

# Immunological and hematological effects of IL-5(R $\alpha$ )-targeted therapy: An overview

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## Abstract

IL-5 is an important cytokine for priming and survival of mature eosinophils and for proliferation and maturation of their progenitors. Hence, IL-5(R $\alpha$ ) targeting will be increasingly used in diseases where eosinophils are the key immune effector cells such as eosinophilic asthma (EA), hypereosinophilic syndrome (HES), eosinophilic esophagitis (EE), and eosinophilic granulomatosis with polyangiitis (EGPA). Therefore, several neutralizing monoclonal antibodies directed against IL-5 (mepolizumab and reslizumab) and its receptor IL-5R $\alpha$  (benralizumab) have found or will find their way to the clinic. While the clinical effect of these drugs has been extensively investigated and reviewed, the understanding of the underlying immunological and hematological mechanisms remains less clear. This review will discuss the translational outcomes of treatment with these monoclonal antibodies in humans to shed light on the mechanisms underlying the main immunological and hematological findings from these clinical trials in humans.

## KEYWORDS

anti-IL-5, asthma, asthma therapy, eosinophils, IL-5

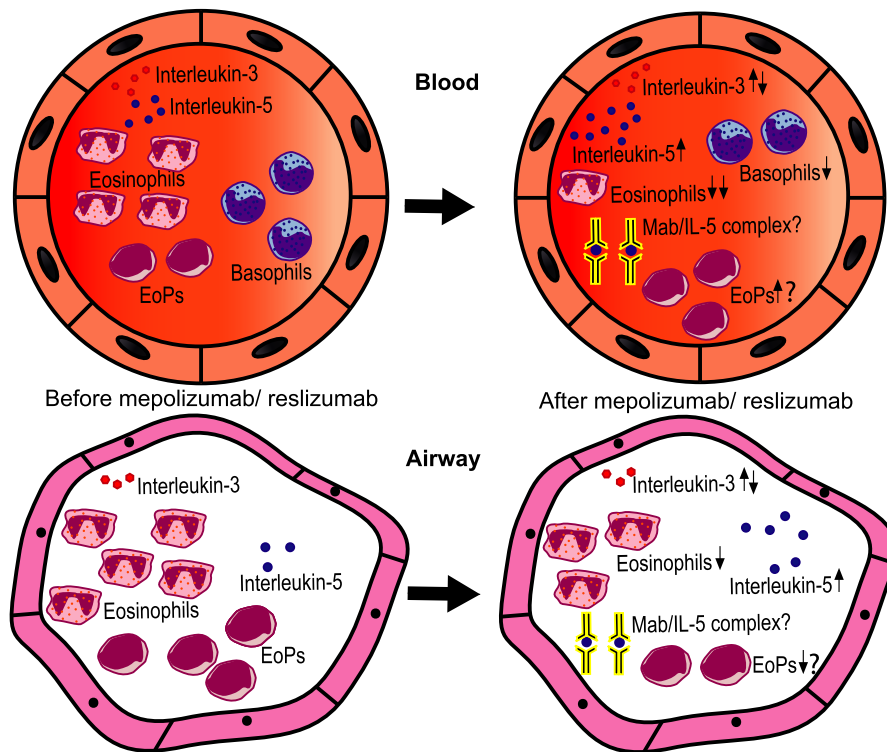
## 1 | INTRODUCTION

Therapies targeted on the IL-5 pathway are a good extension in the treatment of patients with severe eosinophilic asthma.<sup>1</sup> This therapeutical approach has also shown promising results in the treatment of nasal polyps, hypereosinophilic syndrome, eosinophilic granulomatosis with polyangiitis, and other hypereosinophilic disorders.<sup>2-4</sup> For patients with severe eosinophilic asthma treatment with IL-5-targeting drugs result in a lower rate of exacerbations and a decrease in use of glucocorticoids. However, the quality of life and prebronchodilator FEV1 hardly improve in a clinically relevant way.<sup>1,5-13</sup> In addition, only half of the patients with eosinophilic granulomatosis with polyangiitis reached a remission.<sup>3</sup> The lack of a complete clinical response in both diseases is difficult to understand as long as some important immunological and

hematological issues of the therapy remain to be established. One of the key questions is whether eosinophils that remain in the body during IL-5-targeted therapy are an intrinsically different nonresponsive subset or residual "normal" cells, supporting the view that IL-5 is not critical in human eosinophilopoiesis. A third possibility is that residual eosinophils are found because of under dosing of the monoclonal antibodies such as recently suggested.<sup>3,14,15</sup> To gather translational data on IL-5 inhibition: A systemic literature search in PubMed was performed on October 2017 with the following query: (mepolizumab OR nucala OR reslizumab OR benralizumab OR cinquaero OR "anti-il-5" OR "anti-il-5r" OR "anti il-5" OR "anti il 5" OR "anti interleukin 5" OR "anti-interleukin 5" OR "anti-interleukin-5"). This query yielded 749 articles that were assessed for relevance and validity on the basis of title and abstract firstly and on full text secondly.

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## GRAPHICAL ABSTRACT

IL-5 targeted therapy results in an attenuation of eosinophils and basophils in blood and an attenuation of eosinophils and EoPs in the airways. Plasma and sputum levels of IL-5 increase, possibly due to the formation of immune complexes between IL-5 and anti-IL-5.

## 2 | THE FUNCTION OF INTERLEUKIN-5 IN HOMEOSTASIS

### 2.1 | Receptor of IL-5 and its signal transduction

Interleukin-5 is a cytokine which is produced as a dimer and secreted by multiple cells such as Th2 cells, mast cells, ILC2 cells, and eosinophils.<sup>16</sup> It binds to the IL-5-specific  $\alpha$  subunit—interleukin-5R $\alpha$  (CD125)—that is part of a heterodimeric receptor with the common  $\beta$  subunit (CD131). This latter subunit is shared with the heterodimeric IL-3 receptor (CD123) and GM-CSF receptor (CD116).<sup>17</sup> The common  $\beta$  subunit does not express any ligand binding site but confers high-affinity ligand binding to intracellular signaling.<sup>18</sup> Upon binding of IL-5 to its receptor, juxtamembranous tyrosine kinases phosphorylate the  $\beta$  receptor upon which at least 3 major signaling pathways are activated: JAK/STAT, MAPK, and PI-3K.<sup>19</sup> All 3 pathways eventually lead to rapid reprogramming of gene expression and a plethora of cellular responses ranging from proliferation of eosinophil progenitors to priming of cytotoxicity by mature cells.<sup>20</sup> Interestingly, IL-5 with a charge reversal mutation at position 12 (E12K)<sup>21</sup> and GM-CSF with a similar mutation at position 21 (E21R),<sup>22</sup> which are important for receptor binding, fail to stimulate tyrosine phosphorylation but can still affect survival.<sup>21</sup> This finding reveals the existence of 2 distinct mechanisms of receptor activation, one of which is  $\alpha$ -chain specific.

### 2.2 | Expression of IL-5 receptors

In humans, the effects of IL-5 are restricted to basophils and eosinophils.<sup>23</sup> The expression of IL-5R $\alpha$  on basophils is threefold lower compared to mature eosinophils and their differentiation is not dependent on this cytokine.<sup>24</sup> Therefore, the receptor is best characterized in the context of eosinophils. The receptor is both expressed on mature eosinophils and their progenitors including the eosinophil lineage-specific myeloblast (CD34<sup>+</sup> and IL-5R $\alpha$ <sup>+</sup> cells).<sup>25</sup> As stimulation of CD34<sup>+</sup>/IL-5R $\alpha$ <sup>+</sup> cells only produces eosinophils, it is yet not clear at what stage the IL-5R $\alpha$  is upregulated on basophils.<sup>25</sup>

In tissue, the level of IL-5R $\alpha$  on eosinophils is lower compared to blood eosinophils.<sup>26,27</sup> It seems plausible that the receptor is shed after migration to the tissue, because the amount of soluble IL-5R $\alpha$  is increased in tissue. This hypothesis is supported by the finding that IL-5R $\alpha$  is shed from the surface of healthy control eosinophils *in vitro* upon interaction with IL-5. This is probably due to proteolytic cleavage.<sup>28</sup> Whether IL-5R $\alpha$  expression is also found on type 2 innate lymphoid cells (ILC2) is still a subject of debate, because the results in literature are inconsistent.<sup>29,30</sup>

### 2.3 | The role of IL-5R in differentiation and proliferation

IL-5 stimulates eosinophil colony formation in bone marrow samples *in vitro*. IL-3 and GM-CSF can also give rise to eosinophil colonies

in vitro, but unlike the other cytokines, IL-5 is the most eosinophil specific.<sup>31,32</sup> The concentrations of IL-3 and GM-CSF in vitro need to be 10-fold higher in order to create eosinophils from marrow mononuclear cells instead of solely neutrophils or monocytes.<sup>32</sup> The formation of basophil colonies is also stimulated by IL-5 in HL-60 cells albeit to a lesser extent.<sup>32,33</sup>

In vivo, the situation is more complex. Murine experiments show a central role of IL-5 for reactive eosinophilia rather than differentiation per se, as IL-5 knockout mice do contain mature eosinophils in peripheral blood. They, however, do not exhibit eosinophilia after parasite infection. In culture systems of human CD34<sup>+</sup> progenitor cells, IL-5 only induces transient proliferation and maturation of eosinophil precursors, which also suggests that IL-5 is a relatively late-acting factor for eosinophil proliferation rather than a factor for early differentiation.<sup>34</sup> All these experiments imply that other factors drive differentiation of eosinophil precursors and IL-5 is a major growth factor for IL-5R expressing cells, at least in mice.<sup>34</sup>

## 2.4 | The IL-5R and survival

In vitro the presence of IL-5 and GM-CSF increases mature eosinophil survival up to 10 days by inhibition of apoptosis.<sup>35-37</sup> The apoptotic eosinophils are normally recognized and phagocytosed by macrophages or to a lesser extent by small-airway epithelial cells in a process generally referred to as efferocytosis.<sup>38,39</sup> In vivo the average circulatory lifespan of eosinophils was estimated to be between 11 and 63 hours. This large range is probably due to ex vivo manipulation of cells,<sup>40</sup> the use of toxic label,<sup>40-42</sup> and the difference in kinetics between homeostasis and pathological conditions.<sup>43</sup> The effect of IL-5 on these kinetics is unknown, but a clear negative correlation between eosinophil apoptosis and sputum IL-5 levels was found in asthmatic patients, which suggests that IL-5 is involved in inhibition of apoptosis in eosinophils in vivo.<sup>44</sup> Another important receptor involved in eosinophil survival is Siglec-8. In contrast to IL-5, GM-CSF, and IL-3, activation of Siglec-8 by Mab or glycan ligands induced cell death.<sup>45</sup> Interestingly, this ROS-dependent regulated cell death only occurred after priming of cells with IL-5, GM-CSF, or IL-33.<sup>46</sup> This finding suggests that steady state and resident eosinophils are less susceptible for Siglec-8-induced cell death.<sup>45</sup>

## 3 | IL-5-TARGETED THERAPY: HOW AND WHAT ARE THE OPTIONS OR ALTERNATIVES?

Mepolizumab and reslizumab are both humanized monoclonal antibodies (Mab) that bind to and block the function of circulating IL-5 and consequently prevent binding of IL-5 to its receptor. Mepolizumab is an IgG1 kappa Mab registered to be administered via a subcutaneous injection with a fixed dose of 100 mg, whereas reslizumab is a IgG4 kappa Mab which is registered to be injected intravenously with a weight-adjusted concentration of 3 mg/kg.<sup>47</sup> Benralizumab is an IgG1 kappa antibody directed against the IL-5R $\alpha$ . The published data imply

### Highlights

- IL-5 targeted therapy (mepolizumab or reslizumab) results in a clear attenuation of eosinophils in blood and a less distinct attenuation of eosinophils in (bronchial) tissue. Basophils are attenuated in blood.
- Plasma and sputum IL-5 levels are increased after treatment. A possible source of elevated levels of IL-5 might be due to immune complexes between IL-5 and anti-IL-5.
- Early eosinophil progenitors (CD34<sup>+</sup> and IL-5R $\alpha$ <sup>+</sup> cells) might increase in blood and decrease in sputum after treatment.

that benralizumab can completely deplete eosinophils and their (late) bone marrow progenitors by induction of antibody-dependent cell-mediated cytotoxicity (ADCC) executed by natural killer cells and/or macrophages both in vitro and in nonhuman primates in vivo.<sup>24</sup> As phase 3 trials show promising results, this drug will probably be approved soon in the treatment of severe eosinophilic asthma as well.<sup>1</sup> Omalizumab is a humanized monoclonal antibody that binds to C $\epsilon$ 3 of IgE and blocks this protein from binding to its receptor that is located on mast cells and basophils.<sup>48</sup> The required doses are dependent on body weight and serum levels of IgE. As IgE is important in the Th2 allergic immune response, omalizumab is prescribed for patients with severe allergic asthma with high levels of aero-allergen specific IgE.<sup>49</sup> Both anti-IL-5 and anti-IgE target Th2-mediated inflammation and are therefore eligible for treating patients with eosinophilic and allergic asthma. A systematic review of available literature has shown that mepolizumab and omalizumab have similar therapeutic effects and tolerability in responsive patients with severe asthma.<sup>50</sup> As eosinophilic inflammation is not necessarily mediated through the "classical" IgE pathway,<sup>51</sup> anti-IL-5 seems to be favorable in all patients with signs of eosinophilic inflammation, but a direct clinical comparison has not been performed yet.

## 4 | KEY THERAPEUTIC EFFECTS OF IL-5 INHIBITION IN EOSINOPHILIC-DRIVEN DISEASES

All asthma trials have in common that the effect of IL-5(R)-targeted therapy primarily leads to a decrease in disease exacerbations and steroid dependence rather than a direct effect on asthma characteristics such as changes in BHR and lung function.<sup>1</sup> For chronic rhinosinusitis with polyposis, both reslizumab and mepolizumab led to an improvement in symptoms for most, but not all patients.<sup>52,53</sup> Besides this clinical effect, surgery was no longer required in 30% of patients treated with mepolizumab.<sup>54</sup> For eosinophilic esophagitis, anti-IL-5 did not lead to improvement of symptoms or histological remission.<sup>55</sup> Treating patients with hypereosinophilic syndrome (HES) with mepolizumab resulted in a lower dependence of prednisolone in less

**TABLE 1** Mean eosinophil numbers in circulation or sputum before and after treatment with IL-5-targeted therapy

	Mean blood eosinophil before-after treatment million/mL (N)	Mean blood eosinophil before-after placebo million/mL (N)	Duration of treatment (frequency)	Mean % sputum eosinophil before-after treatment (N)	Mean% sputum eosinophil before-after placebo (N)	Treatment concentration (road of administration)
Haldar (2009) <sup>5</sup>	0.32-0.048 (29)	0.32-0.35 (32)	52 wks (Q4W)	6.8%-0.95% (29)	5.46%-2.78% (32)	Mepolizumab- 750 mg (IV)
Pavord (2012) <sup>6</sup>	0.25-0.03 (156)	0.28-ND (155)	52 wks (Q4W)	5.8%-0.48% (21)	6.8%-ND (24)	Mepolizumab- 750 mg (IV)
Pavord (2012) <sup>6</sup>	0.25-0.06 (153)	0.28-ND (155)	52 wks (Q4W)	13.9%-8.9% (18)	6.8%-ND (24)	Mepolizumab- 75 mg (IV)
Bjerner (2016) <sup>7</sup>	0.59-0.06 (102)	0.60-0.57 (103)	16 wks (Q4W)	-	-	Reslizumab- 3.0 mg/kg (IV)
Castro (2011) <sup>8</sup>	0.50-0.10 (52)	0.50-0.50 (50)	12 wks (Q4W)	10.7%-0.49% (16)	8.5%-5.2% (15)	Reslizumab- 3.0 mg/kg (IV)
Castro <sup>a</sup> (2015) <sup>9</sup>	0.62-0.14 (477)	0.66-0.52 (476)	52 wks (Q4W)	-	-	Reslizumab- 3.0 mg/kg (IV)
Corren (2016) <sup>11</sup>	0.28-0.021 (344)	0.28-0.28 (83)	16 wks (Q4W)	-	-	Reslizumab- 3.0 mg/kg (IV)
Bleecker <sup>b</sup> (2016) <sup>10</sup>	0.45-0.00 (202)	0.46-0.38 (202)	48 wks (Q4W)	-	-	Benralizumab- 30 mg (SC)
Fitzgerald <sup>b</sup> (2016) <sup>12</sup>	0.47-0.00 (234)	0.47-0.39 (238)	56 wks (Q4W)	-	-	Benralizumab- 30 mg (SC)
Nair <sup>b</sup> (2017) <sup>13</sup>	0.46-0.00 (63)	0.54-0.34 (66)	28 wks (Q4W)	4.8%-0.15% (8)	4.9-12.15% (4)	Benralizumab- 30 mg (SC)

IV, intravenous; Q4W, every 4 wks; ND, not determined; SC, subcutaneous.

<sup>a</sup>Mean of 2 cohorts.

<sup>b</sup>Median instead of mean.

than half of the patients.<sup>4</sup> Similarly, less than half of the eosinophilic granulomatosis and polyangiitis (EGPA) patients treated with mepolizumab reached complete remission.<sup>3</sup> Finally, in atopic dermatitis, mepolizumab did not lead to any clinical significant results, even though eosinophils may play a role in this disease.<sup>56</sup>

## 5 | INTERLEUKIN-5-TARGETED THERAPY: DIFFERENCE BETWEEN MICE AND MEN

There are key differences between murine and human data regarding pathogenesis of asthma and IL-5-targeted therapy.<sup>23,57-59</sup> Therefore, this review focusses on human data rather than murine data.

## 6 | EFFECT OF ANTI-IL-5(R) ON EOSINOPHIL AND BASOPHIL NUMBERS IN PERIPHERAL BLOOD AND TISSUES

There has not been a study performed that directly compared mepolizumab and reslizumab in a randomized controlled clinical trial. However, the results in the separate trials were comparable when it comes to reducing the number of circulating eosinophils (Table 1).<sup>5-11</sup> Still, it was speculated that in a certain group of patients with steroid unresponsive asthma, the fixed lower concentration of mepolizumab (100 mg) might be insufficient to reduce the number of eosinophils in the tissues. It was even speculated based on preliminary studies that this low concentration might lead to formation of immune complexes between IL-5 and mepolizumab potentially activating tissue eosinophils.<sup>14,15</sup> It was indeed found that lower concentrations of antibody were associated with lower reduction of both circulating and tissue eosinophils (Pavord et al 2012 Table 1).<sup>6,60</sup> However, no data to date have directly measured the formation of immune complexes under these conditions and it remains to be established whether immune complexes are a worry in IL-5-targeted therapy.

Benralizumab depletes circulating eosinophils completely, likely through ADCC, although in some patients, small number circulatory cells still remained.<sup>10,12,13,61</sup> However, the number of mucosal and/or submucosal airway eosinophils was less affected by the treatment when compared to the effect on the number of circulating cells.<sup>13,62</sup> It is too early to speculate how to interpret this counterintuitive finding, but the concept of the parallel presence of homeostatic and inflammatory eosinophils in the airways of eosinophilic asthmatics might shed some light on the tissue-dwelling cells in the future.<sup>63</sup>

Next to altering eosinophil levels, 750 mg of mepolizumab also reduced the number of basophils in the tissue and bone marrow, although this reduction was less pronounced compared to eosinophils.<sup>64</sup> It is, therefore, likely that basophils rely on different cytokines such as IL-3.<sup>65,66</sup>

The effect of mepolizumab on mast cells in tissue remains uncertain, because inconsistent results have been published.<sup>24,64,67</sup> Evidence that mast cells express the IL-5R $\alpha$  receptor has only been provided in vitro in very limited number of studies.<sup>68</sup>

Surprisingly, even though the expression of IL-5R $\alpha$  is threefold lower on basophils, still a complete depletion of also basophils in peripheral blood is seen after treatment with benralizumab.<sup>24,62</sup> This suggests that even though basophils rely on IL-3, a low membrane expression of IL-5R $\alpha$  can already induce clearance through ADCC.

## 7 | IL-5 IS NOT ESSENTIAL FOR EOSINOPHIL AND BASOPHIL DIFFERENTIATION AND PRODUCTION DURING HOMEOSTASIS

It is now tempting to speculate why in response to all above-mentioned therapies a small amount of eosinophils can still be found in sputum or bronchial tissue in patients with asthma.<sup>5,6,8,13,62</sup> Also treatment of patients with eosinophilic esophagitis with

mepolizumab induced a reduction in the number of eosinophils in circulation and in esophageal tissue when compared to placebo but did not lead to complete depletion of the cells. Interestingly, the number of homeostatic eosinophils in the duodenum was not altered by mepolizumab even after treatment with a high concentration of mepolizumab (1500 mg).<sup>69</sup> It is possible that IL-5R $\alpha$  expression can be redundant by co-expression of IL-3 and GM-CSF receptors and the presence of sufficient amounts of IL-3 or GM-CSF. It might even be that certain tissue eosinophils lose their IL-5R $\alpha$  such as described for cells found in the BAL after segmental allergen challenge.<sup>70</sup> To date, the data in the literature are not yet sufficient to reach such conclusion, but it is relevant to emphasize that tissue eosinophils are found in homeostasis without any indication that IL-5 is driving this response. The IL-5R $\alpha$  expression on these resident eosinophils of the duodenum seems lower, compared to circulating eosinophil.<sup>69</sup> This is not likely caused by homologous desensitization as it is completely unclear what the source of IL-5 would be under these conditions, but ILC2 cells are a putative source.<sup>16</sup>

## 8 | EFFECT OF ANTI-IL-5(RECEPTOR) MAB'S ON EOSINOPHIL PROGENITORS IN AND OUTSIDE THE BONE MARROW

Eosinophilopoiesis takes place in the bone marrow particularly under homeostasis. Under pathological conditions such as found in severe asthma, eosinophil progenitors (CD34<sup>+</sup> and IL-5R $\alpha$ -positive progenitors) have also been found in tissue and blood. This supports the hypothesis that extramedullary eosinophilopoiesis can take place.<sup>71</sup> In fact, in severe prednisolone-dependent asthma, the amount of eosinophil lineage-committed progenitor cells in sputum was over 700-fold higher compared to healthy controls, supporting a role of extramedullary hematopoiesis in eosinophil-driven disease.<sup>72</sup>

In the bone marrow, mepolizumab (administered 750 mg intravenously) reduced both the mature and late immature eosinophils (myelocytes and metamyelocytes) significantly compared to placebo while the number of EoPs (early progenitors) was not attenuated in blood or bone marrow.<sup>73</sup> It can be hypothesized that IL-5 mainly influences eosinophil proliferation and maturation of relatively late progenitors rather than the reduction of early progenitors themselves.<sup>73</sup> However, the relative amount of late immature and mature eosinophils in the bone marrow was still high after 2 months of therapy (mean of 1.9% and 1.1%, respectively). This lack of complete depletion of relatively late progenitors might be due to a role of other cytokines, such as IL-3 and GM-CSF.<sup>73</sup> Indeed, treatment with GM-CSF also leads to eosinophilia.<sup>74</sup>

Sehmi et al<sup>72</sup> showed an increase in blood levels of eosinophil lineage-committed progenitors compared to basal levels in patients who received 6 months of 100 mg mepolizumab by subcutaneous injection and not in patients who received placebo. This finding contradicts the fact that IL-5 can upregulate its own receptor on CD34<sup>+</sup> cells, because a reduction rather than an increase in EoPs would be expected.<sup>72,75</sup> Another finding of the same study was

that the number of EoPs and mature eosinophils in sputum was not attenuated in these patients with severe asthma. The authors hypothesized that this might be due to insufficient bioavailability within the tissue of 100 mg mepolizumab opposed to 750 mg. Indeed, in a comparable cohort of patients with severe asthma and prednisolone dependence, the same investigators showed that the number of early progenitors (EoPs) in blood was not attenuated by a low dose of mepolizumab in contrast to a weight-adjusted relatively high dose of intravenous reslizumab.<sup>14</sup> The authors speculated that if also mepolizumab would have been administered high enough (weight-adjusted), the number of EoPs would in fact be diminished with a consequent attenuation of sputum eosinophils. This hypothesis is, however, contradicted by the finding of an earlier study in which unaltered levels of EoPs in blood and bone marrow were found after treatment with 750 mg mepolizumab.<sup>73</sup> In sputum, the amount of CD34<sup>+</sup>/IL-5R $\alpha$ <sup>+</sup> cells was also attenuated after treatment with 750 mg mepolizumab, but this was also seen with placebo.<sup>73</sup>

In contrast to the findings obtained with anti-IL-5 antibodies, a complete depletion of eosinophils and their early progenitors in the bone marrow was seen in a very small cohort of 4 patients with asthma that were treated with a single dose of 1 mg/kg benralizumab (anti-IL-5R $\alpha$ ) via an intravenous injection.<sup>62</sup> It is possible that this difference in the amount of bone marrow eosinophils and late progenitors in comparison with mepolizumab is caused by antibody-dependent cell-mediated cytotoxicity.<sup>24</sup> Similarly, EoPs are also strongly attenuated in blood and sputum after treatment with benralizumab.<sup>61</sup>

## 9 | EFFECT OF ANTI-IL-5(RECEPTOR) ON CELLULAR PRIMING AND ACTIVATION

IL-5-targeted therapy also affects the release of eosinophil basic proteins in these compartments. For instance, it has been described that eosinophil cationic protein (ECP) is found in enhanced levels in serum of asthma patients.<sup>76</sup> Also increased concentrations of major basic protein (MBP),<sup>77</sup> eosinophil cationic protein (ECP),<sup>78</sup> and eosinophilic peroxidase (EPO) were found in tissue in this patient group.<sup>70,76</sup> These cytotoxic proteins can affect extracellular matrix (ECM) proteins in the reticular basement membrane (RBM).<sup>79</sup> In addition, they can modulate TGF- $\beta$ 1 expression by airway eosinophils. The concentrations of these mediators are blunted in BAL-fluid of patients with atopic asthma after treatment with mepolizumab.<sup>79</sup> This suggests that anti-IL-5 also reduces the degree of activation of eosinophils in the tissue. This hypothesis is supported by the finding that treatment with anti-IL-5 leads to inhibition of the activation of eosinophils by anti-IL-5 in a segmental antigen challenge model.<sup>80</sup> In this study, the intermediate upregulation of  $\beta$ <sub>2</sub>-integrin and the upregulation of P-selectin glycoprotein ligand-1 (PSGL-1) on circulating eosinophils was decreased after mepolizumab.<sup>80</sup> Eosinophils of patients treated with mepolizumab have also shown a reduced eotaxin-induced shape change *ex vivo*.<sup>81</sup>



Treatment with anti-IL-5R $\alpha$  antibodies (benralizumab) also caused a decreased concentration of ECP and eosinophil-derived neurotoxin (EDN) in serum, meaning that despite ADCC, no harmful proteins are released in blood.<sup>82</sup>

All of the above suggest that anti-IL-5(R $\alpha$ ) not only attenuates eosinophil numbers but also reduces priming and/or activation of eosinophils and can therefore diminish inflammation and remodeling as well.

Despite these findings, the situation with priming and activation in vivo is more complex than these in vitro data suggest. This complexity is illustrated by the finding that the expression of activation-associated markers on eosinophils is not all pointing at complete suppression of eosinophil priming by IL-5(R $\alpha$ )-targeted therapy. This is particularly shown by the expression of  $\beta_1$ -integrin in their active configuration in blood eosinophils, which was not altered by treatment with mepolizumab.<sup>80</sup>

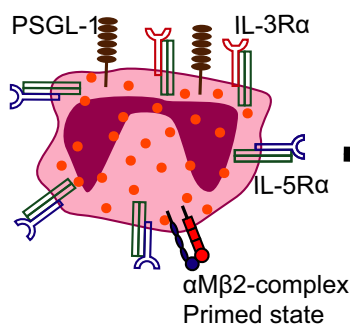
Also, the amount of major basic protein (MBP) in tissue was not found to drop with reduction in eosinophils.<sup>64</sup> A possible explanation could be that the remaining eosinophils are still able to produce sufficient amounts of MBP or there is a different source of MBP-1.<sup>83</sup> Alternative tissue sources of MBP other than placenta are poorly defined although mRNA of the MBP gene (PRG1) has been found in multiple tissues.<sup>84</sup> Similarly, unexpected was the finding that the rise of bronchoalveolar eosinophils after an allergen challenge was strongly reduced after administration of 750 mg of mepolizumab intravenously, whereas the remaining eosinophils still showed an IL-5 signature (enhanced expression of CD23, CD44, and CD69).<sup>70</sup>

## 10 | EFFECT OF IL-5(R $\alpha$ )-TARGETED THERAPY ON IL-5 LEVELS IN BLOOD AND TISSUE

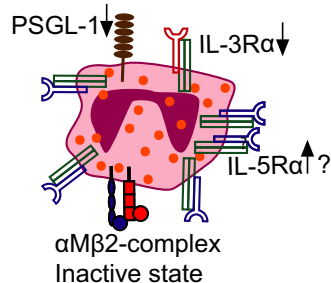
Treatment with anti-IL-5 (mepolizumab or reslizumab) eventually results in an increase in plasma levels of IL-5 (bound to Mab?) in patients with asthma, hypereosinophilic syndrome, and eosinophilic gastroenteritis even though there might be a slight decrease just after the start of treatment.<sup>81,85</sup> This increase was also partially found in sputum of patients with asthma treated with mepolizumab

but was surprisingly not seen in patients treated with reslizumab.<sup>14</sup> The authors of the latter article concluded that this difference was probably due to a low local concentration of mepolizumab (and not of reslizumab) which consequently would have resulted in the formation of immune complexes because the target antigen would still be in excess. These presumably long-lived immune complexes would then be a stable source of IL-5. Although this may be a plausible hypothesis, direct proof is lacking. An increase in the plasma level of IL-5 was also seen with high doses of mepolizumab (750 mg) or an intermediate dose with reslizumab (1 mg/kg) in a small study.<sup>81,85</sup> These data do not rule out the formation of immune complexes, but alternative hypotheses such as the existence of a feedback/feedforward mechanism are equally plausible. Indeed, after treatment with mepolizumab, there was a suggestion that both IL-5-producing CD4<sup>+</sup> and CD8<sup>+</sup> cells increased in peripheral blood.<sup>81</sup> This result has not been reproduced in another smaller study with reslizumab.<sup>85</sup> It is, therefore, unfortunate that in the study of Mukherjee et al,<sup>14</sup> no plasma levels of IL-5 were presented. An argument against a dominant role of immune complexes in modulating IL-5 levels in vivo came from data of a study on treating patients with benralizumab. Also in these patients, treatment with this antibody independently of the dose (25-200 mg) resulted in a clear increase in serum level of IL-5 while treating a similar cohort of patients with placebo did not.<sup>82</sup> The increase in serum/plasma IL-5 regardless of its cause during treatment with anti-IL-5(R $\alpha$ ) might have clinical consequences. For, there is a possibility that after termination of therapy, a rebound eosinophilia may arise. This has been suggested before for patients with hypereosinophilic syndrome, eosinophilic esophagitis, and nasal polyps receiving a single dose of reslizumab (varying dose of 1-3 mg/kg).<sup>53,85-87</sup> Remarkably, this rebound eosinophilia was absent in asthma patients treated with a higher single dose of reslizumab: 1 mg/kg instead of 0.3 mg/kg.<sup>87</sup> Moreover, in 2 studies in which patients were treated with multiple doses of mepolizumab at a higher dose of 10 mg/kg or 750 mg, a clear rebound phenomenon was also absent.<sup>81,88</sup> Apparently, multiple pathways are in control of eosinophilia. Interesting in this regard is the finding that treatment with GM-CSF induces eosinophilia but only transiently.<sup>89</sup> Nonetheless, these data implicate that discontinuation of the anti-IL-5 therapy should always be well monitored, because in some patients, aggravation of symptoms has been

### Before mepolizumab/reslizumab



### After mepolizumab/reslizumab



**FIGURE 1** Treatment with anti-IL-5 results in a decrease in IL-3R $\alpha$  expression on eosinophils in blood, while the expression of IL-5R $\alpha$  might increase. Besides eosinophils change from a primed state to a more inactive state, which is characterized by a reduced eotaxin-induced shape change and smaller size of eosinophils; downregulation of PSGL-1 and a change of configuration of the ( $\alpha$ M) $\beta$ 2-complex

described, particularly when steroids were tapered before cessation of the IL-5(R $\alpha$ )-targeted therapy.<sup>81,88</sup>

## 11 | EFFECT OF IL-5(R $\alpha$ )-TARGETED THERAPY ON IL-5R $\alpha$ AND IL-3R $\alpha$ EXPRESSION LEVELS ON EOSINOPHILS

A next level of complexity in IL-5(R $\alpha$ )-targeted therapy is the influence on the expression of cytokine receptors on eosinophils *in vivo*. Unfortunately, the effect of IL-5-targeted therapy on IL-5R $\alpha$  expression on eosinophils has not been sufficiently established. The only data available imply that anti-IL-5 therapy resulted in a small increase in the IL-5R $\alpha$  on eosinophils, but the results were not convincing.<sup>81</sup> This study of Stein et al<sup>81</sup> evaluated patients with different forms of eosinophilic inflammation that were treated with a high dose of mepolizumab. They found an 18% increase in IL-5R $\alpha$ -expression on blood eosinophils together with an increase rather than a decrease in plasma IL-5. However, in 2 other placebo-controlled studies performed in different cohorts, this effect of anti-IL-5 on the expression of IL-5R was absent.<sup>76,90</sup> A last relevant study was performed by Kelly et al<sup>70</sup> showing the same but not statistically significant trend of an increase in IL-5R $\alpha$  on eosinophils in asthma patients that were treated with a single dose of 750 mg of mepolizumab after an antigen challenge. It is not completely clear why IL-5R $\alpha$  expression on eosinophils would increase after anti-IL-5, but an autoregulatory pathway could be a possible explanation. Another explanation could be that IL-5 induced receptor shedding is naturally inhibited when less IL-5 is available, but as there is an increase in IL-5 after IL-5 inhibition, this explanation is less likely.

Surprisingly and still not fully understood in the study of Kelly et al<sup>70</sup> was the downregulation of IL-3R $\alpha$  expression on blood eosinophils in asthma patients after treatment with mepolizumab. The authors speculated that this change might be induced by mepolizumab during eosinophilopoiesis by yet poorly understood mechanism(s). It might also be possible that IL-5 neutralization could have led to selective homing of IL-3R $\alpha$ <sup>high</sup> cells into the tissue.<sup>70</sup> Supporting this hypothesis was the finding that IL-3R $\alpha$  expression on BAL eosinophils after the antigen challenge was not attenuated. Alternatively, one might argue that the low expression was caused by *in situ* eosinophilopoiesis from mobilized (early) progenitors under the influence of IL-3 and/or GM-CSF instead of IL-5 leading to cells with specific expression profiles. This hypothesis is supported by a recent study<sup>63</sup> potentially showing the existence of 2 distinct eosinophil subsets also in humans. This study of Mesnil et al<sup>63</sup> found difference in IL-3R $\alpha$  expression between lung parenchymal and peribronchial eosinophils of healthy human subjects and asthmatic patients. It is therefore tempting to speculate that these resident eosinophils are more dependent on IL-3 instead of IL-5. This might explain why tissue eosinophilia persists in patients treated with IL-5(R $\alpha$ )-targeted therapy despite low levels of eosinophils in peripheral blood.<sup>69</sup>

## 12 | CONCLUSION

### 12.1 | How to link the clinical action of IL-5(R $\alpha$ )-targeted therapy with the cell biology of IL-5?

Mechanisms of anti-IL-5 treatment are diverse and not fully understood (see graphical abstract and figure 1).

It is tempting to speculate why IL-5-targeted therapy is only beneficial for a part of the disease spectrum in patients with eosinophil-driven diseases. Firstly, eosinophils seem, especially in the tissue, only partially dependent on IL-5. It is still unclear whether these cells are the resident cells<sup>63</sup> or that redundancy with IL-3 and GM-CSF is present in the system. Several important issues need to be addressed before robust conclusions can be drawn. These include the following: (i) demonstration of the relative importance of local eosinophilopoiesis in affected tissues outside the bone marrow; (ii) direct identification of IL-5/anti-IL-5 immune complexes during anti-IL-5 therapy; and (iii) local survival of eosinophils due to other cytokines than IL-5 such as IL-3 or GM-CSF. It is also important to emphasize that inhibiting IL-5 does not lead to complete disarmament of eosinophils which means that remaining eosinophils are still able to release toxins that can cause tissue damage. Lastly, it is possible that eosinophil-dominated inflammation in the tissue is not exclusively mediated by eosinophils. This latter observation is illustrated for eosinophilic asthma where patients might still experience symptoms and exacerbations because of neutrophils.<sup>91</sup> Also a (near to) complete depletion of eosinophils in patients with eosinophilic asthma receiving benralizumab does not clearly lead to a better improvement in the reduction in asthma exacerbations compared to the treatment with anti-IL-5.<sup>1</sup> Interestingly, patients with severe "eosinophilic" asthma that are treated with dupilumab (anti-IL-4 receptor  $\alpha$ -subunit shared between IL-4 and IL-13 receptors) show a comparable or maybe an even better clinical improvement while the number of eosinophils in circulation and in sputum remains unchanged.<sup>92</sup> This indicates that even within the spectrum of Th2-driven diseases, the pathogenesis of inflammation leading to tissue damage is complex. It is, therefore, not particularly surprising that single mediator antagonists only affect part of the disease spectrum in patients with complex immune-mediated diseases.

### CONFLICTS OF INTEREST

Both authors declare that they have no conflict of interests.

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