Eosinophils represent approximately 1% of peripheral blood leukocytes in normal donors and their maturation and differentiation in the bone marrow are mainly regulated by interleukin (IL)-5 [Broughton et al. 2015]. IL-5, a cytokine that belongs to the β common-chain family, together with IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF), stimulates also the activation and survival of eosinophils and, to some extent, of basophils. IL-5 binds to a heterodimer receptor composed of the specific subunit IL-5Rα and a common subunit βc shared with IL-3 and GM-CSF. Human eosinophils express approximately a three-fold higher level of IL-5Rα compared with basophils. Major sources of IL-5 are T-helper 2 (Th2) cells, mast cells, CD34+ progenitor cells, invariant natural killer (NK) T-cells, group 2 innate lymphoid cells (ILC2s), and eosinophils themselves. ILC2s control not only eosinophil number but also their circadian cycling through the production of IL-5.

**Keywords:** asthma, eosinophilia, exacerbations, IL-5, mepolizumab, personalized medicine, severe asthma, targeted therapy

**Introduction**

Paul Ehrlich announced the discovery of the eosinophil in a presentation to the Physiological Society of Berlin on 17 January 1879 [Ehrlich, 1879a]. His next paper contained an extensive description of these cells [Ehrlich, 1879b]. Ehrlich identified peripheral blood eosinophils thanks to their capacity to be stained by eosin. He suggested that eosin interacted, like a ‘magic bullet’, with a specific eosinophil receptor. Ehrlich’s hypothesis of ‘chemical affinities’ in biological processes is epitomized in his maxim *corpora non agunt nisi fixata*, namely, a substance is not biologically active unless it is bound by a receptor. This led him to the use of a magic bullet to treat a given disease. Thus, Ehrlich was not only the founder of modern immunology, but he was also a pioneer in pharmacological sciences.

Eosinophils represent approximately 1% of peripheral blood leukocytes in normal donors and their maturation and differentiation in the bone marrow are mainly regulated by interleukin (IL)-5 [Broughton et al. 2015]. IL-5, a cytokine that belongs to the β common-chain family, together with IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF), stimulates also the activation and survival of eosinophils and, to some extent, of basophils. IL-5 binds to a heterodimer receptor composed by the specific subunit IL-5Rα and a common subunit βc shared with IL-3 and GM-CSF. Human eosinophils express approximately a three-fold higher level of IL-5Rα compared with basophils. Major sources of IL-5 are T-helper 2 (Th2) cells, mast cells, CD34+ progenitor cells, invariant natural killer (NK) T-cells, group 2 innate lymphoid cells (ILC2s), and eosinophils themselves. ILC2s control not only eosinophil number but also their circadian cycling through the production of IL-5.
Mepolizumab in adults with eosinophilic asthma

Given the critical role of IL-5 in influencing several activities of eosinophils, this cytokine and its receptor attracted the attention of pharmaceutical industries as a possible target in the treatment of hypereosinophilic diseases including eosinophilic asthma [Varricchi et al. 2016]. Mepolizumab (Nucala; GlaxoSmithKline, London, UK) was the first anti-IL-5 humanized monoclonal antibody described over 15 years ago [Zia-Amirhosseini et al. 1999]. Mepolizumab binds to IL-5 with high specificity (maximal inhibitory concentration <1 nm) and affinity (approximately 4.2 pM), thus preventing its binding to the α chain of the IL-5R complex on eosinophils and basophils. A preclinical study on the pharmacology and safety of mepolizumab in naïve and *Ascaris suum*-sensitive *Cynomolgus* monkeys demonstrated that a single intravenous (iv) dose reduced blood eosinophilia for 6 weeks without affecting acute bronchoconstriction [Hart et al. 2001]. Two initial studies evaluated, in a randomized, double-blind, parallel group, the effects of iv anti-IL-5 in a small group of mild asthmatic patients (Table 1). Although anti-IL-5 produced a decrease in blood eosinophils and partial reduction of airway and bone marrow eosinophils, there were no effects on airway hyperresponsiveness (AHR) and late response to inhaled allergens [Flood-Page et al. 2003; Leckie et al. 2000]. Similarly, in a multicenter study to evaluate safety and efficacy of iv mepolizumab in patients with moderate persistent asthma, the treatment produced a rapid and marked reduction in blood eosinophils, without improving lung functions and symptoms [Flood-Page et al. 2007]. These initial studies produced frustrating results, and several investigators questioned the efficacy of this targeted therapy on asthma treatment [Flood-Page et al. 2003; Wenzel,

| Study                  | Disease severity                  | Dosage/delivery                      | Outcome summary                                                                 |
|------------------------|-----------------------------------|--------------------------------------|---------------------------------------------------------------------------------|
| [Menzies-Gow et al. 2003] | Mild asthmatics                    | 750 mg iv every 4 weeks for 3 months | ↓ Eosinophils within bronchial mucosa                                           |
| [Flood-Page et al. 2003] | Mild asthmatics                    | 750 mg iv every 4 weeks for 3 months | ↓ Blood eosinophils, ↓ Airway eosinophils by 50%, No effect on PEF, FEV₁, and bronchial hyperresponsiveness |
| [Flood-Page et al. 2007] | Moderate asthmatics                | 250 or 750 mg iv every 4 weeks for 3 months | ↓ Blood and sputum eosinophils, No effect on PEF, FEV₁, and AQLQ               |
| [Haldar et al. 2009]   | Severe eosinophilic asthmatics     | 750 mg iv every 4 weeks for 1 year   | ↓ Blood eosinophils, ↓ Exacerbations, ↑ AQLQ                                   |
| [Nair et al. 2009]     | Prednisone-dependent eosinophilic asthmatics | 750 mg iv every 4 weeks for 5 months | ↓ Blood and sputum eosinophils, ↓ Exacerbations, Prednisone-sparing effect      |
| [Pavord et al. 2012]   | Severe eosinophilic asthmatics     | 1 of 3 doses (750, 250 or 75 mg) iv every 4 weeks for 13 months | ↓ Blood and sputum Eosinophils, ↓ Exacerbations, No effect on FEV₁, and AQLQ |
| [Ortega et al. 2014b]  | Severe eosinophilic asthmatics MENSA STUDY | 100 mg sc every 4 weeks for 8 months | ↓ Blood eosinophilia, ↓ Exacerbations, ↑ FEV₁, ↑ ACQ-5 score                  |
| [Bel et al. 2014]      | Severe eosinophilic asthmatics SIRIUS STUDY | 100 mg sc every 4 weeks for 6 months | ↓ Blood eosinophils, ↓ Exacerbations, Glucocorticoid sparing-effect, ↑ ACQ-5 score |
| [Haldar et al. 2014]   | Severe eosinophilic asthmatics     | 750 mg iv every 4 weeks Outcome after cessation | Rapid increase in blood and sputum eosinophils followed by increased of asthma symptoms and exacerbations |

ACQ-5, Asthma Control Questionnaire-5; AQLQ, Asthma Quality of Life Questionnaire; FEV₁, forced expiratory volume in one second; iv, intravenously; PEF, peak expiratory flow; sc, subcutaneously.
Therapeutic Advances in Respiratory Disease 11(1)

2009]. In fact, no significant effects were found in terms of AHR, peak expiratory flow (PEF), and forced expiratory volume in one second (FEV₁) despite a remarkable reduction in blood eosinophilia [Flood-Page et al. 2003; Leckie et al. 2000].

Retrospectively, two major factors could explain these preliminary results. First, an incorrect selection of patients with mild or moderate asthma without significant eosinophilia and airway eosinophilic inflammation; second, perhaps, the iv administration of mepolizumab. The latter observation is relevant because there is evidence that subcutaneous (sc) administration of human polyclonal immunoglobulins provides more prolonged serum levels of immunoglobulins compared with iv infusion [Spadaro et al. 2016]. The two subsequent studies in patients with refractory eosinophilic asthma demonstrated some efficacy of mepolizumab in the control of severe asthma. The first one was a study on patients who had refractory eosinophilic asthma and a history of recurrent severe exacerbations [Haldar et al. 2009]. Patients received iv infusion of mepolizumab at monthly intervals for one year. This treatment caused fewer severe exacerbations than placebo with a significant improvement in the glucocorticoid dose and exacerbations, while improving the control of asthma symptoms. Another conclusive research was the MENSA trial, a multicenter, double-blind, placebo-controlled study recruiting a large number of patients with severe eosinophilic asthma [Ortega et al. 2014a]. The predictors identified were blood eosinophils, airway reversibility and body mass index.

The latter study represented an important progress in the selection of subgroups of patients affected by severe eosinophilic asthma with frequent exacerbations. A supervised cluster analysis with recursive partitioning approach was applied to the DREAM study to identify characteristics able to maximize the differences among subgroups [Ortega et al. 2014a]. The predictors identified were blood eosinophils, airway reversibility and body mass index.

Two studies evaluated the effects of sc mepolizumab in patients with severe asthma with more than two exacerbations in the previous year and a blood eosinophil count greater than 0.15 × 10⁹/l at screening. In the SIRIUS trial, the primary outcome was the degree of the glucocorticoid-sparing effect of mepolizumab (100 mg sc every 4 weeks for 20 weeks) [Bel et al. 2014]. Anti-IL-5 reduced the glucocorticoid dose and exacerbations, while improving the control of asthma symptoms. Another conclusive research was the MENSA trial, a multicenter, double-blind, placebo-controlled study recruiting a large number of patients with severe eosinophilic asthma [Ortega et al. 2014b]. Mepolizumab (100 mg sc every 4 weeks for 8 months) reduced asthma exacerbations, eosinophilia and improved FEV₁ and QoL. Importantly, a pharmaco-economic evaluation of the latter study showed that mepolizumab is cost effective in that specific context [Basu et al. 2016]. A recent post-hoc analysis of data from the DREAM and MENSA trials has shown a close relationship between baseline blood eosinophil count and clinical efficacy of mepolizumab in patients with severe eosinophilic asthma and a history of exacerbations. The authors noted a clinically-relevant reduction in exacerbation frequency, defined as at least a 30% decrease in this endpoint, in patients starting from a baseline count of 150 cells/µl and showing better outcomes in patients with over 300 eosinophils/µl [Ortega et al. 2016]. Even if the blood eosinophilic threshold is still under discussion, the use of this baseline biomarker could help to select patients who are likely to achieve more benefits in asthma control with mepolizumab. Based on the results of the SIRIUS and MENSA
trials, first the United States (US) Food and Drug Administration, and subsequently, the European Medicines Agency approved mepolizumab as an add-on maintenance treatment for severe eosinophilic asthma in adults. Nucala® (mepolizumab) is currently licensed in the US, Japan and more than 30 countries worldwide.

**Conclusion**

Several studies demonstrated that mepolizumab is well tolerated and efficacious in adults with severe eosinophilic asthma treated for up to 1 year. A recent study examined the outcome of patients with severe asthma after cessation of mepolizumab [Haldar et al. 2014]. In fact, there was some concern about a possible risk of ‘rebound’ of eosinophilic airway inflammation after stopping mepolizumab [Kim et al. 2004]. In addition, mepolizumab was associated with up-regulation of IL-5 synthesis by Th2 cells and overexpression of IL-5R by eosinophils [Stein et al. 2008]. Cessation of mepolizumab resulted in a rapid increase of blood eosinophils followed by a gradual increase in asthma symptoms and exacerbations [Haldar et al. 2014]. This observation emphasizes the importance of maintaining suppression of eosinophilic inflammation in these patients. Although eosinophil deficiency appears to have no effects on normal health [Gleich et al. 2013], these cells have been implicated in cancer rejection [Carretero et al. 2015] and several cancers are associated with eosinophilia [Simson et al. 2007]. It has been suggested that ‘targeted anti-eosinophilic strategies may unmask or even accelerate progression’ of certain tumors in patients with hypereosinophilic syndrome [Roufosse et al. 2010]. Consequently, long-term studies are required to evaluate the safety of targeted anti-eosinophilic treatments.

In conclusion, targeted therapy with mepolizumab appears to be effective in the treatment of severe eosinophilic asthma thereby echoing the concept of ‘magic bullets’ epitomized by Paul Ehrlich more than 130 years ago. In the current context, his maxim corpora non agunt nisi fixata can be translated as ‘mepolizumab is pharmacologically active in severe eosinophilic asthma because it binds to IL-5’.

**Conflict of interest statement**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**References**

Bel, E., Wenzel, S., Thompson, P., Prazma, C., Keene, O., Yancey, S. et al. (2014) Oral glucocorticoid-sparing effect of mepolizumab in eosinophilic asthma. *N Engl J Med* 371: 1189–1197.

Bischoff, S., Brunner, T., De Weck, A. and Dahinden, C. (1990) Interleukin 5 modifies histamine release and leukotriene generation by human basophils in response to diverse agonists. *J Exp Med* 172: 1577–1582.

Broughton, S., Nero, T., Dhagat, U., Kan, W., Hercus, T., Tvorogov, D. et al. (2015) The βc receptor family - structural insights and their functional implications. *Cytokine* 74: 247–258.

Carretero, R., Saktioglu, I., Garbi, N., Salgado, O., Beckhove, P. and Hammerling, G. (2015) Eosinophils orchestrate cancer rejection by normalizing tumor vessels and enhancing infiltration of CD8(+) T cells. *Nat Immunol* 16: 609–617.

Ehrlich, P. (1879a) Beiträge zur Kenntniss der granulirten Bindegewebszellen und der eosinophilen Leukocythen. *Arch Anat Physiol (Leipzig)* 3: 166–169.

Ehrlich, P. (1879b) Über die spezifischen Granulationen des Blutes. *Arch Anat Physiol (Leipzig)* 3: 571–579.

Fallon, P., Ballantyne, S., Mangan, N., Barlow, J., Dasvarma, A., Hewett, D. et al. (2006) Identification of an interleukin (IL)-25-dependent cell population that provides IL-4, IL-5, and IL-13 at the onset of helminth expulsion. *J Exp Med* 203: 1105–1116.

Flood-Page, P., Menzies-Gow, A., Kay, A. and Robinson, D. (2003) Eosinophil's role remains uncertain as anti-interleukin-5 only partially depletes numbers in asthmatic airway. *Am J Respir Crit Care Med* 167: 199–204.
(2007) A study to evaluate safety and efficacy of mepolizumab in patients with moderate persistent asthma. *Am J Respir Crit Care Med* 176: 1062–1071.
Gleich, G., Klion, A., Lee, J. and Weller, P. (2013) The consequences of not having eosinophils. *Allergy* 68: 829–835.
Haldar, P., Brightling, C., Hargadon, B., Gupta, S., Monteiro, W., Sousa, A. *et al.* (2009) Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med* 360: 973–984.
Haldar, P., Brightling, C., Singapuri, A., Hargadon, B., Gupta, S., Monteiro, W. *et al.* (2014) Outcomes after cessation of mepolizumab therapy in severe eosinophilic asthma: a 12-month follow-up analysis. *J Allergy Clin Immunol* 133: 921–923.
Hart, T., Cook, R., Zia-Amirhosseini, P., Minthorn, E., Sellers, T., Maleeff, B. *et al.* (2001) Preclinical efficacy and safety of mepolizumab (SB-240563), a humanized monoclonal antibody to IL-5, in Cynomolgus monkeys. *J Allergy Clin Immunol* 108: 250–257.
Hirai, K., Yamaguchi, M., Misaki, Y., Takaishi, T., Ohta, K., Morita, Y. *et al.* (1990) Enhancement of human basophil histamine release by interleukin 5. *J Exp Med* 172: 1525–1528.
Kim, Y., Prussin, C., Martin, B., Law, M., Haverty, T., Nutman, T. *et al.* (2004) Rebound eosinophilia after treatment of hyper eosinophilic syndrome and eosinophilic gastroenteritis with monoclonal anti-IL-5 antibody SCH55700. *J Allergy Clin Immunol* 114: 1449–1455.
Kolbeck, R., Koizhic, A., Koike, M., Peng, L., Andersson, C., Damaschroder, M. *et al.* (2010) MEDI-563, a humanized anti-IL-5 receptor alpha mAb with enhanced antibody-dependent cell-mediated cytotoxicity function. *J Allergy Clin Immunol* 125: 1344–1353.
Leckie, M., ten Brinke, A., Khan, J., Diamant, Z., O’Connor, B., Walls, C. *et al.* (2000) Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 356: 2144–2148.
McKenzie, A. (2006) Identification of an interleukin (IL)-25-dependent cell population that provides IL-4, IL-5, and IL-13 at the onset of helminth expulsion. *J Exp Med* 203: 1105–1116.
Menzie-Gow, A., Flood-Page, P., Schmi, R., Burman, J., Hamid, Q., Robinson, D. *et al.* (2003) Anti-IL-5 (mepolizumab) therapy induces bone marrow eosinophil maturational arrest and decreases eosinophil progenitors in the bronchial mucosa of atopic asthmatics. *J Allergy Clin Immunol* 111: 714–719.
Nair, P., Pizzichini, M., Kjarsgaard, M., Inman, M., Efthimiadis, A., Pizzichini, E. *et al.* (2009) Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. *N Engl J Med* 360: 985–993.
Nussbaum, J., van Dyken, S., von Moltke, J., Cheng, L., Mohapatra, A., Molofsky, A. *et al.* (2013) Type 2 innate lymphoid cells control eosinophil homeostasis. *Nature* 502: 245–248.
Ortega, H., Li, H., Suruki, R., Albers, F., Gordon, D. and Yancey, S. (2014a) Cluster analysis and characterization of response to mepolizumab. A step closer to personalized medicine for patients with severe asthma. *Ann Am Thorac Soc* 11: 1011–1017.
Ortega, H., Liu, M., Pavord, I., Brusselle, G., FitzGerald, J., Chetta, A. *et al.* (2014b) Mepolizumab treatment in patients with severe eosinophilic asthma. *N Engl J Med* 371: 1198–1207.
Ortega, H., Yancey, S., Mayer, B., Gunsoy, N., Keene, O., Bleekere, E. *et al.* (2016) Severe eosinophilic asthma treated with mepolizumab stratified by baseline eosinophil thresholds: a secondary analysis of the DREAM and MENSA studies. *Lancet Respir Med* 4: 549–556.
Pavord, I., Korn, S., Howarth, P., Bleekere, E., Buhl, R., Keene, O. *et al.* (2012) Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo-controlled trial. *Lancet* 380: 651–659.
Phillips, C., Coward, W., Pritchard, D. and Hewitt, C. (2003) Basophils express a type 2 cytokine profile on exposure to proteases from helminths and house dust mites. *J Leukoc Biol* 73: 165–171.
Rosas, M., Dijfers, P., Lindemans, C., Lammers, J., Koenderman, L. and Coffer, P. (2006) IL-5-mediated eosinophil survival requires inhibition of GSK-3 and correlates with beta-catenin relocalization. *J Leukoc Biol* 80: 186–195.
Roufosse, F., de Lavareille, A., Schandene, L., Cogan, E., Georgelas, A., Wagner, L. *et al.* (2010) Mepolizumab as a corticosteroid-sparing agent in lymphocytic variant hypereosinophilic syndrome. *J Allergy Clin Immunol* 126: 828–835.
Simson, L., Ellyard, J., Dent, L., Matthaei, K., Rothenberg, M., Foster, P. *et al.* (2007) Regulation of carcinogenesis by IL-5 and CCL11: a potential role for eosinophils in tumor immune surveillance. *J Immunol* 178: 4222–4229.
Spadaro, G., Pecoraro, A., De Renzo, A., Della Pepa, R. and Genovese, A. (2016) Intravenous versus subcutaneous immunoglobulin replacement in secondary hypogammaglobulinemia. *Clin Immunol* 166–67: 103–104.
Stein, M., Villanueva, J., Buckmeier, B., Yamada, Y., Filipovich, A., Assa’ad, A. et al. (2008) Anti-IL-5 (mepolizumab) therapy reduces eosinophil activation ex vivo and increases IL-5 and IL-5 receptor levels. *J Allergy Clin Immunol* 121:1473–1483.

Takatsu, K. (2013) Interleukin-5 and its receptor molecules. In: Lee, J. and Rosenberg, H. (eds), *Eosinophils in Health and Disease*. Waltham, MA: Academic Press, pp. 97–105.

Varricchi, G., Bagnasco, D., Borriello, F., Heffler, E. and Canonica, G. (2016) Interleukin-5 pathway inhibition in the treatment of eosinophilic respiratory disorders: evidence and unmet needs. *Curr Opin Allergy Clin Immunol* 16: 186–200.

Wenzel, S. (2009) Eosinophils in asthma – closing the loop or opening the door? *N Engl J Med* 360: 1026–1028.

Yamaguchi, Y., Suda, T., Ohta, S., Tominaga, K., Miura, Y. and Kasahara, T. (1991) Analysis of the survival of mature human eosinophils: interleukin-5 prevents apoptosis in mature human eosinophils. *Blood* 78: 2542–2547.

Zia-Amirhosseini, P., Minthorn, E., Benincosa, L., Hart, T., Hottenstein, C., Tobia, L. et al. (1999) Pharmacokinetics and pharmacodynamics of SB-240563, a humanized monoclonal antibody directed to human interleukin-5, in monkeys. *J Pharmacol Exp Ther* 291: 1060–1067.