and EGPA patients. IL-5 may not be the sole determinants of persistent airway eosinophilia. Recently described immune cells such as the ILC2s and epithelium-derived type-2 alarmins that release TGF2 cytokines like IL-13 may also play important roles. Hence, targeting IL-5 and IL-13 to curb the eosinophil-derived clinical symptoms needs to be investigated in select patient-subtypes. Finally, while blood eosinophil count or other indirect assessments such as the eosinophil/lymphocyte ratios may help to identify an “eosinophilic phenotype” to initiate therapy with an anti-eosinophil biologic drug in patients with moderate asthma, this strategy may not be as effective as measuring sputum eosinophils to monitor response to therapy particularly in more severe systemic corticosteroid-dependent asthmatic patients. The key to successful therapy would be to select the appropriate patient population. The mere presence of eosinophils in blood or sputum may not be sufficient. The patients who are likely to respond are those whose disease is truly largely dependent on eosinophil biology. Identification of these patients require clinical acumen, clinical criteria and demonstration of persistent (not transient) systemic and airway eosinophilia.

Abbreviations
mAb: Monoclonal antibodies; ILSRα: Interleukin-5 receptor alpha; HES: Hypereosinophilic syndrome; EGPA: Eosinophilic granulomatosis and polyangiitis; COPD: Chronic obstructive pulmonary disorder; FEV1: Forced expiratory volume in 1 second; ACQ: Asthma control questionnaire; OCS: Oral corticosteroid; IL: Interleukin; ECP: Eosinophilic cationic protein; CCR: Chemokine chemokine receptor; TSLP: Thymus stromal lymphopoietin.
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