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1 **Pharmacological approaches to target type 2 cytokines in asthma**

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21 **Abstract**

22

23 Asthma is the most common chronic lung disease, affecting more than 250 million people
24 worldwide. The heterogeneity of asthma phenotypes represents a challenge for adequate
25 assessment and treatment of the disease. However, approximately 50% of asthma patients
26 present with chronic type 2 inflammation initiated by alarmins, such as IL-33 and thymic
27 stromal lymphopoietin (TSLP), and driven by the T_H2 interleukins IL-4, IL-5 and IL-13.
28 These cytokines have therefore become important therapeutic targets in asthma. Here, we
29 discuss current knowledge on the structure and functions of these cytokines in asthma. We
30 review preclinical and clinical data obtained with monoclonal antibodies (mAbs) targeting
31 these cytokines or their receptors, as well as novel strategies under development, including
32 bispecific mAbs, designed ankyrin repeat proteins (DARPs), small molecule inhibitors and
33 vaccines targeting type 2 cytokines.

34 **Keywords**

35 Interleukin, Allergy, Asthma, Antibody, DARPin, Vaccine, Dupilumab, Mepolizumab,
36 Reslizumab, Benralizumab, Tezepelumab

37

38 **Abbreviations**

39

40 ADCC: antibody-dependent cell-mediated cytotoxicity

41 AHR: airway hyperreactivity

42 CRM₁₉₇: diphtheria ‘cross-reactive material 197’

43 DARPins: designed ankyrin repeat proteins

44 DC: dendritic cell

45 FeNO: fractioned exhaled nitric oxide

46 FEV₁: forced-expiratory volume in 1 second

47 γ c: common gamma chain

48 GM-CSF: granulocyte-macrophage colony-stimulating factor

49 Ig: immunoglobulin

50 IL: interleukin

51 IRS: insulin receptor substrate

52 JAK: Janus kinase

53 KLH: keyhole limpet hemocyanin

54 mAb: monoclonal antibody

55 NKT: natural killer T cell

56 STAT: signal transducer and activator of transcription

57 T_{FH}: follicular helper T cell

58 TGF: transforming growth factor

59 T_H2: type 2 helper T cell

60 TSLP: thymic stromal lymphopoietin

61 VCAM: vascular cell adhesion molecule

62 VEGF: vascular endothelial factor

63 VLP: virus-like particles

64 WT: wild type

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98

99 **Introduction**

100

101 Asthma is the most common chronic lung disease, affecting over 250 million people in
102 2019 and causing 461,000 deaths (Vos, et al., 2020). It is well recognized that inhaled
103 corticosteroids (ICS) are the cornerstone of asthma treatment (Reddel, et al., 2015).
104 Although ICS has played a crucial role in the decrease in asthma mortality, this treatment
105 has some limitations. The low adherence (Stempel, Roberts, & Stanford, 2004) and
106 frequent technical errors (Sanchis, Gich, Pedersen, & Team, 2016) in the use of ICS are
107 associated with asthma exacerbations (Engelkes, Janssens, de Jongste, Sturkenboom, &
108 Verhamme, 2015). Moreover, ICS is sometimes not sufficient for the control of severe
109 asthma patients who still experience frequent exacerbations and hospitalizations with high
110 morbidity and mortality (Chung, et al., 2013; Hossny, Caraballo, Casale, El-Gamal, &
111 Rosenwasser, 2017; Lang, 2015). On the other hand, oral steroids have too many side
112 effects for their use in clinical practice. Consequently, other treatments are needed in
113 severe asthma, but also in mild-to-moderate asthma.

114

115 While asthma is an heterogenous disease with a variety of phenotypes triggered by different
116 inflammatory responses, type 2 inflammation occurs in about half of asthma patients (Barnes,
117 2018; Fahy, 2015). Type 2 immune responses are initiated by alarmins, such as IL-33 and
118 thymic stromal lymphopietin (TSLP), and characterized by high levels of IgE antibodies and
119 type 2 cytokines, such as interleukin-4 (IL-4), IL-5 and IL-13, and eosinophilia (Barnes,
120 2018; Fahy, 2015). It is therefore not surprising that most biotherapies developed to date
121 for asthma target type 2 cytokines, alarmins or IgE.

122

123 Recent reviews have focused on IgE targeted therapies. Therefore, this review will focus
124 on strategies to inhibit alarmins and type 2 cytokines in asthma. We will first discuss
125 current knowledge on the structure and functions of these cytokines. We will then review
126 preclinical and clinical data obtained with monoclonal antibodies (mAbs) targeting these
127 cytokines or their receptors, as well as novel strategies under preclinical development.

128

129 **1. IL-4, IL-13 and their receptors**

130

131 *1.1. IL-4 expression and functions*

132

133 Human IL-4 was first isolated from a concanavalin A-activated human T-cell cDNA library
134 (Yokota, et al., 1986). IL-4 is a 15 kDa globular protein with 129 amino acids, formed by 4 α
135 helices and 3 disulfide bonds (**Fig. 1A**). It is mainly produced by CD4⁺ T_H2 cells, but also by
136 basophils, eosinophils, mast cells and natural killer T (NKT) cells (Paul, 2015; Sokol, Barton,
137 Farr, & Medzhitov, 2008; Yoshimoto & Paul, 1994). Type 2 innate lymphoid cells (ILC2) can
138 probably also produce small amounts of IL-4 (Doherty, et al., 2013; Pelly, et al., 2016).
139 Several groups have shown that type 2 follicular helper T cells (T_{FH}2) can also release IL-4 in
140 the context of helminth infection (King & Mohrs, 2009; Meli, Fontes, Leung Soo, & King,
141 2017). More recently, it was reported that a distinct T_{FH} population, named T_{FH}13, can
142 produce both IL-4 and IL-13, and is induced in response to allergens but not helminths
143 (Gowthaman, et al., 2019).

144

145 Increased IL-4 levels have been observed in the serum of allergic patients and in the
146 bronchoalveolar lavage fluid of asthmatic patients (Walker, et al., 1994). IL-4 regulates a
147 wide spectrum of cellular functions, such as T cell proliferation and T_H2 polarization (Kopf,
148 et al., 1993). The central role of IL-4 in type 2 immune responses was first demonstrated
149 using mouse models of parasite infection, where treatment with neutralizing anti-IL-4
150 antibodies abrogated protective immunity to *Heligmosomoides polygyru* infection (Urban,
151 Katona, Paul, & Finkelman, 1991). In addition, treatment with recombinant IL-4 was able to
152 cure gastrointestinal nematode infections in mice (Urban, Maliszewski, Madden, Katona, &
153 Finkelman, 1995).

154 Around the same time, Brusselle et al. demonstrated that IL-4-deficient mice develop
155 markedly reduced eosinophilia and peribronchial inflammation in a model of asthma as
156 compared to wild-type controls (Brusselle, et al., 1994). In agreement with that, Lukacs and
157 colleagues demonstrated that the administration of anti-IL-4 neutralizing antibodies induces a
158 ten-fold reduction in airway eosinophilia in a model of asthma (Lukacs, Strieter, Chensue, &
159 Kunkel, 1994). IL-4 was initially described as a B cell growth factor and an IgG1-inducing
160 factor in mice (Noma, et al., 1986). Subsequent work indicated that IL-4 is a key inducer of
161 class switching to IgE in B cells (Cohn, Homer, Marinov, Rankin, & Bottomly, 1997; Oettgen
162 & Geha, 2001). Although IL-13 can also induce class switching to IgE, data from mice
163 deficient for IL-4 or IL-13 indicate that IL-4 plays a predominant role in IgE production
164 (Gour & Wills-Karp, 2015). Moreover, IL-4 also regulates the expression of the two IgE
165 receptors FcεRI and CD23 (FcεRII) (Novak, Kraft, & Bieber, 2001; Toru, et al., 1996). In
166 addition to its role on B cells, IL-4 is also a major T cell growth factor and promotes the
167 differentiation of naïve T cells into T_H2 cells, which play an important role in allergic asthma.
168 Many additional functions of IL-4 have been described on a broad range of cells which could
169 also play important roles in asthma. This includes the polarization of macrophages into an M2
170 phenotype, characterized by anti-inflammatory and tissue regeneration functions (Junttila,
171 2018).

172

173 A second isoform of IL-4, called IL-4δ2, has also been described. It is produced by alternative
174 splicing of exon 2. Although early *in vitro* experiments suggested that IL-4δ2 could act as a
175 natural antagonist of IL-4 (Atamas, Choi, Yurovsky, & White, 1996), more recent *in vivo* data
176 in mice indicate that overexpression of IL-4 or IL-4δ2 induces the recruitment of T and B
177 cells. However, unlike IL-4, IL-4δ2 does not induce pulmonary eosinophilia or goblet cell
178 hyperplasia (Luzina, et al., 2011).

179

180 *1.2. IL-13 expression and functions*

181

182 IL-13 is a 13 kDa globular protein. It has a secondary structure similar to that of IL-4, with 4
183 α helices (**Fig. 1C**). However, it only has 25% sequence homology to IL-4 (Rael & Lockey,
184 2011). IL-13 is produced by activated T_H2 cells, NKT cells, mast cells, basophils, eosinophils,
185 B cells, macrophages and activated type 2 innate lymphoid cells (ILC2) (Hajoui, et al., 2004;
186 Hancock, Armstrong, Gama, & Millar, 1998; Marone, et al., 2019; Shimokawa, et al., 2017).
187 Similar to IL-4, studies in mice have revealed the key role of IL-13 in type 2 immunity, by
188 showing that the cytokine plays a pivotal role in host defense against intestinal parasites
189 (Urban, et al., 1998). The same year, Grünig and colleagues demonstrated that the
190 neutralization of IL-13 using a soluble IL-13 receptor α_2 -human Fc fusion protein (IL-13R-
191 Fc) can reduce key features of asthma in mice, including airway hyperresponsiveness,
192 eosinophilia and mucus production (Grünig, et al., 1998). Moreover, they also showed in the
193 same study that the administration of recombinant IL-13 or IL-4 induced an asthma-like
194 phenotype in mice (Grünig, et al., 1998). The year after, Zhu et al. reported that transgenic
195 mice expressing IL-13 under the dependency of the promoter of the lung-specific Clara cell
196 10-kDa protein develop spontaneous lung eosinophilia and goblet cell hyperplasia (Zhu, et al.,
197 1999).

198

199 Some of the functions of IL-13 are similar to those of IL-4. This includes inducing
200 proliferation of B cells and promoting class switching to IgE (Oettgen & Geha, 2001). IL-13
201 also induces polarization of macrophages into a M2 phenotype (Martinez-Nunez, Louafi, &
202 Sanchez-Elsner, 2011). IL-13 also promotes the survival and activation of eosinophils and
203 their recruitment to the site of inflammation (Luttmann, et al., 1996). This can be explained in
204 part by the fact that IL-13 induces the expression of IL-5 and eosinophil chemokines such as

205 eotaxin, as well as the expression of VCAM-1 on endothelial cells (Bochner, Klunk,
206 Sterbinsky, Coffman, & Schleimer, 1995; Pope, et al., 2001).

207

208 In addition, IL-13 has important functions on non-hematopoietic cells. It is also a potent
209 inducer of VCAM-1 expression on human lung fibroblasts, boosting muscular contraction and
210 enhancing smooth muscle cell proliferation (J. C. Laporte, et al., 2001). IL-13 also induces
211 collagen production by fibroblasts (O'Reilly, Ciechomska, Fullard, Przyborski, & van Laar,
212 2016). IL-13 has also been described as one of the major cytokines implicated in mucus
213 hypersecretion and goblet cell metaplasia (Kuperman, et al., 2002), in airway
214 hyperresponsiveness and in airway narrowing observed in patients with asthma (Manson, et
215 al., 2020). Finally, IL-13 induces vascular endothelial factor (VEGF) expression in the lungs,
216 leading to angiogenesis (Corne, et al., 2000). Recently, it has also been demonstrated that IL-
217 13 and IL-4 directly activate sensory neurons that express IL-4R α and IL-13R α 1 chains
218 (Oetjen, et al., 2017). This could have important functions in the context of asthma, as a study
219 demonstrated that sensory neurons are required for the development of airway
220 hyperresponsiveness in a mouse model of asthma (Trankner, Hahne, Sugino, Hoon, & Zuker,
221 2014).

222

223 *1.3. IL-4 and IL-13 receptors*

224

225 IL-4 signaling is regulated via two receptors, both signaling via the Janus kinase (JAK) /
226 signal transducer and activator of transcription (STAT) pathway (Maes, Joos, & Brusselle,
227 2012). The type 1 IL-4 receptor comprises the high affinity IL-4 receptor alpha chain (IL-
228 4R α) and the common gamma chain (γ c) and exclusively binds IL-4 (**Fig. 1B**). The IL-4R α
229 chain is widely expressed, whereas the γ c subunit is more restricted to specific hematopoietic
230 cell types, including lymphocytes and myeloid cells (Junttila, 2018). IL-4R α can also

231 associate with IL-13R α 1 to form a type 2 IL-4 receptor (**Fig. 1B**). Engagement of both type 1
232 and type 2 receptors induces phosphorylation of the tyrosine residues in the two GYKxF
233 motifs present in IL-4R α , which leads to the recruitment of signal transducer and activator of
234 transcription factor 6 (STAT6) (Pernis, et al., 1995). Subsequent phosphorylation of STAT6
235 results in its dimerization and translocation into the nucleus, where it induces the transcription
236 of IL-4 responsive genes involved in T_H2 response, including that of the transcription factor
237 GATA-3 (Goenka & Kaplan, 2011). Type 1 receptors can also activate the insulin receptor
238 substrate (IRS)-2 pathway (Heller, et al., 2008).

239
240 IL-13 can also activate the type 2 IL-4 receptor. However, unlike IL-4, IL-13 does not bind to
241 IL-4R α but interacts with IL-13R α 1, which also induces the interaction and activation of the
242 IL-4R α -IL-13R α 1 heterodimer. Interestingly, IL-13 can also bind a type 2 IL-13 receptor
243 composed of IL-13R α 2 (**Fig. 1B**). Traditionally, this type 2 receptor was always considered a
244 decoy receptor, as the cytoplasmic tail is composed of only 7 amino acids. However, recent
245 studies have shown that the receptor exists in both soluble and membrane forms encoded by
246 distinct transcripts generated by alternative splicing (Tabata, et al., 2006). In addition, one
247 study has suggested that engagement of this type 2 receptor by IL-13 can promote TGF- β 1
248 synthesis in macrophages (Fichtner-Feigl, Strober, Kawakami, Puri, & Kitani, 2006) .

249
250 Thus, although IL-4 and IL-13 share signaling through the type 2 IL-4R, some of the non-
251 overlapping functions of these two cytokines could be explained by the fact that each cytokine
252 also binds an additional receptor. Moreover, IL-13 is more expressed in lung effector sites,
253 whereas IL-4 is mainly produced in the lymph nodes, and the two cytokines can thus activate
254 different effector cells (Liang, et al., 2011). Finally, it has been shown that the two cytokines
255 have different potencies at inducing the activation of the type 2 IL-4 receptor. IL-4 binds IL-

256 4R α with a higher affinity than that of IL-13 for IL-13R α 1. Thus, IL-4 is able to signal more
257 effectively at lower concentrations than IL-13. However, the overall higher concentration of
258 IL-13R α 1 as compared to IL-4R α appears to allow a high concentration of IL-13 to signal
259 more strongly than IL-4 (S. L. LaPorte, et al., 2008).

260

261 **2. IL-5 and IL-5R**

262

263 *2.1. IL-5 expression and functions*

264

265 IL-5 is a homodimer made up of two monomers of 115 (in human; 113 in mice) amino acids
266 linked by disulfide bonds (Broughton, et al., 2012; Milburn, et al., 1993) (**Fig. 2A**). The IL-5
267 dimer has a molecular mass that varies from 50 to 60 kDa due to heterogeneous glycosylation
268 levels. However, glycosylation does not appear to be required for the biological activity of IL-
269 5 (Kunimoto, et al., 1991). It was long thought that IL-5 is mainly produced by CD4⁺ T_H2
270 cells, and to a lesser extent by mast cells, NK cells, eosinophils and basophils (reviewed in
271 (Brussino, Heffler, Bucca, Nicola, & Rolla, 2018)). However, more recent work using IL-5
272 reporter mice demonstrated that long-lived type 2 innate lymphoid cells (ILC2) resident in
273 peripheral tissues are the predominant source of circulating IL-5 (Ikutani, et al., 2012;
274 Nussbaum, et al., 2013). IL-5 is a key factor for the differentiation, growth, survival and
275 recruitment of eosinophils. This is supported by early studies in mice showing that the
276 overexpression of IL-5 leads to eosinophilia (Dent, Strath, Mellor, & Sanderson, 1990). Mice
277 lacking IL-5 or IL-5R α have normal levels of circulating eosinophils, but they do not develop
278 eosinophilia in models of asthma and allergic inflammation, or upon infection with parasites
279 (Matthaei, Foster, & Young, 1997). In humans, most diseases accompanied by eosinophilia

280 are also associated with increased IL-5 production (Roufosse, 2018). For example, increased
281 IL-5 levels are detected in the bronchoalveolar lavage of patients with asthma (Sur, Gleich,
282 Swanson, Bartemes, & Broide, 1995), or in the skin of subjects with atopic dermatitis (Jeong,
283 et al., 2003). For this reason, IL-5 and its receptor represent major pharmacological targets in
284 asthma and other eosinophilic diseases. These approaches and the beneficial effects of
285 blocking IL-5 or IL-5R in asthma are discussed in detail in part 4. However, the functions of
286 IL-5 are not limited to eosinophils, since IL-5 deficient mice also have reduced numbers of B-
287 1 cells, a population of B cells that play an important role in innate immune responses (Kopf,
288 et al., 1996; Yoshida, et al., 1996). IL-5 can also prime human blood basophils for histamine
289 release (Bischoff, Brunner, De Weck, & Dahinden, 1990) and cord blood-derived human mast
290 cells for cytokine production (Ochi, De Jesus, Hsieh, Austen, & Boyce, 2000).

291

292 *2.2. IL-5 receptor*

293

294 The IL-5 receptor is made of a ligand-binding α subunit (IL-5R α , also known as CD125) and
295 a signal-transducing β chain (β c, also named CD131), which is shared with the granulocyte-
296 macrophage colony-stimulating factor (GM-CSF) and IL-3 receptors (Milburn, et al., 1993)
297 (**Fig. 2, B and C**). Kusano and colleagues reported that the crystal structure of IL-5 bound to
298 the IL-5R α subunit and suggested a structural model for the complete IL-5 receptor complex
299 based on the GM-CSF/GM-CSFR α / β c crystal structure (Kusano, et al., 2012). In this model,
300 IL-5 dimers initially bind IL-5R α to form a binary complex. The ligand-binding pocket
301 formed by the three extracellular domains of IL-5R α interacts with residues from both IL-5
302 monomers. This binary complex subsequently recruits the β c dimer to form an octomeric
303 ternary complex with a 4:2:2 (IL-5:IL-5R α : β c) stoichiometry. The IL-5R α binds IL-5

304 homodimers with a relatively low affinity (0.2 nM). However, the presence of the βc converts
305 this binding to high affinity (100 pM) (Milburn, et al., 1993).

306

307 **3. Epithelial cell-derived cytokines regulating type 2 immune responses**

308

309 The epithelium in the skin, lung and gut forms a physical barrier providing a first line of
310 defense against many pathogens and can also first sense many allergens encountered in these
311 organs. Upon contact with allergens, the epithelium releases an array of mediators among
312 which the three cytokines IL-25, IL-33 and TSLP play a key role in initiating and driving type
313 2 immune responses. These cytokines are often referred to as “alarmins” because they are first
314 released in response of external insults, and their biology has been reviewed recently (Roan,
315 Obata-Ninomiya, & Ziegler, 2019). Not surprisingly, various therapeutic approaches targeting
316 these cytokines are under preclinical and clinical development (Adhikary, Tan, Page, &
317 Hedtrich, 2021; Pelaia, Pelaia, Longhini, et al., 2021). Among these, therapeutic mAbs
318 targeting TSLP recently gave very promising results in asthma (Menzies-Gow, et al., 2021),
319 and will thus be discussed in more detail in part 4.1.5.

320

321 IL-33 is expressed by epithelial cells but also by fibroblasts and endothelial cells and acts
322 through its receptor ST2. This receptor is highly expressed on ILC2s and T_H2 cells
323 (Molofsky, Savage, & Locksley, 2015). In physiological conditions, IL-33 resides in the
324 nuclei of resting epithelial cells, and is released after allergen exposure (Martin & Martin,
325 2016).

326

327 IL-25 is also released by activated epithelial cells and might play a role in enhancing T_H2
328 phenotype in the lung. Elevated levels of IL-25 and IL-25R transcripts were observed in lung

329 tissues from asthmatic patients (Wang, et al., 2007). The IL-25 receptor is expressed on the
330 surface of T_H2 cells, and IL-25 can promote T_H2 cell expansion and release of type 2
331 cytokines.

332

333 The biology of thymic stromal lymphopoietin (TSLP) and its role in asthma and atopic
334 diseases was the topic of a recent review in this journal (Adhikary, et al., 2021). It is produced
335 in the epithelium of many organs, including the lungs (Lee, et al., 2012; Lee & Ziegler, 2007),
336 as well as by some immune cells, including dendritic cells, mast cells, monocytes and
337 macrophages (Adhikary, et al., 2021). TSLP signals through a heterodimeric receptor
338 composed of TSLPR and IL-7R α (also known as CD127), which is expressed by many cell
339 populations, including dendritic cells, mast cells, epithelial cells, macrophages, basophils,
340 type 2 innate lymphoid cells or T cells (Adhikary, et al., 2021; Lambrecht, Hammad, & Fahy,
341 2019).

342

343 TSLP exists in two variants, which are regulated by distinct gene promoters: a short isoform
344 named sfTSLP of 60 or 63 amino acids, and a long isoform, lfTSLP, of 159 amino acids
345 (Harada, et al., 2009). sfTSLP is constitutively expressed under steady-state conditions,
346 including in the oral epithelium, and is down-regulated in inflammatory conditions. By
347 contrast, lfTSLP is inducible and up-regulated in conditions like allergic asthma (Adhikary, et
348 al., 2021). As reviewed extensively, TSLP is a key driver of asthma and other type 2 immune
349 responses, mainly through its ability to induce the expression of IL-4, IL-5, IL-9 and IL-13
350 (Adhikary, et al., 2021; Lambrecht, et al., 2019).

351

352 **4. Pharmacological approaches to target type 2 cytokines or epithelial cell-** 353 **derived cytokines in asthma**

354

355 Various strategies to target type 2 cytokines or epithelial cells-derived cytokines have been
356 developed and are under preclinical or clinical evaluation for asthma. These strategies are
357 summarized in **Fig. 3** and detailed below. The main advantages and disadvantages of each
358 pharmacological approaches are summarized in **Table 1**.

359

360 **4.1. Therapeutic monoclonal antibodies (mAbs)**

361

362 Given the crucial role of type 2 cytokines in the pathophysiology of severe asthma, several
363 mAbs targeting those cytokines have been developed. Nowadays, the use of these therapeutic
364 mAbs has revolutionized severe asthma management. Before their development, the
365 therapeutic strategy of severe asthma was limited to the daily use of oral steroids with
366 frequent side effects. Monoclonal antibodies targeting type 2 cytokines already on the market
367 or under development are listed in **Table 2**.

368

369 **4.1.1 Anti-IL-5 and anti-IL-5R mAbs**

370

371 Several biological therapies targeting IL-5 itself or its receptor IL-5R α have been developed.
372 Mepolizumab is a humanized IgG1 mAb that binds IL-5 with high affinity (Zia-Amirhosseini,
373 et al., 1999). The first clinical studies showed that mepolizumab had no significant effect on
374 asthma outcomes in unselected patients with moderate-to-severe asthma (Flood-Page, et al.,
375 2007). Given its capacity to reduce blood and sputum eosinophils (Flood-Page, et al., 2007),

376 mepolizumab was thereafter tested with promising results in patients with severe eosinophilic
377 asthma and a history of recurrent severe exacerbations (Haldar, et al., 2009; Nair, et al.,
378 2009). Later, the efficacy of mepolizumab was confirmed in larger phase III studies
379 conducted in severe eosinophilic asthma. Compared to placebo, mepolizumab significantly
380 reduces the annualized rate of exacerbation in patients with recurrent asthma exacerbations
381 and evidence of eosinophilic inflammation despite high doses of inhaled steroids (Ortega, et
382 al., 2014; Pavord, et al., 2012). A significant reduction in the oral steroid dose was also
383 observed with mepolizumab in severe eosinophilic asthma in patients requiring the use of
384 daily oral steroids (Bel, et al., 2014). Mepolizumab is associated with significant
385 improvements in quality of life compared to placebo (Chupp, et al., 2017). Although post-hoc
386 analysis found a clinical efficacy of mepolizumab in patients with a blood eosinophil count of
387 150 cells per μL or greater at baseline, no significant reduction of exacerbation was observed
388 in patients with 150 to 300 cells per μL in the phase III MENSA study (Ortega, et al., 2014;
389 Ortega, et al., 2016). In the open-label COLUMBA study, a 3-year follow-up confirmed the
390 long-term efficacy and safety of mepolizumab in severe eosinophilic asthma patients (Khatri,
391 et al., 2019).

392

393 Reslizumab is a humanized IgG4 mAb specifically targeting IL-5 at its IL-5R α binding site
394 (Zhang, et al., 1999). Compared to placebo, reslizumab administered intravenously (IV) led to
395 a significant reduction in exacerbation frequency in two duplicate randomized controlled trials
396 in patients with severe eosinophilic asthma (blood eosinophil count $\geq 400/\mu\text{l}$) (Castro, et al.,
397 2015). A significant improvement of pre-bronchodilator forced expiratory volume 1 (FEV1)
398 at 16 weeks was also observed with IV reslizumab in eosinophilic asthma compared to
399 placebo (Bjermer, et al., 2016). However, subcutaneous reslizumab was not effective
400 regarding the reduction of exacerbation frequency in patients with eosinophilic uncontrolled

401 asthma (≥ 300 cells/ μL) in a randomized clinical trial (Bernstein, et al., 2020). No reduction
402 of the daily maintenance oral corticosteroid dose was found with reslizumab compared to
403 placebo in patients with severe oral corticosteroid-dependent eosinophilic asthma, either
404 (Bernstein, et al., 2020).

405

406 Finally, benralizumab is an IgG1 mAb that binds with high affinity to an epitope in domain 1
407 of the α chain of IL-5R (Koike, et al., 2009). Afucosylation of the oligosaccharide core of the
408 constant Fc domain of this mAb results in a high affinity to human Fc γ R11A, the main
409 activating Fc γ receptor (Fc γ R) expressed on NK cells, macrophages and neutrophils (Shields,
410 et al., 2002). Consequently, benralizumab induces antibody-dependent cell-mediated
411 cytotoxicity (ADCC) of both eosinophils and basophils that express IL-5R, leading to a
412 suppression of peripheral eosinophils (Kolbeck, et al., 2010; Laviolette, et al., 2013).
413 Benralizumab significantly reduces annual exacerbation rates in patients with severe,
414 uncontrolled eosinophilic asthma (blood eosinophils 300 cells per μL or greater) compared to
415 placebo (Bleecker, et al., 2016; FitzGerald, et al., 2016). This mAb also has a significant
416 beneficial effect as an oral steroid-sparing therapy in asthma patients relying on oral steroids
417 (Nair, et al., 2017). As observed with anti-IL5 therapies, blood eosinophil count is a good
418 predictive biomarker of response to benralizumab (Jackson, Humbert, Hirsch, Newbold, &
419 Garcia Gil, 2020). Oral steroid use, nasal polyposis and pre-bronchodilator forced vital
420 capacity are also associated with greater benralizumab responsiveness for a reduced
421 exacerbation rate (Bleecker, et al., 2018). A 2-year follow-up confirmed the good safety
422 profile and the long-term efficacy of benralizumab in patients with severe eosinophilic asthma
423 (Busse, Bleecker, et al., 2019). However, benralizumab did not lead to achieving the
424 minimum clinically important difference of FEV1 improvement in mild-to-moderate asthma
425 patients compared to placebo (Ferguson, et al., 2017).

426 All three drugs are now FDA-approved for treatment of severe asthma with an eosinophilic
427 phenotype.

428

429 *4.1.2 Anti-IL-4 mAbs*

430

431 A variety of mAbs and soluble receptors blocking IL-4 and/or IL-13 have been developed for
432 the treatment of severe asthma. Pascolizumab is a humanized mAb that neutralizes both
433 human and cynomolgus monkey IL-4 bioactivity and was well tolerated in monkeys (Hart, et
434 al., 2002). However, even if it was also well tolerated in adults with asthma, it did not produce
435 a significant reduction in circulating IgE in phase I/II clinical trials and its development was
436 discontinued (Shames, et al., 2001).

437

438 *4.1.3 Anti-IL-13 mAbs*

439

440 Given the key role of IL-13 in mediating goblet cell hyperplasia, airway smooth muscle
441 contractility, collagen deposition and fibrosis, several anti-IL13 mAbs were developed a
442 decade ago for the treatment of severe asthma (Gour & Wills-Karp, 2015).

443

444 Lebrikizumab is a humanized IgG4 mAb that blocks the signaling pathway through the IL-
445 4R α /IL-13R α 1 heterodimer by binding soluble IL-13 and preventing its link to the receptor
446 (Ultsch, et al., 2013). Phase II studies have suggested that treatment with lebrikizumab can
447 lead to a reduction of asthma exacerbation and an improvement in lung function in patients
448 with poorly controlled asthma, and in particular in patients with high levels of serum periostin
449 (Corren, et al., 2011; Hanania, et al., 2015). However, a significant reduction in asthma
450 exacerbations was only observed in biomarker-high patients (high levels of periostin) in one

451 of the two duplicate phase III clinical trials (LAVOLTA I and II) (Hanania, et al., 2016).
452 Although a significant improvement in FEV1 was observed with lebrikizumab in LAVOLTA
453 I and II, its lack of efficacy in the reduction of asthma exacerbation led to the discontinuation
454 of its development. Treatment with lebrikizumab does not have an impact on the number of
455 eosinophils in the tissue and blood (Corren, et al., 2011). This could explain the lack of effect
456 on asthma exacerbations (Austin, et al., 2020).

457

458 Tralokinumab is another human IgG4 mAb against IL-13 that inhibits downstream IL-13-
459 mediated effects by preventing cytokine binding to both IL-13R α 1 and IL-13R α 2 (Popovic,
460 et al., 2017). Tralokinumab has also been evaluated in two duplicate phase III studies
461 (STRATOS 1 and 2) in patients with severe asthma (Panettieri, et al., 2018). A significant
462 reduction in the asthma exacerbation rate was observed in participants with baseline
463 fractionated exhaled nitric oxide (FeNO) of 37 parts per billion (ppb) or higher in STRATOS 1,
464 but not in STRATOS 2. FENO is a non-invasive, safe and simple method of quantifying
465 airway inflammation. In another study, lebrikizumab has no oral steroid-sparing effect in
466 patients with severe asthma (Busse, Brusselle, et al., 2019). These inconsistent effects led to
467 the discontinuation of the development of tralokinumab, despite its significant positive impact
468 on FEV1. As observed with lebrikizumab, tralokinumab's lack of effect on asthma
469 exacerbation is probably due to the fact that anti-IL13 antibodies do not significantly affect
470 eosinophilic inflammation in bronchial submucosa, blood or sputum (Russell, et al., 2018).

471

472 *4.1.4 Anti-IL-4R mAbs*

473

474 The fact that IL-4 and IL-13 share a receptor explains their large functional overlap (see
475 section 1.3). Targeting both IL-4 and IL-13 or their common receptor appears to be more
476 effective than addressing a single cytokine.

477

478 Dupilumab is a recombinant human IgG4 mAb that targets the IL-4R α chain, the common
479 chain shared by IL-4 and IL-13 receptors (Kim, et al., 2019). Dupilumab blocks the
480 transduction signal activated by IL-4 and IL-13. In phase III trials, dupilumab significantly
481 lowered the rate of asthma exacerbations in patients experiencing at least one exacerbation in
482 the past 12 months despite a medium-to-high dose of inhaled steroids in combination with a
483 second controller (Castro, et al., 2018). Patients were recruited irrespective of a minimum
484 baseline blood eosinophil count or biomarkers of type 2 inflammation. However, dupilumab
485 was not effective in patients with a blood eosinophil count less than 150/ μ l or FeNO less than
486 25 ppb. Dupilumab has also been evaluated in patients with severe glucocorticoid-dependent
487 asthma (Rabe, et al., 2018). A significant reduction of the daily dose of oral steroids was
488 observed compared to placebo. The long-term efficacy and safety were demonstrated in a
489 study with a 3-year follow-up (Wechsler, Ford, et al., 2021). Dupilumab has been approved in
490 severe asthma with type 2 inflammation, but also in atopic dermatitis in Europe. Interestingly,
491 this antibody improves asthma outcomes even in patients with comorbid moderate-to-severe
492 atopic dermatitis (Boguniewicz, et al., 2021).

493

494 AMG317, another monoclonal antibody targeting IL-4R α , did not demonstrate clinical
495 efficacy across unselected asthma patients, and its development was discontinued (Corren, et
496 al., 2010).

497

498 *4.1.5 Anti-epithelial cytokine mAbs*

499

500 Secreted by epithelial cells, IL-25, IL-33 and TSLP act as alarmins and therefore represent
501 important factors in the early phase of asthma. Consequently, decreased production of these
502 cytokines constitutes an important target for asthma with type 2 inflammation. IL-25-related
503 studies have not yet reached clinical evaluation. However, one study in mice indicate that a
504 neutralizing anti-IL-25 mAb can reduce airway hyperreactivity (AHR), eosinophilia, goblet
505 cells hyperplasia and IgE levels in a model of ovalbumin-induced allergic airway
506 inflammation (Ballantyne, et al., 2007). Antibodies targeting IL-33 or TSLP are now at
507 various stages of clinical evaluation and represent promising candidates for the treatment of
508 asthma.

509

510 The TSLP-targeting therapeutic mAb tezepelumab met its primary endpoint in the recent
511 NAVIGATOR phase III clinical trial (Menzies-Gow, et al., 2021). On the basis of these data,
512 tezepelumab was approved in the US at the end of 2021 for severe asthma (Astrazeneca,
513 2021). Its beneficial effect on the annualized rate of asthma exacerbations was obtained in
514 patients with or without type 2 inflammation. This might be explained by the fact that type 2
515 responses could still play important functions at lower biomarker thresholds, and/or because
516 tezepelumab acts through mechanisms beyond type 2 inflammation (Diver, et al., 2021;
517 Puzzovio, Eliashar, & Levi-Schaffer, 2022). Studies in mice and *in vitro* models have point to
518 a potentially important function of TSLP in driving Th17-dependent neutrophilic airway
519 inflammation (Pelaia, Pelaia, Crimi, et al., 2021; Tanaka, et al., 2009). However, results from
520 the CASCADE phase II clinical trial indicated that tezepelumab treatment reduced eosinophil
521 numbers in the airway submucosa without affecting the numbers of neutrophils, T cells or
522 mast cells (Diver, et al., 2021). In addition, according to a press release, the phase III study

523 SOURCE conducted in severe, oral corticosteroid-dependent asthma did not meet its primary
524 end point. Indeed, tezepelumab did not significantly reduce the dose of oral steroids in a
525 cohort of 150 severe asthma patients (Astrazeneca, 2020).

526

527 Therapeutic strategies targeting IL-33 are being explored for their potential in asthma but are
528 still in development. Etokimab, an anti-IL33 mAb, is currently being tested in severe
529 eosinophilic asthma in a phase IIa clinical trial (NCT03469934). Itepekimab is a new human
530 IgG4 monoclonal antibody against IL-33. Itepekimab was tested in patients with moderate-to-
531 severe asthma in a placebo-controlled phase IIa clinical trial (Wechsler, Ruddy, et al., 2021).
532 By 12 weeks, events indicating loss of asthma control occurred in 22% of patients treated
533 with itepekimab versus 41% for the placebo. The combination with dupilumab does not seem
534 to provide additional effects (Wechsler, Ruddy, et al., 2021). Astegolimab, a human IgG2
535 mAb that selectively inhibits the IL-33 receptor ST2, reduced asthma exacerbation rates
536 (AER) in a broad population of patients with severe, inadequately controlled asthma,
537 including those who were eosinophil-low (phase IIa) (Kelsen, et al., 2021).

538

539 *4.1.6 Bispecific mAbs*

540

541 Type 2 cytokines have some non-overlapping functions in asthma and/or can produce and
542 exert their functions in different tissues and with different timing. Thus, one interesting novel
543 approach in development is to simultaneously target multiple cytokines using bispecific
544 mAbs. In 2013, Genentech reported the generation of a bispecific anti-human IL-4/IL-13
545 mAb (Spiess, et al., 2013). The anti-IL-13 arm of the bispecific mAb was based on the V_H and
546 V_L sequences of lebrikizumab (described in part 4.1.3). In parallel, the authors generated
547 hybridomas secreting anti-human IL-4 mAbs from mice immunized with human IL-4. Among

548 these, clone 19C11 bound to human IL-4 with an affinity of 10 pM and was humanized. The
549 bispecific anti-IL-4/IL-13 mAb was generated using a knob-in-hole approach (as reviewed
550 recently in (Labrijn, Janmaat, Reichert, & Parren, 2019) using a human IgG4 isotype. To the
551 best of our knowledge, this bispecific mAb has not been tested in humans.

552

553 As a proof of concept, Kasaian et al. generated a bispecific anti-mouse IL-4/IL-13 antibody
554 derivate by fusing the N-terminal part of the constant domain of mouse IgG2c to the murine
555 IL-13Ra2 extracellular domain. In addition, they added an anti-mouse IL-4 single-chain
556 variable fragment (ScFv) at the C-terminal part (Kasaian, et al., 2013). This bispecific IL-
557 4/IL-13 antagonist reduced IgE levels and AHR in a mouse model of ovalbumin-induced
558 asthma.

559

560 To simultaneously inhibit the IL-4, IL-13 and IL-5 pathways, Godar and colleagues
561 developed a bispecific mAb-targeting mouse IL-4R α and mouse IL-5. They found that mAbs
562 targeting IL-4R α or IL-5 alone could suppress eosinophilia, IgE synthesis or both, whereas
563 only the IL-4R α /IL-5-bispecific mAb or the combination of monospecific mAbs additionally
564 inhibited goblet cell hyperplasia and AHR in a mouse model of house dust mite-induced
565 asthma (Godar, et al., 2018).

566

567 In 2018, Boehringer Ingelheim reported the development of two bispecific mAbs, named
568 zweimab and doppelmab, which simultaneously inhibit the TSLP and IL-13 pathways with
569 picomolar affinities (Venkataramani, et al., 2018). To our knowledge, the efficacy of these
570 mAbs in asthma and allergies has not been reported.

571

572 **4.2. Pitrakinra, a mutated recombinant IL-4**

573

574 Pitrakinra is a recombinant IL-4 containing two targeted point mutations and acting as an IL-
575 4R α antagonist by preventing IL-4R α assembly with IL-13R α . Pitrakinra delivered via
576 inhalation route did not have a significant impact on lung function (FEV1) in response to
577 allergen challenges in asthma patients (Wenzel, Wilbraham, Fuller, Getz, & Longphre, 2007).
578 Interestingly, in a large pharmacogenetic study, pitrakinra treatment significantly lowered the
579 asthma exacerbation frequency in a subgroup of patients carrying specific single-nucleotide
580 polymorphisms in the gene encoding IL-4R α (Slager, et al., 2012). To our knowledge, no
581 phase III study is ongoing.

582

583 **4.3. DARPins**

584

585 Designed ankyrin repeat proteins (DARPins) are small engineered proteins that can recognize
586 targets with high specificity and with affinity in the low nanomolar range (Binz, et al., 2004;
587 Seeger, et al., 2013). Although no DARPins has yet been approved in humans, several
588 candidates are now at various stages of clinical trials for COVID-19 and different cancers. A
589 DARPins targeting human IL-4 was designed and named DARPins_44. It neutralizes the
590 activity of recombinant human IL-4 with an IC₅₀ of 5 pM (Obmolova, et al., 2015). The
591 crystal structure of DARPins_44 bound to human IL-4 reveals that such binding occurs on the
592 same IL-4 face that interacts with the junction of the D1 and D2 domains of the IL-4R α
593 receptors, thereby neutralizing binding of IL-4 to its receptor (Obmolova, et al., 2015).
594 However, to the best of our knowledge, the efficacy of DARPins_44 in asthma or allergies has
595 not been reported.

596

597 Similarly, a DARPin targeting human IL-13 was designed and named DARPin 6G9. It binds
598 human IL-13 with a K_D in the picomolar range and exhibits cross-reactivity towards
599 cynomolgus monkey IL-13 (Teplyakov, et al., 2017). The crystal structure of DARPin 6G9 in
600 complex with cynomolgus monkey IL-13 revealed that the neutralization effect of DARPin is
601 due to the blocking of the IL-13 interaction with the receptor chain IL-13R α 1 (Teplyakov, et
602 al., 2017). Here again, to the best of our knowledge, the efficacy of this DARPin in asthma or
603 allergies has not been reported.

604

605 *4.4. Small molecules inhibitors*

606

607 Small molecule inhibitors can offer the advantage of better tissue penetration and easier large-
608 scale production as compared to mAbs (Imai & Takaoka, 2006). Moreover, while most mAbs
609 are delivered intravenously or subcutaneously, small molecules may offer the advantage of
610 oral or pulmonary administration. However, it is very challenging to develop small molecules
611 able to neutralize cytokines or their receptors with high efficacy and selectivity.

612

613 *4.4.1. Small molecule inhibitors of IL-4*

614

615 Recently, the first compound able to inhibit human IL-4 activity was described (Quinnell, et
616 al., 2020). The authors tested a bank of 50,000 compounds to identify molecules able to bind
617 IL-4. From this screening, they isolated one candidate, named compound 52, displaying low
618 micromolar affinity and able to disrupt the binding of IL-4 to the type 2 IL-4R. While
619 compound 52 also has some affinity for IL-13, it is however 10 times more selective for IL-4
620 (Quinnell, et al., 2020). To the best of our knowledge, there is currently no report on the
621 efficacy of compound 52 in asthma or other allergic conditions.

622

623 *4.4.2. Small molecule inhibitors of IL-5*

624

625 England and colleagues identified a 19-amino-acid peptide that has no primary sequence
626 homology to IL-5, but which binds to the extracellular domain of human IL-5R α with an
627 affinity equal to that of human IL-5 (England, et al., 2000). The authors demonstrated that this
628 peptide is a potent and specific antagonist of IL-5 activity using a human eosinophil adhesion
629 assay. The peptide spontaneously associates into dimers and binds simultaneously to the two
630 receptor α chains in the solution (England, et al., 2000).

631

632 *4.4.3. Small molecule inhibitors of TSLP*

633

634 A total of 16 peptides were designed spanning the TSLPR binding site. Among these, 3
635 peptides display > 50% inhibitory activity at a concentration of 0.3 mM. While the activity of
636 such peptides is somewhat low and their selectivity has not been reported, the authors
637 suggested these molecules could be used as hit compounds for the further development of
638 peptide-based TSLP inhibitors (S. Park, et al., 2017). The same year, a total of 3,500
639 compounds were screened using a virtual docking method to identify molecules able to
640 disrupt the TSLP:TSLPR complex. From this virtual docking, six compounds were selected
641 and tested *in vitro*, and 4 molecules were able to inhibit TSLP binding by more than 75%
642 (Van Rompaey, et al., 2017). More recently, Park and colleagues found that baicalein, a major
643 component of the plant *Scutellaria baicalensis*, was able to inhibit human TSLP activity.
644 Repeated intraperitoneal administrations of baicalein also reduced eosinophilia in a mouse
645 model of asthma (B. B. Park, et al., 2019).

646

647 *4.5 Vaccine approaches*

648

649 Therapeutic mAbs have validated the clinical efficacy of targeting type 2 cytokines in asthma.
650 However, the current cost of such mAbs and the need for frequent reinfusion still limit the use
651 of these drugs in asthma and other chronic allergic diseases. To bypass this, several groups
652 are working on novel vaccine strategies to promote long-term endogenous production of
653 neutralizing antibodies against these type 2 cytokines.

654

655 *4.5.1. Anti-IL-5 vaccines*

656

657 Different vaccine strategies have been proposed to target IL-5. Hertz et al. first showed that
658 the immunization of mice with a DNA vaccine encoding murine IL-5 modified to contain a
659 promiscuous foreign T-helper epitope can bypass B cell tolerance to IL-5 and induces
660 neutralizing polyclonal anti-IL-5 antibodies (Hertz, et al., 2001). Prophylactic immunization
661 with this anti-IL-5 DNA vaccine reduced airway inflammation and eosinophilia in a model of
662 ovalbumin-induced allergic airway inflammation (Hertz, et al., 2001). Zou and coworkers
663 coupled mouse IL-5 to virus-like particles (VLPs) derived from bacteriophage Q β (Zou, et al.,
664 2010). Immunization of mice with this conjugate vaccine induced neutralizing antibodies
665 against IL-5. Moreover, the vaccine was also able to reduce eosinophilia in the lungs and
666 blood in a model of ovalbumin-induced allergic airway inflammation (Zou, et al., 2010). The
667 same group then developed a vaccine against equine IL-5 by coupling this cytokine to a
668 cucumber mosaic VLP containing a universal T cell epitope (CuMV_{TT}). They showed that the
669 vaccine was well tolerated in horses and induced anti-IL-5 antibodies. The antibody response
670 was maximal around two months post-vaccination and continuously dropped thereafter. A
671 vaccine boost after 6 months promoted a second wave of antibody production with about the

672 same kinetic as the first wave. Importantly, this anti-IL-5 vaccine was able to reduce allergic
673 dermatitis and eosinophilia in horses suffering from insect-bite hypersensitivity (Fettelschoss-
674 Gabriel, et al., 2019; Jonsdottir, et al., 2020).

675

676 *4.5.2. Anti-IL-4/IL-13 vaccines*

677

678 A first conjugate vaccine against mouse IL-4 was generated in 2007. It consisted of
679 recombinant mouse IL-4 coupled to the carrier protein keyhole limpet hemocyanin (KLH).
680 Using this conjugate vaccine, the authors demonstrated that vaccination against mouse IL-4
681 can partially reduce IgE levels and eosinophilia, with only minor effects on mucus production
682 in a mouse model of ovalbumin-induced asthma (Le Buanec, et al., 2007). The same year, a
683 peptide vaccine against mouse IL-13 was also generated. It consisted of a 15-amino-acid
684 peptide comprising the IL-13R α receptor binding site (LTLKELIEELSNITQ) coupled to a
685 truncated hepatitis B virus core antigen (HBcAg) used as a carrier protein (Ma, HayGlass, et
686 al., 2007). The vaccine was tested with or without adjuvants, which demonstrated that the
687 vaccine is immunogenic even in the absence of artificial adjuvants. Prophylactic subcutaneous
688 vaccination with this peptide vaccine reduced eosinophilia and mucus production in a model
689 of ovalbumin-induced asthma. However, the vaccine had no effect on IgE levels (Ma,
690 HayGlass, et al., 2007). In a follow-up study, the authors showed that intranasal immunization
691 with the vaccine induced IL-13-specific IgA responses in multiple mucosal tissues and higher
692 serum titers of IgG than subcutaneous vaccination and was more potent at reducing asthma
693 features (Ma, Ma, & Peng, 2007).

694

695 These first two vaccine candidates demonstrated the efficacy of immunization against IL-4 or
696 IL-13 in models of asthma, but they also highlighted the fact that the two cytokines play some

697 non-redundant functions. This is very consistent with the results from clinical trials in human
698 asthma, which demonstrated the superior efficacy of dupilumab, targeting both IL-4 and IL-
699 13 pathways, over mAbs targeting IL-4 or IL-13 alone (Bagnasco, Ferrando, Varricchi,
700 Passalacqua, & Canonica, 2016) (as detailed in part 4.1). It was thus decided to test the
701 efficacy of combined vaccination against IL-4 and IL-13 in asthma models. Two vaccine
702 candidates were produced, consisting of recombinant mouse IL-4 or IL-13 coupled to the
703 carrier protein diphtheria ‘cross-reactive material 197’ (CRM₁₉₇, a non-toxic mutant of the
704 diphtheria toxin used in a number of approved conjugate vaccines (Malito, et al., 2012)).
705 Subcutaneous immunization with these vaccine candidates alone or in combination, together
706 with a squalene oil-in-water emulsion adjuvant (SWE) (Ventura, et al., 2013), induced high
707 titers of neutralizing antibodies against the respective cytokines. These vaccines were tested
708 in a model of house dust mite-induced asthma. Prophylactic vaccination against IL-4 induced
709 a more pronounced reduction of IgE levels and lung mast cell numbers than with the IL-13
710 vaccine. By contrast, airway hyperreactivity and mucus levels were reduced to a greater
711 extent with the IL-13 vaccine. Combined vaccination against IL-4 and IL-13 nearly abrogated
712 all these key asthma features, including airway eosinophilia, confirming the benefit of
713 targeting both cytokines (Conde, Bertrand, et al., 2021; Conde, Bruhns, Serra, & Reber,
714 2021). Importantly, combined vaccination against IL-4 and IL-13 remained efficient at
715 reducing asthma features in a therapeutic vaccination protocol. Finally, a human IL-4/IL-13
716 vaccine was designed using the same approach and tested in humanized mice expressing
717 human IL-4, IL-13 and IL4R α in place of the respective mouse proteins. This human vaccine
718 also elicited an efficient neutralizing response against IL-4 and IL-13 and was able to reduce
719 both circulating IgE and IgE on the surface of basophils and mast cells (Conde, Bertrand, et
720 al., 2021).

721

722 Altogether, these results illustrate the fact that long-lasting vaccine strategies against type 2
723 cytokines might be an efficient strategy to promote long-term protection in asthma. Given the
724 importance of protective type 2 immune responses, it is possible that such vaccine strategies
725 might reduce immunity against parasites or other pathogens. However, it is important to note
726 that data on the long-term effects of drugs targeting type 2 cytokines in humans are still
727 limited, but several large clinical studies are now available for the anti-IL-4R α mAb
728 dupilumab with treatment periods from 52 to 96 weeks in asthma (Castro, et al., 2018;
729 Pavord, et al., 2020). Overall, these studies demonstrate a good safety profile, which would
730 argue in favor of the feasibility of the long-term targeting of type 2 cytokines with a vaccine.

731

732

5. Concluding remarks

734

735 Several therapeutic mAbs targeting type 2 cytokines or their receptors have been approved for
736 the treatment of asthma. A major challenge is to now identify biomarkers that will help
737 predict the patients most likely to respond to such therapies. Many asthma patients also do not
738 develop strong type 2 inflammation, and better knowledge of the mechanisms of type 2 low
739 asthma endotypes will help design effective biologics for these patients. In this regard, the
740 recent finding that anti-TSLP mAbs have beneficial effects in patients with or without a type
741 2 signature is very promising. The cost of antibody-based therapies remains high, which still
742 precludes their use for many patients. Although still at an early pre-clinical stage, small
743 molecule inhibitors and conjugate vaccines might represent promising new strategies to
744 induce cost-effective and long-term beneficial effects in asthma.

745

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747

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752

753 **7. Conflict of interest statement**

754

755 L.G. has been an investigator in clinical trials for AstraZeneca, MSD and Novartis, reports
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757 Regeneron and fees for consulting from ALK, Bayer, Chiesi, MSD, not related to the
758 submitted work. E.C reports a patent related to asthma and allergy therapy, WO2019228674
759 (A1). L.L.R. reports grants or fees for consulting from Argenx, Novartis and Neovacs, as well
760 as patents issued or pending related to asthma and allergy diagnosis or therapy: EP20315224-
761 4, WO2019197607 (A1) and WO2019228674 (A1).

762

763

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1365 **Figure legends**

1366

1367 **Fig. 1. Structure of IL-4, IL-13 and their receptors. A.** IL-4 is a 15 kDa globular protein
1368 with 129 amino acids, formed by 4 α helices and 3 disulfide bonds (PDB ID: 2B8U). **B.**
1369 Structure of IL-4 bound to the type 1 IL-4 receptor composed of the high affinity IL-4
1370 receptor alpha chain (IL-4R α , depicted in yellow) and the common gamma chain (γ c,
1371 depicted in red) (PDB ID: 3BPL). IL-4R α can also associate with IL-13R α 1 (in green) to
1372 form a type 2 IL-4 receptor (PDB ID: 3BPN). **C.** IL-13 is a 13 kDa globular protein
1373 composed of 4 α helices (PDB ID: 1IJZ). **D.** Structure of IL-13 bound to the type 2 IL-4
1374 receptor. Unlike IL-4, IL-13 does not bind to IL-4R α , but instead interacts with IL-13R α 1,
1375 which induces subsequent interaction and activation of the IL-4R α -IL-13R α 1 heterodimer
1376 (PDB ID: 3BPO). IL-13 can also bind a type 2 IL-13 receptor composed of IL-13R α 2 (in
1377 grey) (PDB ID: 3LB6).

1378

1379 **Fig. 2. Structure of the IL-5/IL-5R complex. A.** IL-5 forms homodimers made up of two
1380 individual monomers (depicted in orange and green), each containing four α helices and two
1381 β sheets (represented by arrows) (PDB ID: 1HUL). **B.** Structure of the IL-5 dimer bound to
1382 the IL-5R α subunit with a 1:1 stoichiometry. The extracellular portion of IL-5R α (depicted in
1383 purple) is made up of three domains (labeled D1-3), each composed of seven β sheets
1384 (represented by arrows). The ligand-binding pocket formed by the three IL-5R α domains
1385 interacts with residues from both IL-5 monomers (PDB ID: 3VA2). **C.** Structural model for
1386 the IL-5/IL-5R α / β c complex based on the GM-CSF/GM-CSFR α / β c crystal structure (PDB
1387 ID: 3CXE) (Kusano, et al., 2012). In this model, IL-5 dimers initially bind IL-5R α , and this

1388 binary complex subsequently recruits the βc dimer (depicted in grey) to form an octomeric
1389 ternary complex with a 4:2:2 (IL-5:IL-5R α : βc) stoichiometry.

1390

1391 **Fig. 3. Therapeutic strategies directed against type 2 cytokines in asthma.** The epithelial
1392 cell-derived cytokines TSLP and IL-33 can initiate type 2 responses in asthma. Tezepelumab,
1393 an anti-TSLP mAb, has recently met its primary endpoint in severe asthma and should be
1394 commercialized soon. Small molecule inhibitors (SMI) that target TSLP are also under
1395 preclinical development. Etokimab and itepekimab, two anti-IL33 mAbs, are under clinical
1396 evaluation. Another mAb, astegolimab, that targets the IL-33 receptor ST2, reduced asthma
1397 exacerbations in a phase IIa study. TSLP and IL-33 can activate T_H2 cells and type 2 innate
1398 lymphoid cells (ILC2) that are involved in the secretion of the main type 2 cytokines: IL-4, -5
1399 and -13. IL-5 is mainly responsible for recruitment and activation of eosinophils.
1400 Mepolizumab and reslizumab are mAbs neutralizing soluble IL-5. Benralizumab is a mAb
1401 that targets the IL-5 receptor and induces depletion of circulating eosinophils and basophils.
1402 These mAbs are now commercialized for asthma. SMI and vaccines directed against IL-5 are
1403 also in preclinical development. IL-4 plays a key role in IgE synthesis and eosinophilia, and
1404 also has effects on mast cells and basophils. IL-13 mainly induces eosinophilia, mucus
1405 production, goblet cell and smooth muscle cell hyperplasia. However, the anti-IL-4 mAb
1406 pascolizumab and the anti-IL13 mAbs, lebrikizumab and tralokinumab, failed to demonstrate
1407 an effect on the reduction of asthma exacerbation. Their development is currently
1408 discontinued. Targeting IL-4R α which is shared by IL-4 and IL-13 receptors focuses the
1409 interest due to the efficacy of the anti-IL-4R α mAb dupilumab in severe asthma with type 2
1410 inflammation. SMI and vaccines targeting IL-4 and IL-13 are also under preclinical
1411 development.

1412 **Table 1. Main advantages and disadvantages of various pharmacological approaches to target type 2 cytokines in asthma.**

Pharmacological approach	Advantages	Disadvantages
Therapeutic mAbs	<ul style="list-style-type: none"> - High specificity and affinity - Good safety profile for humanized and fully human mAbs - Half-life of about 21 days for IgG 	<ul style="list-style-type: none"> - High cost - Need for frequent reinfusions - Potential generation of anti-drug antibodies (ADA)
DARPinS	<ul style="list-style-type: none"> - High specificity and affinity - Low production cost - High stability 	<ul style="list-style-type: none"> - Low half-life (but can be increased <i>e.g</i> by addition of human albumin binding domains)
Small molecule inhibitors (SMI)	<ul style="list-style-type: none"> - Low production cost - Potential for oral or aerosol delivery 	<ul style="list-style-type: none"> - Reduced selectivity as compared to antibodies - Low half-life
Anti-cytokine vaccines	<ul style="list-style-type: none"> - Low cost - Long-term production of endogenous neutralizing antibodies - No need for frequent reinjections 	<ul style="list-style-type: none"> - Potential adverse effect of targeting type 2 cytokines on the long-term? (Also applies to long-term treatments with other drug formats)

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1414

1415 **Table 2: Clinical effect of drugs targeting type 2 cytokines or alarmins according to primary outcomes from proof-of-concept or large**
 1416 **randomized clinical trials.**

Cytokine target	Name	Type of drug	Stage of development	Effect on asthma outcomes from randomized clinical trials
IL-4	Pascolizumab	mAb (IgG1)	Development discontinued	No reduction of serum IgE in asthma patients (phase I/II trial) (Shames, et al., 2001)
IL-13	Lebrikizumab	mAb (IgG4)	Development discontinued	- 51% and 30% reduction of exacerbation rates with lebrikizumab at 37.5 mg and 125 mg doses in biomarker-high patients (LAVOLTA I) (Significant) (Hanania, et al., 2016). - 26% reduction of exacerbation rates with lebrikizumab at both 37.5 mg and 125 mg doses in biomarker-high patients (LAVOLTA II) (Not significant) (Hanania, et al., 2016). - No effect on the number of bronchial eosinophils (Austin, et al., 2020)
IL-13	Tralokinumab	mAb (IgG4)	Development discontinued	- No effect on exacerbation rate in unselected population with severe asthma (Panettieri, et al., 2018) - 44% reduction of exacerbation rates in patients with high FENO (≥ 37 ppb) (STRATOS 1) (Significant) (Panettieri, et al., 2018) - 16% reduction of exacerbation rates in patients with high FENO (≥ 37 ppb) (STRATOS 2) (Not significant) (Panettieri, et al., 2018) - No OCS-sparing effect in patients with severe asthma (Busse, Brusselle, et al., 2019) - No impact on eosinophilic inflammation in bronchial submucosa, blood or sputum (Russell, et al., 2018)
IL-4R	Dupilumab	mAb (IgG4)	Commercialized	- 46% to 48% reduction of annualized rate of exacerbation (Castro, et al., 2018) - 70% reduction of oral steroid dose from baseline in severe eosinophilic asthma patients (Rabe, et al., 2018) - Long-term efficacy and safety of up to 3 years in severe asthma with type 2 inflammation (Wechsler, Ford, et al., 2021)
IL-4R	Pitrakinra	Mutated IL-4 molecule	Development discontinued	- No significant impact on lung function (FEV1) response to allergen challenge in asthma patients (Wenzel, et al., 2007). - Significant reduction of asthma exacerbations in a subgroup of patients with polymorphisms in IL-4R α gene (Slager, et al., 2012).
IL-4R	AMG317	mAb (IgG2)	Development discontinued	No significant clinical efficacy in asthma patients (Corren, et al., 2010).
IL-5	Mepolizumab	mAb (IgG1)	Commercialized	- 53% reduction of annualized rate of exacerbation in severe eosinophilic asthma patients (Ortega, et al., 2014) - 50% reduction of oral steroid dose from baseline in severe eosinophilic asthma patients (Bel, et al., 2014) - Significant improvement of quality of life (Chupp, et al., 2017)

				- Long-term efficacy and safety in severe eosinophilic asthma patients for up to 3 years (Khatri, et al., 2019)
IL-5	Reslizumab	mAb (IgG4)	Commercialized	- 50% and 59% reduction of exacerbation frequency in severe eosinophilic asthma patients (eosinophil count $\geq 400/\mu\text{L}$) (Castro, et al., 2015) - 115 to 160 ml improvement of prebronchodilator FEV1 compared to placebo (Bjermer, et al., 2016) - No significant reduction of subcutaneous reslizumab on exacerbation frequency or the daily dose of oral steroids in patients with uncontrolled eosinophilic asthma (≥ 300 cells/ μL) (Bernstein, et al., 2020)
IL-5R	Benralizumab	mAb (IgG1)	Commercialized	- 28% to 45% reduction of annualized rate of exacerbation in severe eosinophilic asthma patients (blood eosinophils 300 cells per μL or greater) with benralizumab administered every 4 weeks (Bleecker, et al., 2016; FitzGerald, et al., 2016) - 36% to 51% reduction of annualized rate of exacerbation in severe eosinophilic asthma patients (blood eosinophils 300 cells per μL or greater) with benralizumab administered every 8 weeks (Bleecker, et al., 2016; FitzGerald, et al., 2016) - 75% reduction of oral steroid dose from baseline in severe eosinophilic asthma patients (Nair, et al., 2017) - Long-term efficacy and safety of mepolizumab in severe eosinophilic asthma patients for up to 2 years (Busse, Bleecker, et al., 2019)
TSLP	Tezepelumab	mAb (IgG2)	Positive phase III Approved on Dec 2021 in the US for severe asthma	- 56% reduction of exacerbation frequency in unselected severe asthma patients (Menzies-Gow, et al., 2021) - No significant reduction of oral steroid dose from baseline in severe asthma patients (Astrazeneca, 2020)
IL-33	Etokimab	mAb (IgG1)	Ongoing Phase II	NCT03469934
IL-33	Itepekimab	mAb (IgG4)	Phase IIa	- 58% reduction of events indicating a loss of asthma control compared to placebo (Wechsler, Ruddy, et al., 2021)
IL-33R (ST2)	Astegolimab	mAb (IgG2)	Phase IIa	- 22% to 43% reductions of asthma exacerbations compared to placebo (Kelsen, et al., 2021)

Figure 1

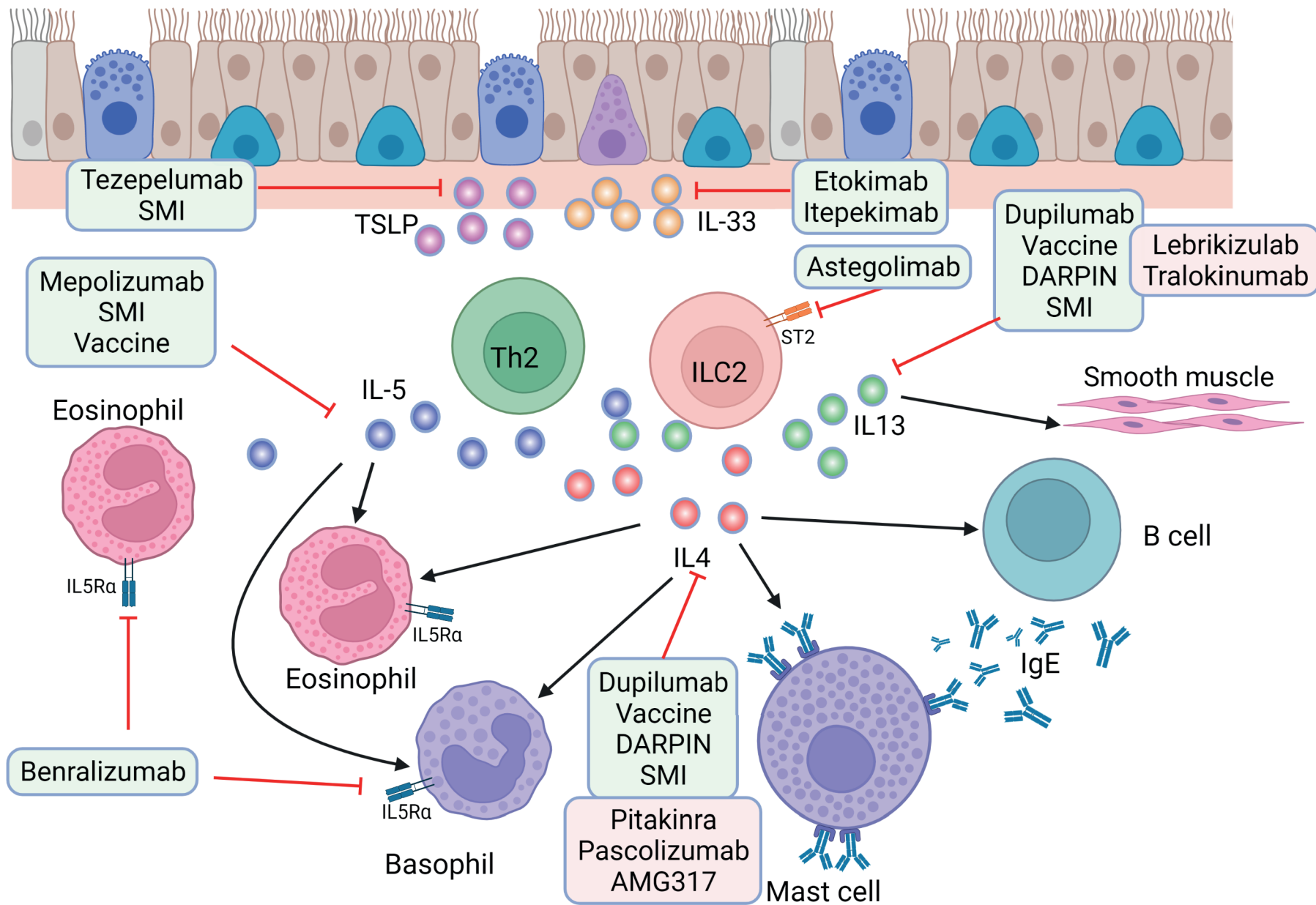
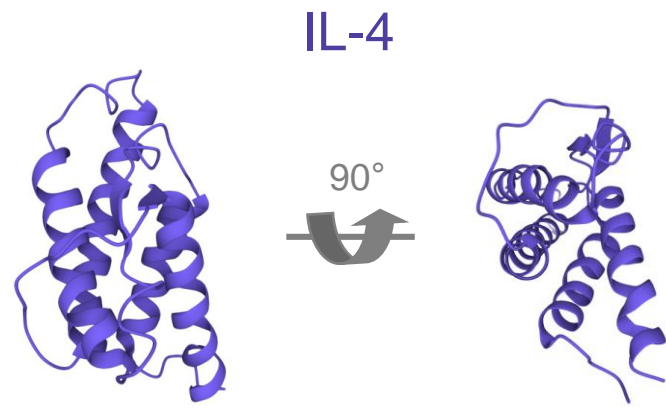
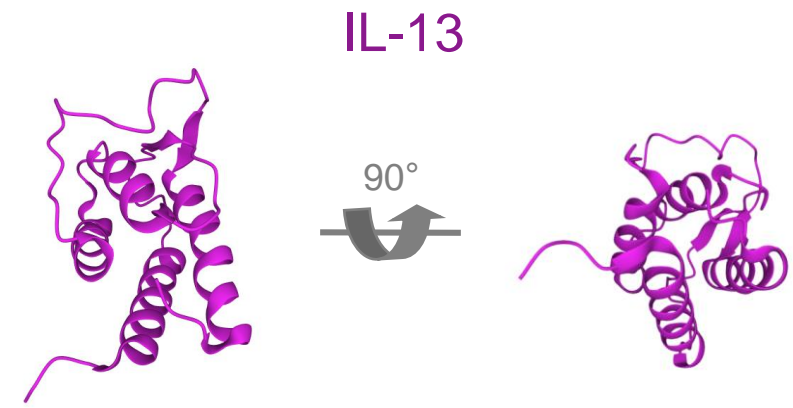


Figure 2

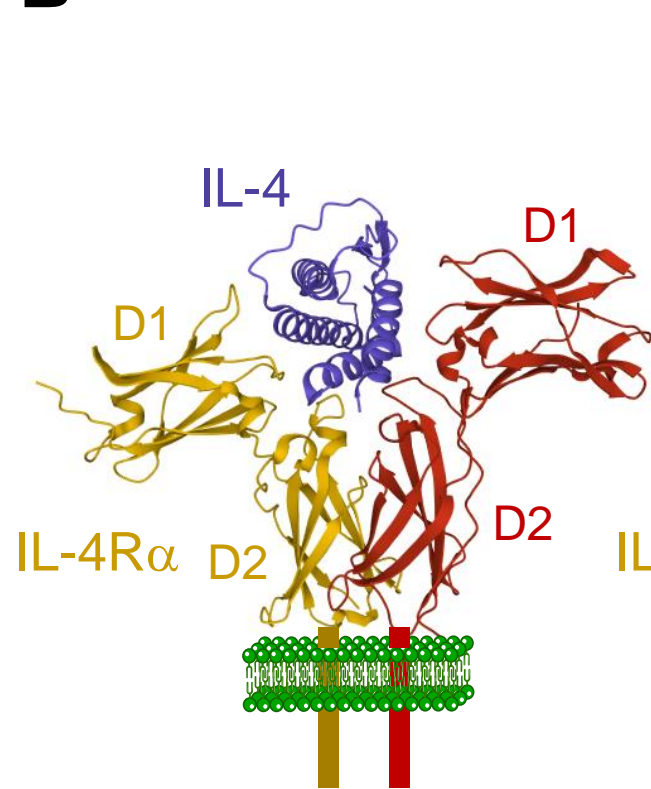
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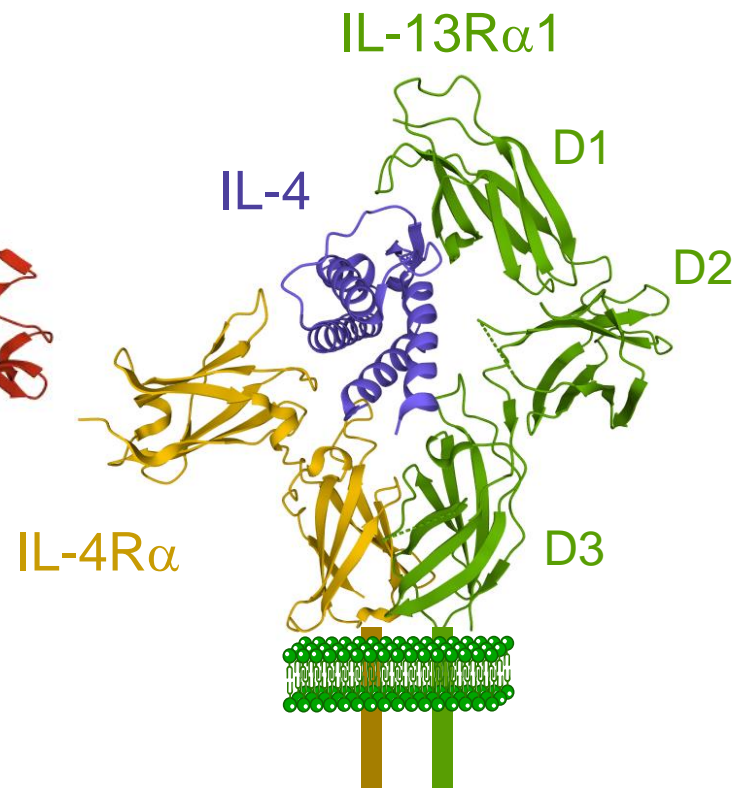
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Type I



Type II

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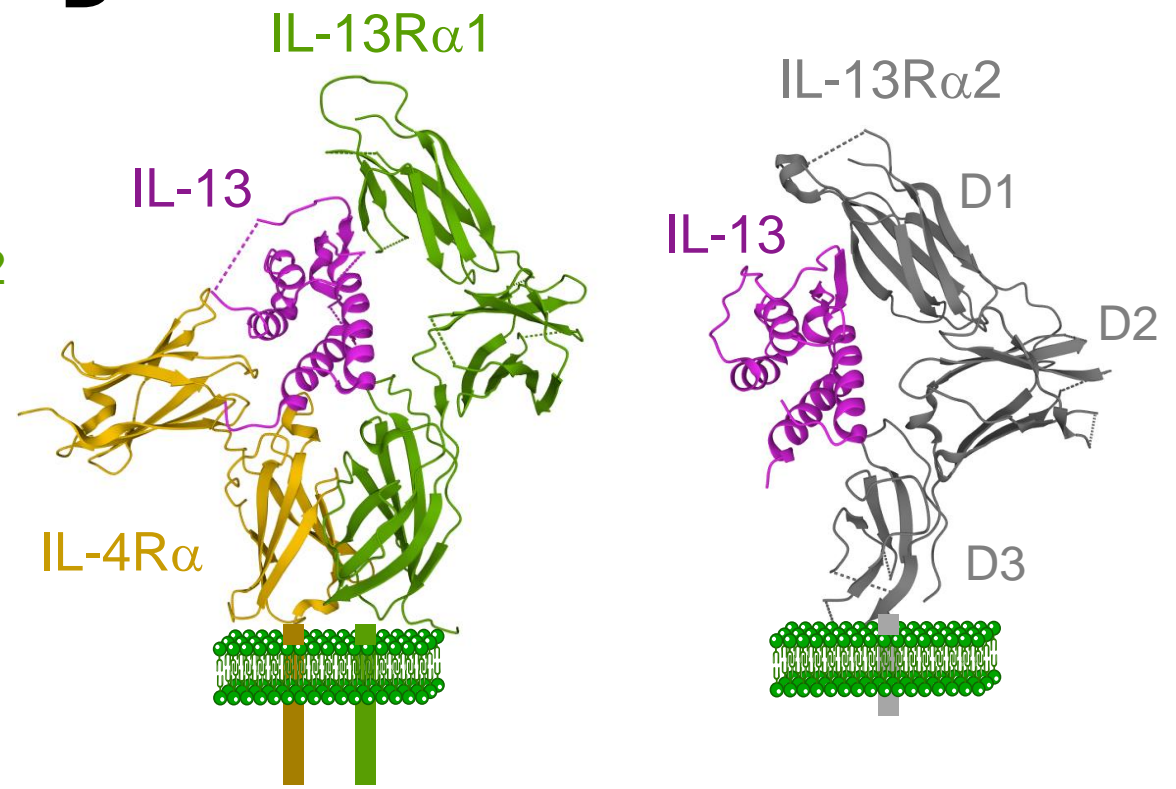


Figure 3

