Pharmacological Therapy of Bronchial Asthma: The Role of Biologicals

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Key Words

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Abstract

Bronchial asthma is a heterogeneous, complex, chronic inflammatory and obstructive pulmonary disease driven by various pathways to present with different phenotypes. A small proportion of asthmatics (5–10%) suffer from severe asthma with symptoms that cannot be controlled by guideline therapy with high doses of inhaled steroids plus a second controller, such as long-acting β_2 agonists (LABA) or leukotriene receptor antagonists, or even systemic steroids. The discovery and characterization of the pathways that drive different asthma phenotypes have opened up new therapeutic avenues for asthma treatment. The approval of the humanized anti-IgE antibody omalizumab for the treatment of severe allergic asthma has paved the way for other cytokine-targeting therapies, particularly those targeting interleukin (IL)-4, IL-5, IL-9, IL-13, IL-17, and IL-23 and the epithelium-derived cytokines IL-25, IL-33, and thymic stromal lymphopoietin. Knowledge of the molecular basis of asthma phenotypes has helped, and continues to help, the develop-

ment of novel biologicals that target a diverse array of phenotype-specific molecular targets in patients suffering from severe asthma. This review summarizes potential therapeutic approaches that are likely to show clinical efficacy in the near future, focusing on biologicals as promising novel therapies for severe asthma.

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Introduction

Bronchial asthma is a heterogeneous, complex, chronic inflammatory and obstructive pulmonary disease characterized by augmented mucus secretion, airway hyper-reactivity, and, in the long term, functional and structural lung tissue alterations [1]. Asthma currently affects an estimated 235 million people worldwide. Most asthmatics are well controlled with asthma guideline therapies. Extrinsic (allergic) asthma is the predominant asthma subtype and usually manifests during childhood, while late-onset asthma is mostly nonallergic [2]. However, a small portion of asthma patients (5–10%) suffer from severe asthma that is refractory to high doses of inhaled steroids plus a second controller. In the uncontrolled situation, long-acting β_2 agonists (LABA) and/or phosphodiesterase inhibitors and/or long-acting anticholinergics (LAMA) are also used to treat severe asthma. Treatment

Table 1. New potential drugs in the pipeline for asthma treatment and their target and developmental stage

Drug name	Drug target	Developmental stage	Estimated time of market launch
Omalizumab	IgE	EMA and FDA approved	Already launched
QGE-031	IgE	Clinical phase III	2019
MEDI-4212	IgE	Clinical phase I	After 2020
Quilizumab	IgE	Clinical phase II	After 2020
Dupilumab	IL-4R α	Clinical phase II	After 2020
Pitrakinra	IL-4 R α	Clinical phase II	NA
Pascolizumab	IL-4	Suspended	–
Altrakincept	IL-4	Suspended	–
Mepolizumab	IL-5	FDA approved	Already launched
Reslizumab	IL-5	Clinical phase II	2016
Benralizumab	IL-5 R α	Clinical phase II	After 2020
MEDI-528	IL-9	Clinical phase II	NA
Tralokinumab	IL-13	Clinical phase II	2017–2018
Lebrikizumab	IL-13	Clinical phase III	2017–2018
QAX-576	IL-13	Clinical phase II	After 2020
Anrukinzumab	IL-13	Clinical phase II	NA
ABT-308	IL-13	Clinical phase I	NA
CNTO 5825	IL-13	Clinical phase I	NA
GSK679586	IL-13	Clinical phase II	NA
Brodalumab	IL-17	Clinical phase II	2019
Secukinumab	IL-17	Clinical phase II	NA
MT203	GM-CSF	Clinical phase II	NA
Golimumab	TNF- α	Suspended	–
Infliximab	TNF- α	Clinical phase II	NA
GW766994	CCR3	Clinical phase II	NA
AMG 761	CCR4	Clinical phase II	NA
GSK2239633	CCR4	Clinical phase II	NA
AM211	CRTH2	Clinical phase I	NA
ARRY-502	CRTH2	Clinical phase I	NA
QAV 680	CRTH2	Clinical phase II	NA
Setipiprant	CRTH2	Clinical phase II	NA
RG7185	CRTH2	Clinical phase I	NA
AMG 853	CRTH2/DPR	Clinical phase III	NA
AZD 1981	CRTH2/DPR	Clinical phase II	NA
AMG 157	TSLP	Clinical phase II	NA
Imatinib	Tyrosine kinases	Clinical phase II	NA ¹

NA = No estimated time of market launch has been announced; – = there will be no market launch (see text).

¹ Already launched/approved to treat chronic granulocyte leukemia.

with systemic steroids is also often required to control severe asthma, which not only affects the patient's quality of life but can also, in rare cases, cause death. Patients with severe asthma account for over 50% of the total healthcare costs associated with bronchial asthma [3] due to frequent hospital admissions, the need for emergency services, and high drug consumption. Therefore, there is an urgent need for novel, more effective strategies to treat severe asthma.

Biologicals (or biologics) are mostly genetically synthesized proteins that exert therapeutic effects by activating or inhibiting diverse endogenous target functions. A

number of biologicals are currently being used or tested in clinical lung research, and convincing data has been published showing a reduction in asthma exacerbations and improved lung function in asthma patients treated with biologicals. The monoclonal anti-IgE antibody omalizumab was the first biological to be approved [i.e. by the US Food and Drug Administration (FDA) in 2003 and the European Medicine Agency (EMA) in 2005] for the treatment of severe allergic asthma as an alternative to systemic steroids.

Given the promise of these novel agents and the diverse array of targets, here we review the therapeutic approaches used in patients with bronchial asthma, focusing on biologicals as potential novel therapies for severe asthma (table 1).

The Pathophysiology of Asthma

Bronchial asthma is a chronic inflammatory and obstructive respiratory disease characterized by mucus hypersecretion, bronchial hyperreactivity and obstruction, and airway remodeling. These pathophysiological features contribute to a progressive loss of lung function [4, 5]. The major clinical symptoms of bronchial asthma are episodic cough, wheezing, chest tightness, and paroxysmal dyspnea (intermittent shortness of breath). More recently, asthma has been recognized as a heterogeneous, complex disease that presents with different phenotypes [2, 6, 7].

The inflammatory processes seen in asthma are initiated via interplay between cytokines produced by different cells including B and T lymphocytes, basophils, eosinophils, neutrophils, mast cells, epithelial cells, mesenchymal cells, group 2 innate lymphoid cells (ILC2) [8], and airway neurons. Asthma is also known to have a hereditary etiological component [9], with its development and progression dependent on gene-environment interactions [10].

Dendritic cells (DC) recognize allergens and subsequently process antigen molecules (fig. 1) to expose the antigens on the cell surface for presentation to naive T_H0 cells. The resulting T_H2 activation arises from secretion of the key interleukins (IL) IL-4, IL-5, and IL-13, probably from basophils, eosinophils, mast cells, and T cells [11]. T_H2 cells can release IL-5, which stimulates eosinopoiesis and leads to the recruitment and maturation of eosinophils, leading to severe eosinophilic bronchial asthma. Both IL-4 and IL-13 result in the production and secretion of IgE by inducing maturation of B lymphocytes to plasma cells [12]. IgE is predominantly membranous, being anchored to the surface of mast cells [13]. Contact between allergens and IgE antibodies initiates intracellular signaling cascades [14] and the secretion of histamine, prostaglandins, cytokines, and other inflammatory mediators [15]. These mediators are proinflammatory and affect a number of cell types including epithelial cells, gland cells and airway smooth muscle cells, leading to bronchoconstriction. Eosinophils, in turn, produce cytokines and leukotrienes, further increasing mucus secretion and bronchoconstriction. T_H2 cells are also known to release IL-4 and tumor growth factor (TGF)- β to acti-

vate IL-9-producing T_H9 cells [16]. By triggering the proliferation and recruitment of mast cells, IL-9 results in the release of different inflammatory cytokines. T_H0 cells activate T_H17 cells, a population of CD4-positive lymphocytes, via the release of TGF- β and IL-6 and the consequent production and secretion of IL-17A and IL-17F [17–19]. IL-17A and IL-17F induce the migration and recruitment of neutrophils [20] via the secretion of highly potent chemoattractants such as CXC ligand 1 (CXCL1) and CXCL8 (IL-8). These chemoattractants are released by different airway cells such as subepithelial fibroblasts and respiratory bronchial cells [21].

Immune responses to viral respiratory infections are thought to be initiated by the epithelium-derived cytokine thymic stromal lymphopoietin (TSLP). TSLP is a cytokine secreted by mast cells and bronchial epithelium. It mainly acts on DC and initiates the T_H2 -mediated asthma response [22]. TSLP triggers the release of different cytokines of the CC motif chemokine family. The CC chemokine receptors CC chemokine receptor type 3 (CCR3) and CCR4 are G-protein-coupled receptors that are highly expressed in eosinophils and basophils, T_H1 and T_H2 cells, and airway epithelia. CCR3 and CCR4 act as receptors for a variety of chemokines including the eotaxins CCL5, CCL17, and CCL22, which play a role in asthma pathophysiology [23]. There is evidence that CCR3 inhibition may decrease inflammation, and CCR4 is believed to be able to recruit T_H2 cells to inflammatory sites [24]. CCR4 ligand binding induces the production and release of many cytokines [25] including granulocyte-macrophage colony-stimulating factor (GM-CSF) [26].

Tumor necrosis factor- α (TNF- α) is a cytokine released by macrophages, monocytes, and CD4⁺ T lymphocytes that also plays a role in neutrophilic asthma and promotes different inflammatory effects [25, 27].

Bronchial asthma is characterized not only by inflammatory processes but also by structural lung tissue alterations such as subepithelial fibrosis, increased airway smooth muscle cell proliferation, and extracellular matrix protein deposition. Bronchial membrane thickening decreases lung function by limiting the airflow [5, 25].

Bronchial Asthma Phenotypes

The different phenotypes observed in asthma have been explored in recent years. Although asthma was initially classified into extrinsic and intrinsic types [28], this classification was further characterized as T_H2 or non- T_H2 phenotypes. The T_H2 -induced phenotype includes

Fig. 1. Key players in the T_H2 -mediated pathomechanism of asthma. DC capture allergens within the cytoplasm, expose them on their surface, and present them to naive T_H0 cells, which causes the activation of T_H2 cells and the secretion of IL-4 and IL-13. Both of these IL activate IgE production and secretion by inducing the maturation of B lymphocytes to plasma cells. The predominantly membranous IgE on mast cells initiates a signal cascade if activated by contact with an allergen. This leads to the secretion of histamines, prostaglandins, cytokines, and other inflammatory mediators. T_H2 cells also release IL-5, which starts the maturation of eosinophils

and finally causes increased mucus secretion in epithelial cells. The expression of IL-4 and TGF- β activates T_H9 cells, which in turn release IL-9 and thereby cause the release of different cytokines, histamines, and other inflammatory mediators. The release of TGF- β and IL-6 by T_H0 cells activates T_H17 , which induces the release of IL-17A and IL-17F. Both of these IL initiate the recruitment and expansion of neutrophils via activation of the secretion of chemoattractants (CXCL1 and CXCL8). CXCL1 and CXCL8 are secreted by different cellular components of lung tissue, such as subepithelial fibroblasts and respiratory bronchial cells.

early-onset allergic T_H2 -mediated and late-onset persistent eosinophilic asthma, with a range of severities [2]. Early-onset asthma is often associated with other allergic comorbidities, and its progression can be mild to severe. Late-onset eosinophilic asthma is usually not atopic and is often severely progressive, characterized by increased numbers of sputum and blood eosinophils [2, 29]. Asthma may be triggered by environmental factors and a genetic predisposition [30, 31]. The T_H2 -mediated pheno-

type also includes exercise-induced asthma (EIA), in which symptoms normally occur during and/or after physical activity and increased dry and cold conditions [2]. Asthma symptoms are often relatively mild in EIA patients, although both high and low levels of eosinophilic inflammation have been reported in EIA [32].

Non- T_H2 -mediated asthma is also divided into different subtypes, i.e. women with late-onset asthma, obesity-related asthma, smoking-associated asthma, neutrophilic

Table 2. Asthma classification and therapy: level of severity, associated symptoms, and suggested pharmacotherapy (GINA modified)

Level of asthma severity	Symptoms	Suggested therapy
1 (intermittent)	<1 daytime and 2 nighttime symptoms/week FEV ₁ and PEF ≥80% Diurnal variability of PEF <20%	RABA if needed
2 (low-grade, persistent)	Same but stronger symptoms than in level 1 More restrictions in physical activity and sleep Diurnal variability of PEF between 20 and 30%	Low-dose ICS; alternatively, LTRA (montelukast)
3 (moderate, persistent)	Daily occurring symptoms >1 nighttime symptom FEV and PEF of 60–80% Diurnal variability of PEF >30%	Middle-dose ICS or low-dose ICS plus LABA
4 (high-grade, persistent)	Persistent day-to-day symptoms; Many exacerbations at day- and nighttime; High negative impact on physical activities FEV and PEF ≤60% Diurnal variability of PEF >30%	Middle-to-high-dose ICS plus LABA
5 (uncontrolled, high-grade, persistent)	Same but stronger symptoms than in level of severity 4	Oral corticosteroids; monoclonal antibodies in IgE-mediated asthma

RABA = Rapid-acting β_2 agonists; LTRA = leukotriene receptor antagonist.

asthma, and smooth muscle-mediated, paucigranulocytic asthma [2]. Although these phenotypes represent a substantial proportion of all asthmatics, little is known about their onset [2]. Obesity-related asthma is one of two non- T_H2 -related eosinophilic asthma phenotypes. Obesity has a negative effect on lung function, and obese patients are more susceptible to gastroesophageal reflux, chest tightness, and hypoventilation; therefore, asthma may be misdiagnosed in obese patients. Neutrophil-related asthma is another non- T_H2 -related asthma subtype that has yet to be fully characterized [33]. There is evidence that T_H17 cells may contribute to this neutrophil-related asthma by promoting neutrophilic inflammation. T_H17 cells release IL-17, a major neutrophil chemoattractant, into the airways [34], and it has been shown that IL-17A and IL-17F levels are associated with asthma severity and an increase in neutrophilic inflammation [17].

Current Pharmacotherapy

According to the Global Initiative for Asthma (GINA), asthmatics should receive low- to high-dose inhaled steroids alone or in combination with other controllers such as LABA and/or leukotriene receptor antagonists (montelukast) and/or theophylline. Additionally, the long-act-

ing muscarinic receptor antagonist tiotropium is recommended for the treatment of severe asthma. Systemic steroids are also often needed in patients with severe asthma. The first biological licensed for asthma is an anti-IgE agent (omalizumab), which is recommended as the preferred controller for patients with severe perennial allergic asthma and may be a suitable alternative treatment to systemic steroids (table 2).

Anti-IgE Therapy

IgE levels are often increased in allergic asthmatics and they are sometimes associated with allergic symptoms [35]. To significantly reduce IgE-induced symptoms, monoclonal anti-IgE antibodies have been recommended for the treatment of severe allergic asthma. Anti-IgE antibodies can bind free serous IgE and membranous IgE at the surface of mast cells and B lymphocytes [36].

Omalizumab binds free IgE with a high affinity by interacting with its C ϵ 3 domain [37], thereby reducing asthma exacerbations [38]. Moreover, omalizumab reduces the expression of Fc ϵ RI on basophils and DC [39]. IgE binding and the reduction in expressed Fc ϵ RI leads to inhibition of further interactions with ligands and deactivation of signaling cascades. The reduced interaction be-

tween IgE and FcεRI avoids allergen-induced degranulation. As well as reducing circulating IgE levels, new IgE production can be limited or prevented [40]. Furthermore, inhibition of IgE-FcεRI binding on DC reduces antigen presentation to T lymphocytes.

However, omalizumab use is bound by specific clinical criteria. Apart from being approved only for patients with severe asthma (level 5), patients need to: (a) have a forced expiratory volume in 1 s (FEV₁) under 80%, (b) be prick test or radioallergosorbent test positive for a perennial inhalation allergen (e.g. mold or house dust), (c) have a serous IgE level between 30 and 1,500 IE/ml, and (d) not weigh over 150 kg. Omalizumab side effects include pruritus, headache, syncope, paresthesia, and anaphylaxis, which could ultimately be lethal due to anaphylactic shock, but, in general, the drug is well tolerated [41]. Based on a study to evaluate its postlaunch safety [42], a higher risk of suffering from thrombotic events caused by the therapy with omalizumab was reported.

Ligelizumab (QGE-031; Novartis) is a humanized monoclonal anti-IgE antibody currently in phase III clinical trials. It binds with a higher affinity to IgE than omalizumab, and analyses have shown an almost 50-fold increased binding affinity to human IgE compared to omalizumab [43]. Ligelizumab suppresses free IgE more rapidly and to a greater extent and also significantly reduces the wheal diameter in skin prick tests compared to placebo and omalizumab [44]. Ligelizumab is suggested for the treatment of IgE-induced diseases like severe allergic asthma and atopic dermatitis.

MEDI-4212 (AstraZeneca) is also an antibody directed against human IgE that inhibits IgE-FcεRI binding and IgE-CD23 binding. Due to its strong inhibitory effect on IgE-FcεRI binding, MEDI-4212 is specific for the treatment of asthma patients with high circulating IgE.

Quilizumab (anti-M1 prime mAb; Roche) is a humanized monoclonal antibody currently in phase II clinical trials. Quilizumab binds the M1 prime domain of membranous IgE, thus preventing the differentiation of B lymphocytes into IgE-producing plasma cells. Based on phase II clinical trial data, quilizumab may be suitable for the treatment of moderate to severe asthma. The drug has been retracted due to a lack of effect.

Anti-IL-5 Therapy

IL-5, which is secreted mainly by T_H2 cells but also in smaller amounts by eosinophils and mast cells, plays a key role in the immune reaction by activating eosino-

phils. The IL-5 receptor is the single interaction partner of IL-5, but it also works in synergy with other signaling molecules such as chemokines, eotaxins, and IL-4 and IL-13 to drive the eosinophilic immune response [45]. The IL-5 receptor (IL-5R) is composed of α and β subunits [45, 46]. The α subunit binds peptides and the β subunit is the signal transduction domain. The IL-5 receptor is expressed on the surface of eosinophils, Eos progenitors (CD34+; eosinophil progenitor cells), mast cells, and basophils [45, 46]. The intracellular signal is mainly transduced via the Janus kinase-activated 2 (JAK2)/signal transducer and activator of transcription 3 (STAT-3) pathway [46].

Mepolizumab, a humanized monoclonal anti-IL-5 antibody, reduced eosinophils in patients suffering from eosinophilic asthma, significantly lowering the number of eosinophils in circulation and in the lung and bone marrow [47]. Asthma exacerbations were distinctly decreased [48, 49], and side effects have not been observed to the date of reporting [50]. Mepolizumab has just been approved for asthma therapy by the FDA (11/2015) and the approval from the EMA is launched.

Another humanized monoclonal anti-IL-5 antibody, reslizumab, has been evaluated for the treatment of severe eosinophilic asthma [51]. Animal studies revealed a 75% decrease in eosinophil accumulation, and current clinical trials have shown a nonsignificant decrease in the asthma exacerbation rate [52].

Benralizumab also belongs to the group of monoclonal anti-IL-5 antibodies, and it binds IL-5Rα with a high affinity even in the presence of slight conformational changes of the epitope. This leads to interruption of IL-5R-mediated signal transduction and inhibition of IL-5R-dependent cell proliferation [53]. There is evidence to suggest that benralizumab may be effective in both serum and tissue [54], perhaps increasing the ultimate clinical significance of this drug. Benralizumab is afucosylated, which improves the antibody dependent cell-mediated cytotoxicity [55].

Drugs Targeting IL-9

IL-9, derived from T_H2 and T_H9 cells, and the IL-9 receptor (IL-9R) are overexpressed in the airways of asthmatics [56]. This leads to eosinophilic inflammation associated with airway hyperresponsiveness (AHR), mucus hyperplasia, mast cell proliferation [57], and augmented expression of IgE and other T_H2-related cytokines [21]. Additionally, IL-9 triggers IL-13 secretion.

MEDI-528 is a humanized monoclonal anti-IL-9 antibody currently in phase II trials. Clinical trial results have differed; when MEDI-528 was used in addition to standard asthma medication, there were no significant effects on asthma exacerbation rates, asthma control questionnaire-6 (ACQ-6) scores, or FEV₁ compared to placebo controls [58]. In patients with mild to moderate asthma, there are some data to suggest that MEDI-528 might have clinical relevance. The two studies performed to the date of reporting indicate an improved quality of life with the use of this agent with respect to asthma symptoms and exacerbations [59].

Drugs Targeting IL-13

IL-13 plays a key role in the initiation and maintenance of inflammation in bronchial asthma, where it exerts the following functions: (i) initiation of the IgM-to-IgE isotype switch in B lymphocytes [60, 61], (ii) mediation of the activation and proliferation of mast cells [62] and smooth muscle cells [63], and (iii) triggering of the adhesion of eosinophils to vascular endothelial cells [64]. In contrast to IL-4, which is able to mediate its effects via the type I IL-4R (IL-4 α and IL-4) or the type II IL-4R, evidence suggests that IL-13 binds solely to the type II IL-4R, which consists of 2 subunits, i.e. the IL-13R α 1 and IL-4R α chains. Therefore, it is thought that an IL-4-independent IL-13 signaling pathway exists [65].

Tralokinumab is a humanized anti-IL-13 monoclonal antibody currently in phase II trials. Furthermore, experiments in tralokinumab-treated mice showed that AHR and eosinophilic influx are reduced in an allergic airway inflammation model [66].

In a randomized multicenter study, lebrikizumab, which blocks IL-13, significantly improved lung function in asthmatic patients. The drug seemed to have a marked differential impact depending on the asthma phenotype, being particularly effective in T_H2 phenotypes with high serous periostin levels.

Other anti-IL-13 agents include: QAX-576, an IL-13 antibody, which is being tested in an early-stage study of patients with moderate to severe asthma; anrukinzumab, a humanized monoclonal anti-IL-13 antibody currently in phase II clinical trials and showing significant improvements in patients with mild atopic asthma [25]; ABT-308, a high-affinity anti-human IL-13 antibody that prevents IL-13 binding to the IL-13R α 1 and IL-13R α 2 subunits, currently in phase I trials after showing significant effects in mice; CNTO 5825, a human monoclonal

anti-IL-13 antibody that has exhibited good safety and tolerability profiles in phase I clinical trials [67], and GSK 679586, a humanized anti-IL-13 IgG1 monoclonal antibody that has currently completed phase II testing. GSK 679586 showed dose-dependent pharmacological activity in a randomized, placebo-controlled phase I dose escalation study [68].

Drugs Targeting IL-4

IL-4 and IL-13 share one receptor, i.e. the IL-4 α -subunit. The mechanism via which IL-13 is activated by IL-4R is not fully understood [69]. It is known, however, that IL-4 contributes to the pathomechanism of asthma by initiating T_H2 differentiation, isotype switching of B lymphocytes during IgE synthesis, mast cell development, eosinophil recruitment, and mucus metaplasia [21]. Moreover, IL-4 is a key player in fibronectin and collagen synthesis [70], which ultimately leads to airway remodeling.

Dupilumab, a human monoclonal antibody, is directed against IL-4R α . IL-4 and IL-13 blockade directly affects IL-4R and indirectly affects downstream IL-13R pathways since IL-13 also binds to IL-4R α . A clinical trial in moderate to severe asthmatics revealed a reduced risk of exacerbations and a significant improvement in most lung function tests [71].

Pitrakinra is a recombinant human protein that acts as an IL-4 and IL-13 antagonist by competitively blocking IL-4R α , thereby affecting both pathways simultaneously. By inhibiting IL-4 and IL-13, pitrakinra inhibits T_H2-mediated immune responses [51]. Clinical and preclinical animal studies have shown that pitrakinra is significantly more effective on AHR when inhaled rather than subcutaneously injected [72].

Pascolizumab is a humanized anti-IL-4 antibody that inhibits both upstream and downstream T_H2 pathway events [73]. Pascolizumab was very well tolerated in a phase I trial in adult patients with mild to moderate asthma, but it lacked clinical efficacy in a large-scale multi-dose phase II trial in steroid-naïve patients with symptomatic asthma [25].

Altrakinept, a soluble recombinant human IL-4 receptor, contains the IL-4R α chain but lacks the other domains. Thus, it cannot activate downstream cascades. IL-4 binds to the IL-4R α domain of altrakinept and is therefore removed from the circulation. However, phase II clinical trials revealed no significant improvement in lung function and asthma symptoms [74] with altrakinept and no new studies are currently planned.

Drugs Targeting IL-17

IL-17 is secreted by T_H17 cells and plays an important role in airway remodeling and neutrophilic inflammation. IL-17 molecules comprise several subtypes, i.e. IL-17A, IL-17B, IL-17C, IL-17E (IL-25), and IL-17F [17]. IL-17 appears to be a promising drug target in asthma, with 2 antibodies in particular, i.e. brodalumab and secukinumab, showing promise. However, their therapeutic effect, tolerability, and safety need to be confirmed in further clinical evaluations.

Brodalumab is a human IL-17-specific monoclonal antibody in phase II clinical trials of patients with severe asthma not adequately controlled by inhaled corticosteroids and LABA. Secukinumab targets IL-17A and is in phase II trials. It is suggested for use in patients with severe asthma that cannot be controlled with inhaled corticosteroids and LABA.

Drugs Targeting GM-CSF

GM-CSF is a glycoprotein that plays a role in the transduction of inflammatory reactions. GM-CSF is generated with other proinflammatory mediators, mainly by epithelial cells but also by fibroblasts, macrophages, mast cells, and natural killer cells; it is then released into the circulation [75]. This leads to activation and differentiation of various cell types that induce airway inflammation with resulting damage to the airway epithelium [76]. Because of the role of GM-CSF during inflammatory events, it may represent a potential future drug target for the treatment of asthma [77].

Drugs Targeting TNF- α

TNF- α , a proinflammatory innate cytokine secreted by macrophages, mast cells, T_H1 lymphocytes, and many other cell types, is overexpressed in the lung tissue of asthmatics and stimulates respiratory smooth muscle [25]. During airway inflammation, TNF- α recruits eosinophils and neutrophils [27]. Despite several controversies (regarding the assertion that blocking TNF- α may lead to a higher risk of infection and cancer), clinical studies with infliximab, etanercept, and golimumab have been undertaken.

The human-murine chimeric monoclonal anti-TNF- α antibody infliximab significantly improved lung function parameters (e.g. peak expiratory flow) and exacerbations in patients with moderate asthma [78]. Etanercept is a recom-

binant protein that binds both TNF- α and lymphotoxin (TNF- β) with a high affinity; however, it showed no significant positive effects in a randomized, double-blind, placebo-controlled phase II trial [79]. Golimumab is an anti-TNF- α antibody that should have been a candidate biological for severe asthma. However, a large multicenter study revealed side effects including infection, pneumonia, tuberculosis, and malignancy (breast cancer and B-cell lymphoma) [80]. Understandably, the research community subsequently withdrew from further anti-TNF- α trials.

Drugs Targeting CCR3 and CCR4

Chemokines play a pivotal role in respiratory inflammation [81]. The CC chemokine receptors CC chemokine receptor type 3 (CCR3) and CCR4 are G-protein-coupled proteins that are highly expressed in eosinophils, basophils, T_H1 and T_H2 cells, and airway epithelia. CCR3 and CCR4 act as receptors for a variety of chemokines such as eotaxins and CCL5, which play a role in the pathogenesis of bronchial asthma [23]. There is evidence to suggest that CCR3 inhibition may decrease airway inflammation. CCR4 is believed to be able to recruit T_H2 cells to the site of inflammation [24].

GW 766994 is a selective, competitively binding CCR3 antagonist that has already completed phase I trials in patients with mild to moderate asthma and high sputum eosinophilia [23]. Mogamulizumab (AMG 761) is a humanized monoclonal afucosylated anti-CCR4 IgG1 antibody [82]. It is currently in phase I trials, and no safety and efficiency data has yet been published.

GSK2239633 [N-(3-[(3-[5-chlorothiophene-2-sulfonamido]-4-methoxy-1H-indazol-1-yl)methyl]benzyl)-2-hydroxy-2-methylpropanamide] is a potent CCR4 antagonist that acts by inhibiting CCR4-TARC (thymus and activation-regulated chemokine) binding and interrupting downstream signaling cascades [24]. The drug is currently in phase I trials. In an open-label study and a randomized clinical trial, GSK 2239633 significantly inhibited TARC from activating the CCR4 receptor [24].

Drugs Targeting CRTH2 and CRTH2/DPR

Chemoattractant receptor-homologous molecule expressed on T_H2 cells (CRTH2) and D-type prostanoid receptor (DPR) are G-protein-coupled prostaglandin (PGD₂) receptors. PGD₂ is secreted by mast cells during inflammation, including in asthma [83]. Binding of PGD₂

to CRTH2 and DPR, respectively, mediates basophil, eosinophil, and T_H2 cell chemotaxis, prolongs their survival [84], and stimulates the secretion of different cytokines [85]. Hence, potent CRTH2 and/or DPR antagonists might be a promising strategy in asthma treatment. Several encouraging antagonists are in different stages of development.

The two CRTH2 antagonists in phase I clinical trials are AM211 [(2'-[3-benzyl-1-ethyl-ureidomethyl]-6-methoxy-4'-trifluoromethyl-biphenyl-3-yl)-acetic acid sodium salt] [86] and RG7185. To the date of reporting, three CRTH2 antagonists have been tested in phase II trials, i.e. NAV-QAV680, which showed good bioavailability in rats and rodents [87], setipiprant [2-(2-[1-naphtho-yl]-8-fluoro-3,4-dihydro-1H-pyrido(4, 3-b)indol-5[2H]-yl)acetic acid] [88], and ARRY-502.

Two CRTH2/DPR antagonists are also currently under clinical investigation. AMG853 showed no clinical efficacy in a phase II trial [89], while AZD1981 [4-(acetylamino)-3-([4-chlorophenyl] thio)-2-methyl-1H-indole-1-acetic acid] is a new CRTH2/DPR antagonist in phase II clinical trials. To date, no data on its clinical and pharmaceutical properties have been published [90]. The major symptoms of aspirin-exacerbated respiratory disease (also termed Samter's triad) are nasal polyps, chronic hypertrophic eosinophilic sinusitis, and asthma. Aspirin and other nonsteroidal anti-inflammatory drugs are known to inhibit the cyclooxygenase-1 (COX-1) enzyme. Inhibition of the COX pathway may cause rhinitis, conjunctivitis, laryngospasm, and bronchospasm. This leads to a shift to increased leukotriene production, resulting in airway inflammation and bronchoconstriction. Under physiological conditions, PGD_2 suppresses the release of histamines and prostaglandins from mast cells. In the presence of aspirin and other nonsteroidal anti-inflammatory drugs, the COX-1/ PGD_2 -induced inhibition of mast cell activation is diminished, which leads to the activation of mast cells, including the release of histamines and PGD_2 [91]. As CRTH2/DPR antagonists, AMG853 and AZD1981 may be useful in the therapy of aspirin-induced respiratory diseases.

Drugs Targeting Epithelium-Derived Cytokines

The epithelial cells that line mucosal surfaces not only act as a physical barrier but also participate in immune reactions. Small-airway epithelial cells produce different cytokines and thus affect T_H2 -mediated immune responses. TSLP, IL-25, and IL-33 represent a group of so-

called epithelial cell-derived cytokines. Treatment of patients with mild atopic asthma with the human anti-TSLP monoclonal immunoglobulin AMG 157 in a double-blind, placebo-controlled study showed a decrease in allergen-induced bronchoconstriction [92]. In mice, anti-IL-33 reduced airway inflammation and decreased airway remodeling [93]. IL-25 (also IL-17E) and its receptor were recently shown to be expressed on eosinophils from patients with allergic asthma [94]. Application of a monoclonal anti-IL-25 antibody in mice prevented AHR [95], and IL-25 may represent another promising drug target.

Drugs Inhibiting Tyrosine Kinases

Tyrosine kinases play a pivotal role in the course of asthma, especially in remodeling processes in lung tissue [96]. The activation of tyrosine kinases initiates a broad spectrum of downstream effector molecules and represents a promising target for drug treatment of asthma. A study in an animal model of asthma in guinea pigs showed encouraging results concerning the reduction of inflammatory effects of the broad-range protein tyrosine-kinase inhibitor genistein [97]. The use of imatinib, a tyrosine kinase inhibitor in a phase II trial, which is already approved for the treatment of chronic granulocytic leukemia, showed promising results in a mouse model for allergic airway inflammation concerning the prevention of inflammatory and remodeling events in lung tissue [98]. Another drug against chronic granulocyte leukemia that has already shown promising and even more positive effects than imatinib is nilotinib [99]. Clinical trials concerning safety and efficacy in asthma are yet to come.

Drug Use for an Unapproved Indication

In addition to guideline-driven treatment, some drugs have also been used to treat bronchial asthma as an unapproved indication.

Asthma patients are often also treated with macrolides (azithromycin, roxithromycin, and clarithromycin). Macrolides are often used as antibiotics to treat acute bacterial respiratory infections. There is growing evidence that macrolides not only exert an antibacterial effect but also modulate the immune system to improve inflammatory responses [100]. The long-term use of macrolides may improve several lung function parameters such as the FEV_1 and the peak expiratory flow and decrease symptoms like AHR [101].

Cyclosporine is another off-label drug used to treat asthma. Cyclosporine is immunosuppressive by inhibiting T lymphocyte function [102] and avoiding the release of proinflammatory mediators by basophils and mast cells [103]. Specifically, it inhibits GM-CSF, IL-5, eotaxin, and bronchial eosinophilia [104]. Cyclosporine has been shown to improve several lung function parameters [105], but adverse side effects such as infection, hypertrichosis, tremor, and paraesthesia limit its use [102].

Methotrexate is an immunotherapy that inhibits the enzyme dihydrofolate reductase [103], IL-1, and basophilic histamine release [106]. Since no large-scale studies have been conducted to test its safety and efficacy, methotrexate use for the treatment of severe asthma remains controversial due to potentially severe side effects including a deranged liver function, disordered hematopoiesis, and infections that can ultimately lead to sepsis [107].

Azathioprine is another immunosuppressive agent that is used as an oral corticosteroid-sparing agent [108]. Azathioprine inhibits purine synthesis and thus inhibits lymphocyte proliferation [109]. However, there is only limited efficacy and safety data on azathioprine in the context of asthma, and more studies will need to be performed to evaluate its use in asthma. Likewise, interferon

may also be a potent asthma treatment, particularly interferon- λ , which has been found to modulate the release of T_H1 cytokines and activate T_H1 pathway drift [110].

In general, these off-label drugs can disrupt hematopoiesis, derange liver enzymes, and lead to infections. The outcome of an off-labeled drug therapy is dependent on the individual and therapy is not always successful.

Conclusion

Bronchial asthma is a heterogeneous, complex, chronic inflammatory and obstructive disease with many different phenotypes. The recognition, identification, and characterization of T_H2 and non-T_H2 phenotypes have driven the development of molecular targeted therapies that are likely to become available in the near future. Many promising drugs are currently undergoing clinical trials that not only target the main IL but also receptors (such as chemokine receptors) and other signaling proteins. These are showing promise in meeting the ultimate goal of controlling severe, treatment-refractory asthma.

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