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Commentary

The increasing challenge of discovering asthma drugs

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ABSTRACT

The prevalence of asthma continues to rise. Current drugs provide symptomatic relief to some, but not all, patients. Despite the need for new therapeutics, and a huge research effort, only four novel agents from two classes of drugs – the antileukotrienes and an anti-IgE antibody – have been approved in the last 30 years. This review highlights three particular issues that contribute to the challenge of identifying new therapeutics. First is an over-reliance on animal models of allergy to define targets and expectations of efficacy that has met with poor translation to the clinical setting. While sensitivity to particular aeroallergens is one key risk factor for asthma, atopy and asthma are not synonymous, and while about half of adult asthmatics are atopic the incidence of allergic asthma is probably <50%. The second issue is a fundamental disconnect between the directions of basic research and clinical research. Basic research has developed a detailed, reductive, unifying mechanism of antigen-induced, T helper type 2 cell-mediated airway inflammation as the root cause of asthma. In contrast, clinical research has started to identify multiple asthma phenotypes with differing cellular components, mediators and sensitivities to asthma drugs, and probably varying underlying factors including susceptibility genes. Finally, different features of asthma – bronchoconstriction, symptoms, and exacerbations – respond diversely to treatment; effects that are not captured in animal models which do not develop asthma per se, but utilize unvalidated surrogate markers. Basic research needs to better integrate and utilize the clinical research findings to improve its relevance to drug discovery efforts.

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1. Introduction

The prevalence of asthma is still on the rise, and with it, the number of uncontrolled cases leading to emergency room visits and escalating health care costs [1,2]. Despite the worsening situation, only four drugs from two new classes of therapeutics have made it to market in the last 30 years—three anti-leukotrienes and the anti-IgE monoclonal antibody omalizumab. This review examines why it has proven so challenging to identify new asthma therapeutics. Three specific issues are identified and discussed.

First, animal (particularly mouse) models of the disease have been pivotal in identifying the sequence of key events that follow antigen challenge, and have led to a unifying mechanism of the underlying pathology responsible for the disorder. Unfortunately, clinical evaluation of these critical components has not met the expectations anticipated by the animal model [3]. Despite the translational mismatch, this basic research paradigm continues to drive target identification and results are deemed predictive of clinical efficacy.

It is recognized that asthma is a moniker for a heterogeneous group of disorders that have in common some breathing difficulty. Asthma is generally differentiated from other respiratory ailments such as chronic bronchitis/emphysema or COPD because of a partial reversal of the airway defect with β2-adrenoceptor (β2-AR) agonists, although there is probably some overlap [4]. This heterogeneity has confounded attempts to define key genes involved in asthma susceptibility, although that situation is beginning to change. Moreover, while there is significant co-morbidity, asthma is not synonymous with atopy. Based on IgE levels or a positive skin prick test to various allergens (the two markers that define atopy), approximately 10% of atopic individuals develop asthma, while ~50% of asthmatics have evidence for atopy [5–7]. Basic research relies exclusively on antigen challenger models, which, at best, help define only a subset of asthma

Abbreviations: ACQ, asthma control questionnaire; AHR, airway hyper-responsiveness; AQLQ, asthma quality-of-life questionnaire; BALF, bronchoalveolar lavage fluid; bn, billion; cysLT, cysteinyl leukotrienes; DZ, dizygotic; EAR, early airway response; FDA, Food and Drug Administration; FEV1, forced expiratory volume in 1 second; GPCR, G protein-coupled receptor; ICS, inhaled corticosteroid; IgE, immunoglobulin E; IL, interleukin; LABA, long acting β-agonist; LAR, late asthmatic response; MM, million; mRNA, messenger ribonucleic acid; MZ, monozygotic; PEFR, peak expiratory flow rate; SABA, short acting β-agonist; SMART, Salmeterol Asthma Research Trial; SNP, single nucleotide polymorphism; SRS-A, slow reacting substance of anaphylaxis; TGFβ, transforming growth factor β; Th2, T helper cell type 2; TLR, toll-like receptor; TNF, tumor necrosis factor.

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sufferers. As a heterogeneous group of disorders, the causative factors and key components of asthma can vary, and hence their susceptibility to different treatments.

Finally, are the problems of identifying the right patient group and employing an appropriate clinical trial design, particularly in the early stages of drug development. Unfortunately there is no quick and simple clinical study of asthma, nor any reliable biomarkers to indicate activity. Even the choice of endpoints can be challenging since they can respond differentially to various compounds that have different putative mechanisms of action. Absent any meaningful basic research model to guide selection of patients or clinical endpoints embarking on a clinical trial becomes an expensive and high-risk proposition.

2. Background

2.1. Brief description of asthma

Asthma is a chronic respiratory disease whose natural history is characterized by variable and recurring episodes or attacks of impaired breathing. It is comprised of four cardinal components—variable airflow obstruction (bronchoconstriction), symptoms, airway inflammation, and airway hyper-responsiveness (AHR) [8]. Symptoms are attributed to narrowing of small airways and may include shortness of breath, wheezing, chest tightness, and cough. Disease severity ranges from mild with occasional symptoms, to severe with persistent symptoms that impact quality of life, and progression along this continuum is accompanied by increases in all four asthma components. However, even people with mild disease may suffer severe attacks [8]. Indeed exacerbations, where there is a rapid deterioration in lung function despite regular treatment, probably represent another important and independent feature of asthma, as they can respond to therapy independent of improvements in airflow obstruction, symptoms or AHR.

2.2. Asthma prevalence continues to rise

Asthma is prevalent, affecting approximately 300 million people worldwide, and causing 250,000 deaths. In the US the 2009 prevalence is 8.2% and still rising by an annual rate of 1.2% [1]. The highest rates of asthma are in adolescents aged 5–17 years, where the rate is 10.7% [2]. Aside from age, gender, race, income level and geographical location all impact the statistics for asthma. In 2007 there were 1.75MM emergency room visits and 456,000 hospitalizations for asthma [1]. Asthma is estimated to cost the US $20.7bn per year—$15.6bn in direct health care costs and a further $5.1bn in lost productivity. Deaths, emergency room visits, and urgent care account for over 80% of the asthma healthcare budget [2].

In the US, statistics suggest that the increasing prevalence of asthma is being arrested [1,2]. From 1980 to 1996 the number of asthma sufferers doubled, going from 7.0 to 14.6 million, giving an annualized increase in prevalence of 3.8%, compared to the current 1.2%. [1] Mortality related to asthma also decreased by 22% between 1999 and 2006 [2]. These improvements could well be attributed to improved education and guidelines on standards of care provided by important bodies such as GINA (Global Initiative on Asthma) [4] and the National Heart Lung and Blood Institute's National Asthma Education and Prevention Program, and Expert Panel [9]. Given the detailed efforts to optimize treatment with the current portfolio of asthma drugs, it is clear that therapy is still lacking in some important areas, and new drugs are urgently required.

2.3. Current treatments for asthma

The goal of therapy is to achieve asthma control, which is comprised of two components—limiting the current impairment and reducing the risk for future deterioration and exacerbations. Current treatment guidelines recommend a stepwise approach to increase the number, frequency and dose of medications based on increasing asthma severity until the disease is under control [8]. The mildest, intermittent form of asthma is treated with a short acting β2-AR agonist (SABA), e.g., salbutamol, taken when required. For all other forms of asthma, inhaled corticosteroids (ICS) form the backbone of treatment, with increased dosing in line with disease severity. In moderate and severe asthma, a long acting β2-adrenoceptor agonist (LABA) – salmeterol or formoterol – is co-administered with the steroid. Other combinations can include a leukotriene receptor (CysLT1) antagonist such as montelukast, a muscarinic receptor antagonist like tiotropium, or even theophylline or a cromone such as nedocromil.

Despite the importance of ICS for treating asthma, they do not eradicate the disease. Moreover, a number of studies have found that a substantial proportion of patients do not achieve asthma control with their medications. For example, the Gaining Optimal Asthma Control (GOAL) study compared ICS alone with an ICS/β2-AR agonist combination in over 3400 patients with varying severities of asthma. Despite dose-escalations and treatment for one year, still 41% (ICS alone) to 29% (combination therapy) subjects did not achieve adequate control [10]. In another example, Szefler et al. [11] examined variations in response to the ICS, fluticasone, and the CysLT1 antagonist montelukast, among children with mild-to-moderate persistent asthma. They found that 5% of children responded well to montelukast alone, 23% to fluticasone alone, and 17% to both medications—but 55% did not respond significantly to either medication. Studies have also shown that smokers with asthma treated with ICS do not show significant improvements in FEV1 (forced expiratory volume in one second—a measure of lung function), symptoms or asthma-related quality of life [12]. It is studies such as these that highlight the importance of the continued search for new asthma therapeutics.

3. Why has the identification of new therapeutics proven so challenging?

This review seeks to identify some of the key aspects that have contributed to the challenges in identifying new asthma therapeutics. It is of particular concern that only 4 new drugs, representing 2 novel classes of therapeutics, have come to the US market in the last 30 years, and been adopted (Fig. 1). One class is the anti-leukotrienes that include the cysLT1 antagonists, zafirlukast and montelukast, and the leukotriene inhibitor, zileuton. The second area is anti-IgE, with the monoclonal antibody, omalizumab. Certainly other products have entered the marketplace, however, in general these represent improved versions of old drugs – inhaled steroids, β-AR agonists or anticholinergics – and combinations of these three classes. Inhaled glucocorticoids were first used in the 1950s to treat asthma, while the history of β-AR agonists and anticholinergics goes back much earlier. Even the combination of these drugs has been recognized as superior to either agent alone since the early 1960s. The improved versions of these agents have extended durations of activity, improved specificity, and greater convenience of use [13]. These are not trivial features, since patient compliance and regular use of asthma medications is an essential component of maintaining asthma control, and a well-recognized concern. However, since these improved drugs are based on science now 50–100 years old, they are not included in this list of novel therapies. It would be hoped that 30 years of research, yielding over 100,000 publications and a pharmaceutical/biotechnology R&D expenditure measured in the billions of dollars would translate into something more than an improved version of an old remedy for symptomatic relief.

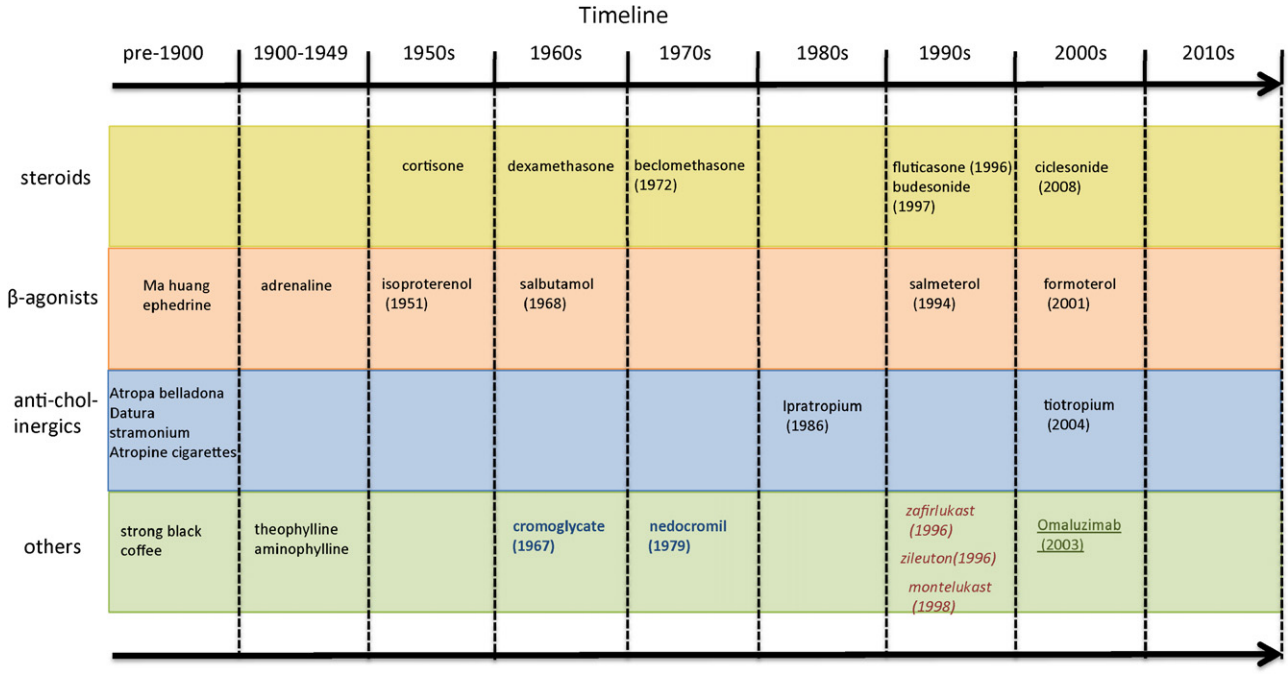

Fig. 1. Timeline of asthma drugs coming to market, indicating successful R&D efforts. (Note that category "others" is color-coded according to class of drug, where black = xanthines, blue/bolded = cromones, red/italcized = anti-leukotriene, green/underlined = anti-IgE antibody). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

The review is divided into three parts. The first deals with the history of how the drugs currently in use were discovered to determine if any lessons can be gleaned from those discoveries that have relevance today. This is followed by a discussion of some of the identified impediments to successful research and development programs today. The final section deals briefly with the implications and future directions.

4. If drug discovery efforts are failing, how were the current drugs identified?

Before detailing the current impediments to asthma drug discovery, it is worthwhile reviewing how the current asthma drugs were discovered, and address why those earlier successes provide few insights into how new therapeutics can be discovered. There are seven classes of drugs that have been adopted widely for the treatment of asthma (Figs. 2 and 3). These include the corticosteroids, β2-AR agonists (sympathomimetics), selective muscarinic antagonists (anti-cholinergics), cromones (cromoglycate and necrodomil), theophylline (a precursor to the search for other phosphodiesterase (PDE) inhibitors, particularly of PDE4), cysteinyl leukotriene antagonists and the anti-IgE monoclonal antibody.

4.1. Corticosteroids

An extract from the adrenal cortex, originally called compound E but later identified as cortisone, was made by Edward Kendal at the Mayo Clinic in 1936. Merck and Co was able to synthesize a few grams in 1948, but the supply was extremely limited. By 1950 cortisone was more readily available and studies in asthmatic subjects commenced. Several studies reported benefits of oral cortisone (for example, [14]), which prompted the first placebo-controlled trial in asthma conducted by the Medical Research Council in the UK in 1956 [15]. Already in the early 1950s studies with inhaled corticosteroids were showing some benefits [16]. However, the effects of cortisone and then prednisone were variable, not very dramatic and accompanied by some severe side effects. Later it was realized that these were prodrugs, reduced in the liver to hydrocortisone and prednisolone, respectively [17]. Using these latter steroids instead was limited by their oxidation via 11β-hydroxy steroid dehydrogenase back to cortisone and prednisone [17].

Dexamethasone was the first corticosteroid to demonstrate clear anti-asthmatic activity by inhalation, and was introduced clinically in the US in the 1960s (Fig. 2). The problem with dexamethasone was its poor lung selectivity by the inhaled route, with a profile similar to that observed with oral administration [17]. Consequently the real breakthrough came with the discovery of beclomethasone, whose effects are confined to the airways when given by inhalation [18]. Although in 1958 Merck had applied for, and received, a patent on beclomethasone, they did not pursue its development and it was Allen & Hanbury who produced an aerosolized version of beclomethasone and began clinical trials in 1970 in patients selected based on sputum eosinophilia [17]. The drug was approved in 1972. Since then various other inhaled steroids have been developed (Fig. 2) that show limited systemic effects, due to molecular features such as decreased absorption from the mucosa (e.g., fluticasone), or a large first-pass metabolism (e.g., budesonide), or as a prodrug activated locally by esterases in the lung (e.g., ciclesonide). Such features are advantageous since up to 80% of an inhaled drug can be swallowed [17].

4.2. Sympathomimetics

The Chinese herb *ma huang* is the dried stem of three species of the *Ephedra* family. One of 365 herbs listed in Shen Nong Ben Cao Jing from the first century AD, it has been used to treat asthma for over 2000 years. The principal active ingredient is the sympathomimetic alkaloid, ephedrine, which acts indirectly by releasing endogenous catecholamines to achieve bronchodilation [19].

In the west, the identification and synthesis of epinephrine at the end of the 19th century led to its use for the treatment of asthma by both the oral, and in particular, subcutaneous routes of

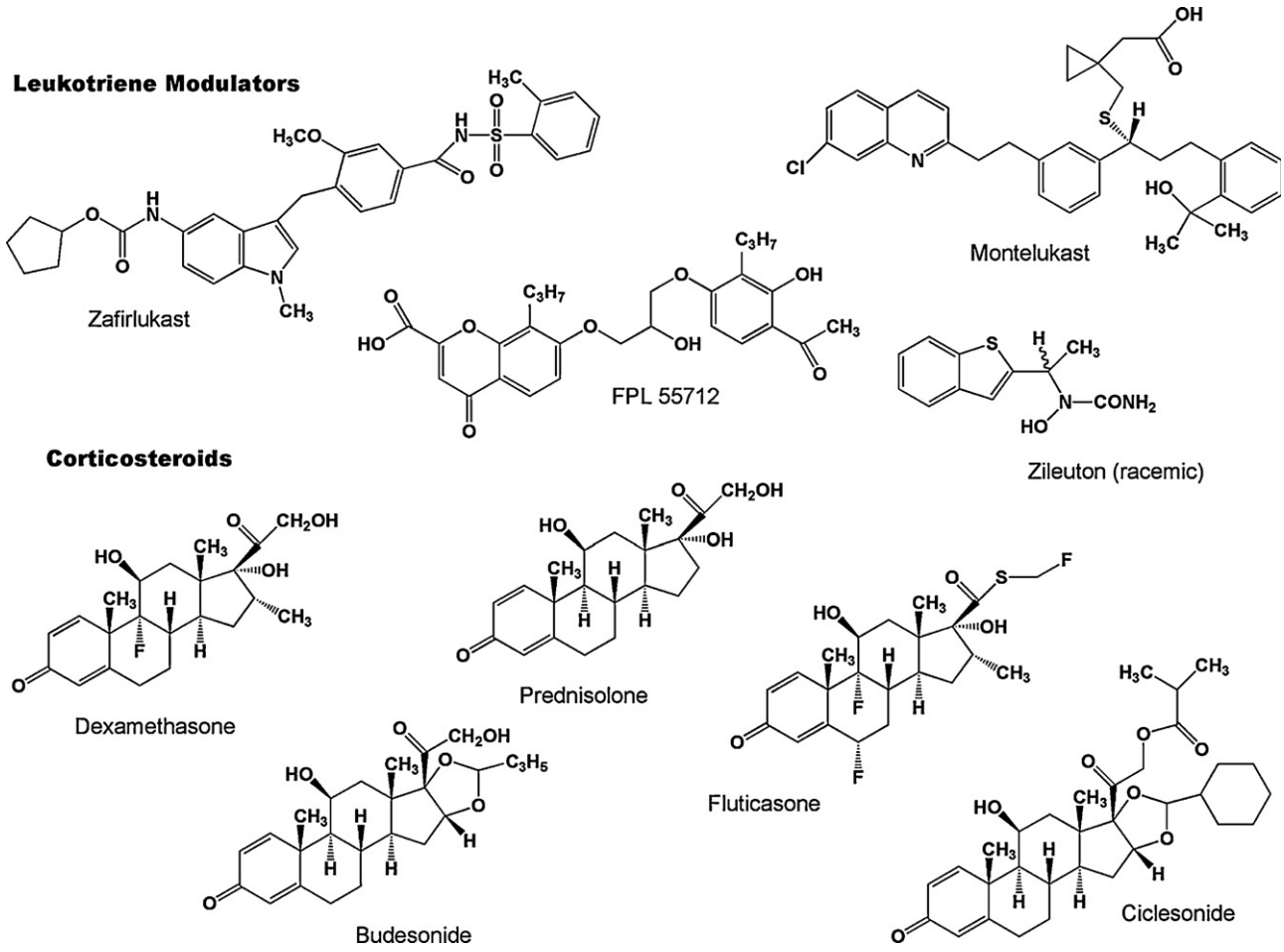

Fig. 2. Chemical structures of several asthma drugs, tracing the development of improved forms. Depicted are the leukotriene modulators, starting from the SRS-A antagonist FPL55712 (a cromone from the same family as cromoglycate) to the cysLT1 antagonists zafirlukast and montelukast, and the leukotriene inhibitor, zileuton; and the development of corticosteroids from prednisolone through dexamethasone to the more recent budesonide, fluticasone and ciclesonide.

administration, before Barger and Dale [20] demonstrated its efficacy by inhalation. The discovery of isoproterenol in the 1930s (Fig. 3), with ten times the potency of epinephrine as a bronchodilator without the hypertensive effects, led to its introduction by Boehringer in 1941 as a treatment for asthma [17]. However, it was not until after World War II that the merits of isoproterenol were recognized more widely and it was introduced into the US as the first β-selective bronchodilator in 1951, following its use to facilitate in the differentiation of adrenoceptors into α- and β-sub-types [21]. For the next 20 years it was the drug of choice to relieve asthma attacks, and patients were encouraged to use it repeatedly until symptoms abated. By the mid-1950s convenient pressurized metered dose inhalers (pMDI) had been developed to deliver isoproterenol and sales rose by 600% between 1959 and 1965 [22]. However, this was coincident with a disastrous 400% rise in deaths in asthma sufferers in the UK in the 5–34 years age group, ultimately attributed to effects of isoproterenol toxicity due to the widespread use of a high strength formulation of isoproterenol in a pMDI [23]. This version of the drug was withdrawn, and a physician education program initiated, stemming the epidemic.

The differentiation of β-adrenoceptors into β1- and β2-subtypes, with β2-AR responsible for mediating bronchodilation, led to the development of the first β2-AR-selective agonist, salbutamol, in the 1960s [24]. Launched by Allen and Hanbury in 1968, it was an instant success and is still in use. Terbutaline, developed by Astra in 1966, has a very similar profile to salbutamol. Subsequent β2-selective agonists including salmeterol, launched in Europe in 1990 and the US four years later, and formoterol, a Japanese drug launched in the US in 2001, were chosen for their improved potency and longer duration of action (Fig. 3). However, concerns regarding the safety of β2-AR agonists, particularly the long acting β-agonists (LABAs), persist and they can only be used in conjunction with an ICS [25]. Currently there are a number of once-daily β2-selective agonists in the late stages of clinical development.

4.3. Anti-cholinergics

Vapor from the leaves of *Datura ferox* (or Thorn apple) inhaled through a hukka have been used for centuries in Ayurvedic medicine to relieve asthma. After a report by Sims in 1812, cigarettes containing the leaf *Datura stramonium* became widely used by asthmatics [26]. Like *Datura* leaves, the plant *Atropa belladonna* contains the anti-cholinergic, atropine. In 1869 Henry Salter described success in treating asthma with *A. belladonna* and with atropine, after which atropine became widely available, again largely in cigarette form [see 26]. Direct spirometry measures showed definitively that inhalation of atropine cigarettes improved asthma [27]. Herxheimer [27] also combined atropine with the sympathomimetic ephedrine as a novel combination bronchodilator therapy, which led on to the study of co-administering inhaled atropine and isoproterenol to produce a greater bronchodilation than could be achieved by either drug alone [28].

The identification of 5 muscarinic receptor subtypes (M1-M5) prompted a search for more selective muscarinic antagonists,

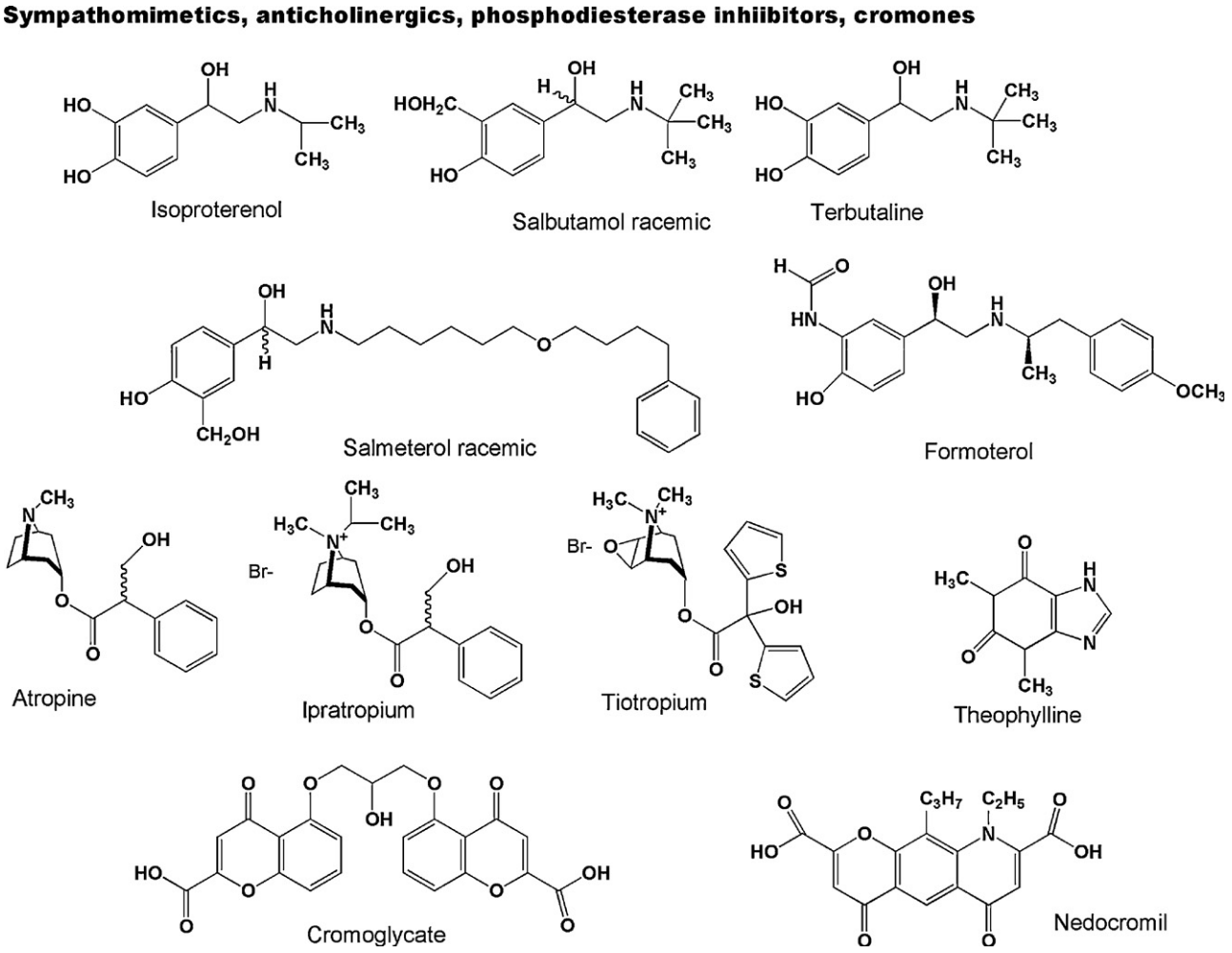

Fig. 3. Chemical structures of several asthma drugs, tracing the development of improved forms. Depicted are the sympathomimetics (β2-AR agonists) from isoproterenol through the short acting salbutamol and terbutaline, to the longer acting salmeterol and formoterol; the anticholinergics from atropine through ipratropium to tiotropium; the cromones, cromoglycate followed by nedocromil; and theophylline, the only phosphodiesterase inhibitor used to treat asthma.

ideally antagonizing only the M1 and M3 receptors implicated in bronchoconstriction, to avoid some of the side-effects of atropine and its congeners [29]. Ipratropium was launched in the US in 1987, but has a short half-life requiring administration four times a day. It was followed by tiotropium, approved in 2004, that is more potent, and, importantly has a half-life suitable for once a day dosing. Both ipratropium and tiotropium are quaternary ammonium salts, in contrast to atropine which is a tertiary ammonium compound (Fig. 3). The significance is that the quaternary salts are poorly absorbed from mucosal surfaces, so when given by inhalation their effects are localized to the airways. Although receptor-binding studies show no difference in affinity of tiotropium for M1, M2 or M3 receptors, dissociation from the M2 receptor is quicker, which translates functionally into relative M1 and M3 selectivity [29]. The addition of tiotropium to an inhaled steroid provided superior benefit to doubling the dose of the steroid and equivalent efficacy to the combination of the long acting β-agonist, salmeterol, with the steroid [30].

4.4. Theophylline

The use of methylxanthines such as theophylline to treat asthma began with the observations of Henry Salter who in 1860 reported that strong black coffee could relieve the breathing problems of asthmatics. The recommended dose was two breakfast cups (see [31]). Theophylline (Fig. 3) was identified as a chemical found in the leaves of the tea bush by Kossel in 1888, and Hirsch described its bronchodilator properties in three asthmatic patients in 1922 (see [31]). Intravenous aminophylline, was found to be effective in treating acute exacerbations, particularly if the patients did not respond well to adrenaline [32], and became a standard treatment for exacerbations for over three decades, until replaced by inhaled β2-AR agonists.

Theophylline has a short half-life, so a slow release oral formulation was developed. Theophylline is a weak bronchodilator whose therapeutic window is narrow, causing it to fall out of use. However, greater benefits have been described when patients are treated with theophylline together with inhaled corticosteroids, and a mechanistic basis for this interaction has been described recently [33].

Many of the benefits of theophylline in the treatment of asthma have been attributed to inhibition of the phosphodiesterase enzymes. Theophylline is a non-specific PDE inhibitor [33], and efforts have been underway for the last 20 years to identify selective inhibitors of PDE4 isozymes in particular, but also in combination with PDE7 or PDE3 inhibition, to improve upon theophylline's efficacy and side-effect profile. One selective PDE4 inhibitor, roflumilast, was approved recently for COPD [34], but results to date with PDE4 inhibitors in asthma have been disappointing.

4.5. Cromones: cromoglycate and nedocromil

Cromones contains a 5:6 benz-1:4-pyrone ring system (Fig. 3). The prototypical example is disodium cromoglycate, discovered by Altounyan at Fisons in the UK and introduced into clinical practice in 1967. The identification of cromoglycate has become a pharmacological legend, and the subject of several publications (see for example, [35]). Altounyan, himself an asthma sufferer, was interested in natural herbal remedies for the treatment of asthma. His attention centered on an extract from the seeds of a medicinal plant, *Amni visnaga*, called khellin. The plant and various decoctions had been in use in eastern Mediterranean countries for centuries as a diuretic and smooth muscle relaxant. The principal active ingredient, khellin, was reported to provide complete and prolonged relief in bronchial asthma upon intramuscular injection [36]. The Fison's team prepared various soluble derivatives of khellin that could be administered either orally or by inhalation. Altounyan, who led the team, was sensitive to guinea pig dander, and tested the compounds on himself in a series of allergen provocation tests, resulting in the identification of cromoglycate. Altounyan even devised the first dry powder inhaler since the required dose of cromoglycate was considered too high for a pMDI [22].

A second cromone, nedocromil, was also developed by Altounyan et al., [35], and introduced in the late 1970s. Nedocromil showed superior efficacy to cromoglycate in the clinic. However, the personal cost was tremendous as by this stage Altounyan had performed over 2000 allergen challenge tests on himself, and his FEV1 had declined from 1.5 l in 1965 to 0.7 l in 1977 [37]. It is not surprising therefore that this drug discovery format, no matter how successful, has little chance of being repeated.

Studies on cromoglycate indicated it acted by inhibiting mast cell degranulation through blocking calcium entry [38]. Since cromoglycate has a short half-life requiring administration four times a day, virtually every major pharmaceutical company rushed to discover an improved version with a longer half-life and oral bioavailability [39]. Many compounds were identified that potently inhibited rat mast cell degranulation, showed efficacy in guinea pig models of asthma and were orally active. Advanced into clinical trials all of the compounds failed; a finding which contributed to the mast cell falling out of favor as the primary source of mediators in asthma. Subsequent studies have shown that cromoglycate and nedocromil have multiple actions on other cells and mediators that continue to challenge researchers (see [40] for example), and recently these cromones were identified as agonists for GPCR-35 [41]. So 43 years after cromoglycate entered the market, its mechanism of action is still being unraveled. However, cromoglycate was withdrawn from the market in 2009 as it was superseded by the inhaled steroids and β2-AR agonists.

Some clinical observations with cromoglycate deserve mentioning. The original clinical study performed by Howell and Altounyan [42] showed marked benefits that were not as profound in subsequent larger clinical studies. It was noted that Howell and Altounyan selected their "bronchial asthma" patients solely on the basis of eosinophilia of the sputum [43], which was not a selection criteria in the other studies. Also it was noted that the conclusions drawn from the trials with cromoglycate depended on the importance attached to FEV1, a key endpoint of asthma studies at that time. It was observed that symptoms seemed to improve to a greater extent than FEV1 [44], suggesting that the connection between airway narrowing and symptoms might not as direct and straightforward as was believed.

4.6. CysLT1 antagonists

Anaphylaxis in guinea pig lungs and human lung fragments produced histamine, and another, unidentified, spasmogen called "slow reacting substance of anaphylaxis" or SRS-A [45]. In 1979, SRS-A was identified as a combination of the cysteinyl leukotrienes (cysLTs), LTC4, LTD4 and LTE4, a new series of eicosanoids discovered by Samuelsson et al. (see [46]) formed via the 5-lipoxygenase enzyme. This led to the search for both enzyme inhibitors of the leukotriene pathway, and receptor antagonists. Two cysLT receptors, CysLT1 and CysLT2, were originally identified pharmacologically based on their sensitivity to CysLT1 antagonists [47]. To date enzyme inhibitors have been associated with some limiting toxicities, while antagonists of the CysLT1 receptor have been more successful as therapeutic agents.

Prior to the identification of SRS-A as the cysLTs, efforts to find antagonists were already underway. The discovery by the Fison's team [48] of another cromone, the carboxylic acid, FPL55712 (Fig. 2), as a potent and specific SRS-A antagonist represented an important landmark. The structural identification of the leukotrienes allowing the synthesis of leukotriene analogs, when coupled with the SAR of FPL55712 analogs, provided medicinal chemists with important information to aid in the identification of potent and selective CysLT1 antagonists.

The first CysLT1 antagonist to market was pranlukast developed by Ono and approved for use in Japan in the middle of 1995. While the core chemical motif was identified through screening company compound libraries, the ultimate structure incorporated structural components present in FPL55712 [49]. Zafirlukast was the first CysLT1 antagonist approved for use by the FDA in September 1996 and marketed by Astra-Zeneca as a twice-a-day oral drug. Zafirlukast incorporated structural elements from both FPL55712 and the leukotrienes [49]. Montelukast, a quinoline modified with LT structural features developed by Merck, was FDA approved in February 1998 (Fig. 2). The once-a-day montelukast quickly became the predominant CysLT1 antagonist in the US. Generic competition is now eroding the sales of zafirlukast, which achieved net sales of $66M and $57M in 2009 and 2010, respectively. Montelukast has been an incredibly successful product with continued growth and sales of $5B in 2010, representing an increase of 7% over the preceding year.

Zileuton is an oral 5-lipoxygenase inhibitor, blocking the formation of all the cysLTs and the chemotactic LTB4 [50]. This broader range of activity than a selective CysLT1 antagonist has not translated into a marked superior clinical profile, and although zileuton was approved in 1996 and launched in January 1997, it has not been adopted widely. Some liver toxicity concerns and the short half-life requiring administration 4 times a day (now replaced by a twice daily extended-release version) contributed to its limited uptake in the market. Sales for zileuton reached a high of $30.6M in 2010, up from $18M in 2009.

4.7. Omaluzimab: anti-IgE

In the early 1920s Küstner noted he developed an allergy after eating fish. His colleague, Prausnitz, injected some of Küstner's serum into his own arm, and noted that after eating fish his skin at the injection site also became hot, red and swollen. The substance present in the serum that could passively transfer sensitivity to a specific agent they called reagin (see [51]). In 1966 reagin was identified as IgE [52], which provided an important link between allergy and asthma.

IgE binds to two Fc receptors—a high affinity FcεRI, and a low affinity FcεRII (also known as CD23) found on several immune cells [53]. The site where IgE binds to FcεRI is located on the Fc fragment in the area where the cε-3 domain adjoins the cε-3 domain. Upon subsequent antigen exposure, there is cross-linking of multiple FcεRI bound IgE molecules on the surface of basophils and mast cells by the antigen, resulting in cell activation, degranulation and release of mediators.

A monoclonal antibody to IgE was identified by conventional somatic cell hybridization techniques [54]. Two companies were competing to produce the first anti-IgE monoclonal antibody, Tanox, partnered with Novartis, and Genentech. The companies decided to pool their resources and co-develop a single antibody. A murine monoclonal anti-human IgE antibody was produced whose paratope was directed towards the FcεRI binding region—called MAE11. MAE11 was humanized, resulting in a human monoclonal anti-human IgE antibody called rhuMAb-E25 [55], later renamed omalizumab.

Omalizumab binds to free IgE with a binding affinity higher than that between IgE and FcεRI, and consequently, free IgE levels drop substantially within 1–2 h of administration. Since it recognizes the same epitope involved in binding to the high affinity receptor, it does not bind to IgE already bound to the receptor, or FcεRI itself. Consequently, it cannot cause cross-linkage and cell degranulation. Since there is a close correlation between free serum IgE levels and the number of FcεRI expressed on basophils [56], removal of the free IgE ultimately feeds back to down-regulate the receptor, which, in turn, serves to further decrease the amount of IgE bound to the immune cell [57].

In June 2003 the FDA approved omalizumab for the treatment of atopic, moderate to severe asthma, inadequately controlled by existing therapy. In November 2006 Genentech acquired Tanox to whom they were paying a royalty on omalizumab sales. In 2008 omalizumab sales totaled $728M, divided between Genentech ($517M) and Novartis ($211M).

4.8. Drug discovery: what has been learnt from the past?

Drug discovery has undergone a profound change over the last 30 years to become a mechanistic, target-driven, high-throughput activity [58,59]. The remarkable success of drugs like the anti-TNFα biologics in a complex, multifactorial inflammatory disease like rheumatoid arthritis [60] speaks to the power of this approach. However, it is equally sobering to reflect on the fact that many of the anti-asthmatic medications currently in use emanated from natural products based on an observed activity and in ignorance of any underlying mechanism of action. Even today, the mechanisms of action of many of these drugs – e.g., ICS, cromones and theophylline – remain active areas of scrutiny. Such limitations have not prevented their effective, and continued use in the clinic. Indeed, the expectation that asthma research would identify the "magic bullet" equivalent of an anti-TNFα drug like etanercept, in the form of an antagonist to a single, critical cytokine—has not been borne out. Mechanistic, target-driven research is made even more difficult when research models that have a history of being misleading and non-predictive remain the primary source for identifying new mechanisms and targets. Also it is inappropriate to expect such a complex disorder with multiple phenotypes to be reduced to a single target.

The ICS, sympathomimetics, anti-cholinergics, methylxanthines and cromones all originated from natural products that had a history of beneficial activities in asthmatics. Two important features of the therapeutic application of these drugs in asthma deserve recognition. One is that their use was not presaged by research studies in animal models, which were only used to account for why these drugs were working. Thus any mechanistic underpinnings for such agents frequently trailed their clinical use by decades. Second, clinical trial design in the 1950s and 1960s was comparatively rudimentary when matched against drug development today, where smaller studies and the inclusion of anecdotal observations was common. This probably aided weaker drugs getting to market, finding their role and allowing improved versions to follow and demonstrate success. The poor safety profile of the early corticosteroids for example, would have convinced many companies to abandon these products before the issues were ultimately resolved 20 years later. A meta-analysis of the benefits of cromoglycate in children could not conclude that the drug was better than placebo [61], while nedocromil, whose identification trailed cromoglycate by a decade, does show efficacy [62].

The discovery of cysLT antagonists was initiated by attempts to block the biological activity of SRS-A before it was identified as a mix of cysLTs, and some of the structure-activity relationships gleaned for SRS-A antagonists were incorporated into the early cysLT antagonists, which contributed to their early success. Omalizumab, the anti-IgE MAb, is representative of the target-based approach to discovering new asthma therapeutics, and remains an important landmark in asthma therapeutics by demonstrating efficacy in a particular patient population (moderate to severe asthmatics with elevated IgE levels) not well controlled with current therapy, and approval based on the endpoint of decreasing asthma exacerbations, rather than improving lung function. It identified a key unmet medical need and marked a new era as more companies saw an opportunity to target severe asthmatics who are refractory to current drugs, and utilize asthma exacerbations as the path to approval.

5. Asthma heterogeneity

Genetic, environmental, epigenetic and other factors all contribute to the heterogeneity of the disease [63–65]. The way in which the patient responds to these factors produces the various phenotypes. One well-recognized risk factor is atopy. However, while ~50% of the US population are atopic, based on positive skin prick tests or IgE levels, only a small proportion develop asthma, indicating it is the response to such risk factors that predispose to the disease. Although historically asthma has been categorized as extrinsic (allergic) or intrinsic (non-allergic), the inflammatory cell repertoire of the airways does not show distinct differences between these two forms of the disease.

5.1. Asthma phenotypes

It is now well recognized that the term asthma is a rubric for a heterogeneous group of disorders, representing different phenotypes, which share the common symptom of partially reversible difficulties in breathing. Ultimately, the phenotypes need to be defined so that medical treatment can be optimized for particular patient groups, and important activities in this regard have begun. To date, different phenotypes have been defined based upon different components of asthma in patients with severe disease. For example, airway inflammation containing either high or low eosinophils [66], high or low neutrophils [67], or high or low T helper 2 (Th2) cells [68] has been used to identify phenotypes. This last study is of particular interest, and is based on the presence of Th2 cytokines in bronchoalveolar lavage fluid (BALF), and Th2 cytokine-induced gene expression in epithelial brushings, occurred in about half of the asthma patients in the study [68]. Interestingly the "Th2 high" group had raised levels of IgE and airway eosinophils, exhibited greater AHR and showed an improvement in FEV1 upon treatment with ICS, when compared to the "Th2 low" group. This study did not characterize the "Th2 low" phenotype or why these asthmatics were unresponsive to ICS. Asthma exacerbations are the single biggest risk factor for future exacerbations and have helped define an "exacerbation-prone" phenotype [69]. AHR and bronchodilator response to salbutamol may help segregate other phenotypes as well [67].

5.2. Atopy

Atopy results from a gene–environment interaction, so has garnered significant attention as a basis for asthma. Atopy is defined as the genetic propensity to develop IgE antibodies in response to exposure to allergen [5], while it is an established risk factor, the amount of asthma attributable to atopy is controversial. The two standard measures to define atopy are IgE levels (both total and allergen specific) and a positive skin prick test to any one of a battery of allergens. Based on these two tests it was concluded that 33% (based on IgE >100 IU/ml) to 38% (positive skin prick) of asthma sufferers are atopic [6].

Two important recent studies addressed the issue by examining a large cohort of subjects representative of the civilian, non-institutionalized US population, and determining the prevalence of atopy, asthma and its combination. In 10,508 subjects aged between 6 and 59 years, the percent atopic was 54.2, while asthma prevalence was 5.2% [5]. Of those with asthma, 56.3% were also atopic. A limitation of this type of study as emphasized by the authors is that there is an inherent assumption that atopy developed before the asthma. Since the sampling represents a later snap-shot in time, any temporal relationship is unknown, and the events could be independent (i.e., the atopy is not the cause of the asthma), so the incidence of atopic asthma could be over-estimated.

In a similar subject cohort (n = 7398), Gerger et al. [7] measured total and specific IgE levels. The mean IgE was 40.8 IU/ml, and asthma prevalence in this population was 8.8%. A 10-fold increase in IgE levels did increase the likelihood of having asthma (odds ratio = 2.18), and asthmatics had higher geometric mean IgE levels (81.1 IU/ml vs. 40.8 IU/ml), but asthma was observed across the range of IgE values, even in those with very low levels. In this large cohort, asthma prevalence was higher in atopic versus non-atopic subjects (12.9% compared to 5.8%). Moreover, 62.1% asthmatics had detectable levels of at least one specific IgE. As in the previous skin prick study [5], Gerger and colleagues caution against concluding that 62.1% of asthmatics have atopic asthma since the two measures (IgE and asthma) might not be causally related in all subjects and could be an over-estimate [7]. However, based on these two studies, it would appear that atopic asthma represents a little more than half of all asthma sufferers. What prompts the disorder in the other half, triggering an airway inflammatory response made up of essentially the same cells as found in atopic asthmatics, is not known.

5.3. Contribution of genetics

That genetic factors contribute importantly to determining asthma susceptibility is well recognized. The analysis of familial aggregation and segregation indicates a heritable component that does not follow a simple Mendelian transmission model. With an asthma prevalence of 5–10% in the general population, one asthmatic parent increases the prevalence in their offspring to 14%, whereas if both parents have asthma the prevalence is 29% [70]. Prevalence decreases with increasing genetic distance to broader family members.

Two approaches demonstrate the contribution of familial transmission to the disorder. One is the study of isolated communities, like the one on the island of Tristan da Cunha in the South Atlantic. When occupied by a British garrison in 1816, the island had only one resident. When the garrison left the following year, 3 soldiers elected to remain, and the population grew slowly from the occasional shipwreck and immigration from St. Helena, an island one thousand miles to the north. By the 1960s it had become a community of nearly 300 people from 90 families, but with only 7 different surnames, when it was discovered that the prevalence of asthma was 50%. The asthma traced back to two sisters who emigrated from St Helena to the island a century earlier, and through cousin-cousin marriages, had spread susceptibility genes among the inhabitants [71].

A second approach is the study of monozygotic (MZ) and dizygotic (DZ) twins [63,70]. By comparing the concordance for a disease in genetically identical monozygotic (MZ) twins with that in dizygotic (DZ) twins with only 50% identical genes, and assuming that environmental conditions are the same for each twin, it is possible to estimate the relative contributions of genetic and environmental factors to asthma. When one member of a MZ pair has asthma there is a 48% likelihood that the other twin will also develop the disorder, while for DZ twins the concordance is 19%. The discordant component is attributed to environmental influences. Particularly intriguing is the finding that, despite having identical genes and environmental influences, 52% of MZ twins do not develop asthma.

5.4. Asthma susceptibility genes

Fourteen years have passed since Sequana Therapeutics, in collaboration with Zamel at the University of Toronto, announced in May, 1997 the discovery of two genes by positional cloning responsible for asthma, that were originally dubbed *wheeze-1* and *wheeze-2* (see [71]). Since then, more than 170 genes located on 10 chromosomes have been either associated with, or found to be in linkage with, asthma and asthma-related phenotypes such as atopy [72]. However, replication between studies of identified genes has been a continual problem, probably due in part to methodological issues, poorly defined phenotypes and genetic heterogeneity among different populations, and where multiple genes each have small effects. One methodological limitation has been sample sizes that provide adequate statistical power for analyzing variants with small effects. To overcome this limitation, samples from different studies and populations are often combined. For example, four asthma populations from Canada and Australia were amalgamated to include samples from 5565 subjects where 93 candidate genes previously associated with asthma were analyzed for 861 single nucleotide polymorphisms (SNPs) and tested for association with asthma, atopy, atopic asthma and AHR. No SNP in any gene showed statistical significance when corrected for all tested SNPs, genes and phenotypes; instead there were a large number of SNPs with an odds ratio of less than 1.4 [73], indicating a small effect that requires a larger sample size to determine significance.

Genome-wide association studies represent a technique that has no bias towards any potential genes and can address complex polygenic disorders. A large consortium study (GABRIEL) was recently published [74] in which 10,365 asthma patients and 16,110 unaffected subjects were each genotyped for 582,892 SNPs, producing ~15 billion genotypes. This study was powered sufficiently to detect variants with an allele frequency of 10% and an odds ratio >1.2, and tested for association in the overall asthma population, but also in particular asthma phenotypes including childhood-onset, later-onset, severe and occupational asthma. For the overall asthma population SNPs at 4 sites were identified, implicating *IL1RL1/IL18R1* on chromosome 2; *HLA-DQ* on chromosome 6; *IL33* on chromosome 9; *SMAD3* on chromosome 15; and *IL2RB* on chromosome 22. Polymorphisms at the *ORMDL3/GSDMB* locus on chromosome 17q21 were specific for childhood asthma, while no significant associations were found specific to either severe or occupational asthma. These candidate genes include a number of cytokines – IL-18, IL-33 and IL-2 – that exhibit both pro-inflammatory and anti-inflammatory activities (see [3]). In addition *SMAD3* is a transcriptional modulator activated by TGFβ, which is implicated in regulatory T cell differentiation and

proliferation among other activities; while changes in expression of the homologous *ORM* gene in yeast interferes with sphingolipid metabolism. Significantly, Moffat and colleagues [74] found little overlap between loci conferring susceptibility to asthma and those regulating IgE levels, leading them to conclude that an elevation in IgE is probably an inconstant secondary effect rather than a cause of asthma.

5.5. Pharmacogenomics

Asthma severity is now being defined based on the response to treatment and the intensity of treatment required to achieve asthma control [8]. Consequently, pharmacogenomics – how variability in the patient's genome can influence the response to therapy [75,76] – is an important component of the assessment. It has been estimated that up to 50% of the variability of response to a therapeutic agent in asthma might be due to genetic variability [75]. For example, one polymorphism that has been studied extensively is at the locus of the 16th amino acid position of the β2-AR gene (*ADRB2*), which can code for either arginine or glycine. Approximately 17–20% of Caucasians and African Americans have the Arg/Arg polymorphism, which is associated with poorer outcomes when using SABAs when compared to those who are Gly/Gly. There is a decrease in SABA-induced bronchodilation (measured as peak expiratory flow rate (PEFR)), worsening symptoms, increased SABA usage and increased frequency of exacerbations [75].

Montelukast has well-established efficacy and safety in the treatment of asthma, either as an alternative to ICS in the treatment of mild asthma, or as an alternative to β2-agonists as an adjunct to ICS in moderate forms of the disorder. However, there is significant variability in the response to montelukast such that 35–78% of patients are classified as non-responders [77]. Promoter polymorphisms in the 5-lipoxygenase (*ALOX5*) and the LTC4 synthase (*LTC4S*) genes have been suggested to account for this variability [76,78]. For example, there is a polymorphism in the promoter region of LTC4S gene (A-444C) where the variant C allele is found in about one third of Caucasians. This polymorphism is associated with a 14% improvement in FEV1 after treatment with pranlukast, compared to a 3% increase in those lacking this polymorphism [79]. The same polymorphism is associated with a 76% reduction in exacerbations in patients treated with montelukast [76]. Repeat variants in the promoter region of ALOX5, in which carriers of the mutant allele (X/X and 5/X) had a 73% reduction in exacerbations over 6 months of treatment with montelukast when compared to the wild-type homozygotes (5/5), are found in approximately one third of Caucasians [76]. Several other polymorphisms in the leukotriene pathway that significantly impact the response to treatment with leukotriene antagonists and inhibitors have been identified [75,78].

Polymorphisms that influence the response to corticosteroids have also been found [75,76]. What is not known at the present time is how multiple polymorphisms differentially affecting the responses to different drugs (e.g., ICS and β2-AR agonists) would translate into an overall response to combination therapy.

6. Safety of β2-AR agonists: pharmacogenomics, masking inflammation or predictable pharmacology?

Since inhaled LABAs were introduced in the US in 1994, there has been an ongoing concern that they may be associated with a greater risk of death from asthma. The Salmeterol Asthma Research Trial (SMART) was stopped due to an increased risk of death in patients receiving salmeterol compared with those taking placebo [80], equating to 1 death in 700 patient-years of treatment. This concern prompted the FDA to issue a "black box" warning for LABAs, requiring that they be administered only in conjunction with an ICS, and for the shortest possible time to effect asthma control and then be discontinued [81]. The FDA also called for 5 new clinical trials comparing the safety of LABA + ICS to ICS alone, with the results expected in 2017.

In SMART [80] some patients were taking ICS together with LABA, while others were not, so it was unclear if the combination therapy was also detrimental. An analysis, conducted within the FDA, of the data from 110 clinical trials with LABA involving 60,954 subjects calculated the risk of asthma-related death in patients on LABA to be 0.4 per 1000—i.e., one extra death for every 4000 patients taking a LABA [82]. However, since 15 of the 16 LABA related deaths occurred in asthmatics *not* taking an ICS (a treatment strategy no longer allowed), the calculated sample size for the FDA mandated study design of LABA + ICS vs. ICS alone is 4,384,000 to show one excess death [83]. Consequently it is concluded that the five ongoing studies are unlikely to provide any greater insights into the risks associated with LABA use [83]. Additionally, any relationship of increased risk to pharmacogenomic variations in the β2-AR, or particular asthma phenotypes, are beyond the scope of the planned studies.

The increased risk of asthma-related mortality in patients receiving LABAs is attributed to a worsening of the underlying inflammation that is masked by the bronchodilator [25], and is reminiscent of the mortality associated with the use of high strength isoproterenol formulations in the 1960s [23]. Both SABAs and LABAs increase inflammatory cells in sputum samples and enhance AHR [25]. Long term activation of β2-AR can also lead to changes in receptor expression and coupling to post-receptor signaling mechanisms, leading to the development of tolerance [25,84]; a common event with GPCRs. One week of treatment reduces the peak FEV1 response to the β2-AR agonist by 17.8%, and enhances the sensitivity to bronchoconstrictor stimuli such as methacholine, causing a 26% decrease in the dose of methacholine required to reduce FEV1 by 20% [85]. The impact of β2-adrenoceptor tolerance on the response to indirect stimuli such as exercise or allergen challenge, is even greater, for reasons that are not clear. One week treatment with salbutamol exacerbates the fall in FEV1 in exercise-induced asthma, that cannot be restored to levels seen in the placebo arm of the study despite the administration of more salbutamol during exercise [86]. While a single dose of salmeterol prevents the early bronchoconstriction (EAR) elicited by allergen challenge in mild asthmatics, after one week of treatment with the β2-AR agonist a significant EAR can be restored in 55% of subjects [87]. Since a SABA is recommended for acute treatment of worsening asthma, the benefits could be blunted in patients taking LABAs, causing the patient to use their reliever SABA more frequently. Difficulty in controlling asthma symptoms and increased SABA usage are hallmarks of an exacerbation [4], the cause of which could be misconstrued.

The situation becomes even more perplexing when examining the role of β2-ARs in AHR in basic research. AHR in β2AR-deficient mice is dramatically reduced (rather than the enhancement expected with removal of a functional antagonist), while the opposite is observed in mice over-expressing β2-AR [88]. Cross-talk between the regular G_s signaling pathway and G_i, whose expression is induced in airway smooth muscle in animal models of asthma, may contribute to changes in β2-AR agonist induced bronchodilation and the development of tolerance [89]. However, what is apparent from these and other related studies (see [90]), is that the long term response to β2-AR stimulation is quite different from the acute effects.

Recognition of the different effects provoked by chronic versus acute β2-AR stimulation led Bond et al. [90] to consider, heretically, that β2-AR *antagonists* could be used long-term to treat asthma. Because the β2-AR is constitutively active (in the

absence of an agonist), the antihypertensive β-blocker nadolol was selected. Nadolol is an inverse agonist, which not only blocks the receptor but also turns off the activated state, as compared to an antagonist that is functionally neutral and would only block the receptor. In research studies nadolol administered for 28 days decreased AHR in a manner very similar to that seen in the β2-AR-deficient mice [90]. Preliminary clinical studies have confirmed the decrease in AHR with nadolol administered over 9 weeks to patients with mild asthma [90]. Clearly there is a lot more to be learnt regarding the benefits and limitations of manipulating β2-AR activity, despite the long history of these drugs.

7. Clinical trial design

Unfortunately there is no quick and simple clinical study of asthma, nor are there any reliable biomarkers to indicate activity of a drug in an early stage development program. Allergen challenge has been used extensively as a "proof-of-concept" and has some compelling benefits, but also some significant limitations. Difficulties in distinguishing particular asthma phenotypes in the clinic mean that the heterogeneity of the patient population recruited for clinical trials is often high. When coupled with confounding issues such as pharmacogenomic variations that can limit responses to certain drugs, and a psychological component that is generally overlooked in clinical trial design but could contribute to a significant placebo effect, then large numbers of subjects need to be enrolled in clinic studies in order to have a chance to see an effect of a drug. Additionally, there is the choice of the appropriate endpoint. Different clinical symptoms of asthma – fixed or variable airflow obstruction, AHR, symptoms (such as wheezing, cough or nighttime awakenings), and exacerbations – are variably responsive to diverse drugs in a manner that can rarely be anticipated.

7.1. Allergen challenge model: utility and limitations

Ideally, early Phase 2 proof-of-concept studies should require relatively few subjects, treated for a brief period of time, and provide relevant, unambiguous signals of activity. Since asthma is a heterogeneous disorder that waxes and wanes over time, and resolution of the underlying airway inflammation can take several weeks, clinical trials often require a relatively large number of patients (~80+ for each variable, such as dose, studied), followed for at least 10–12 weeks. For many small biotechnology companies, the costs of such a study are forbidding at such an early stage. Consequently, many companies have turned to a clinical allergen challenge model to provide an early signal of efficacy [91]. Its utility and success is exemplified by the discovery of cromoglycate and nedocromil, described previously. In this model, mild asthmatics with a known sensitivity to a particular allergen, are challenged by inhaling the allergen to provoke a bronchoconstrictor response. The ensuing reaction occurs in two phases. The first is an immediate bronchoconstriction, beginning within 15 min and lasting around 1–2 h, termed the early airway response (EAR), and attributed to release of mediators from cells resident in the airways such as mast cells and alveolar macrophages. In many cases this is followed by the development of a usually more profound and longer lasting bronchoconstriction – the late asthmatic response (LAR) – which develops over 3–12 h before resolving spontaneously. This LAR is associated with an influx of inflammatory cells and the presence of cytokines and chemokines in the airways, and is generally used as the primary endpoint for such studies. Twenty-four hours after allergen challenge AHR to an agent such as methacholine can be measured, and sputum or BALF samples taken to determine the inflammatory cell number and profile. Consequently, this clinical model allows measurement of a number of the cardinal features of asthma—airflow obstruction, AHR and airway inflammation [91]. It also seems an obvious bridge between basic research antigen challenge models and clinical asthma.

The allergen challenge model is particularly attractive because it requires few subjects per group – usually around 10–12 studied in a cross-over design where each serves as their own control – and while the duration of treatment is drug/target-dependent, it is often ~1 week. The limitations often come when extrapolating the results to the "real world" asthma setting. Allergen challenge is a model of atopic asthma, and since there is a significant proportion of the adult asthma population who do not have atopic asthma (see Section 5.2), it remains unclear if they will respond in a similar manner. Asthma is made up of patients expressing different phenotypes (see Section 5.1) but allergen challenge provides little direction towards selecting appropriate patients for future studies. The mechanism of LAR, usually the primary endpoint, is not known, although it is ablated by a combination of an antihistamine with a CysLTR1 antagonist [92]—a treatment regimen not recommended in asthma. Difficulties in interpretation of an effect on LAR are exemplified further by studies targeting certain cytokines (see [3] for references). For example, the IL-4 mutant protein, pitrakinra, (formerly BAY-16-9996 or AER001) that binds IL-4Rα to block the receptor common to both IL-4 and IL-13, decreases LAR in the allergen challenge setting. However, antisense to mRNA for IL-4Rα, AIR645, has no effect [93]. While there can be many reasons for the negative result – limitations of delivery or dose, for example – when pitrakinra advanced into a large 534 patient study, it showed no effect on measures of lung function or symptoms, affecting only the rate of exacerbations in more severe asthma patients with airway eosinophilia. This "real world" finding is far removed from the profile suggested by the allergen challenge results. Moreover, the anti-IL-5 monoclonal antibody, mepoluzimab, shows no clinical activity in the allergen challenge setting, but also reduces exacerbations in the severe hypereosinophilic asthmatic. Aside from the allergen challenge model potentially missing therapeutic agents that act to decrease exacerbation rates, interpretation of a positive (pitrakinra) or negative (AIR645; mepoluzimab) outcome is difficult.

The unfortunate conclusion from these observations is that there is no "quick and easy" clinical trial for asthma for agents affecting a novel target, until particular asthma phenotypes are better defined and readily identified. Equally unfortunate is that no adequate biomarkers have been identified as surrogate markers of drug efficacy. Among the most popular are exhaled nitric oxide, bronchoprovocation tests with mannitol or histamine, and total IgE, but all have shown significant limitations [3,94]. This has resulted in almost a "standardized clinical protocol", requiring a relatively large number of patients (to account for the heterogeneity) treated for ~12 weeks (based on experience with ICS) to determine efficacy, regardless of mechanism. While taking such a conservative approach might be understandable, it is hardly rational.

7.2. Choice of endpoints in clinical studies

Up until the last decade most clinical trials of new therapeutics used a tried-and-tested measure of lung function, such as FEV1 or PEFR, as the primary endpoint to demonstrate efficacy. There had been an inherent assumption that the underlying defect in asthma was airway obstruction, reversal of which would improve symptoms and reduce long-term risks. The benefits of β2-AR agonists, muscarinic antagonists, and even ICS, supported this notion as they all induced significant improvements in airway function. However, it became increasingly recognized that asthma was not just about bronchoconstriction, and the benefits of the direct-acting bronchodilators were limited and less than could be

achieved with ICS, the latter also having a more pronounced effect on the risk of future exacerbations [4].

Recognition that exacerbations had a profound effect on a patient's quality of life, and were enormously costly to the health care system, directed attention towards these events as an important endpoint for clinical trials in severe asthmatics. The approval of omalizumab in 2003 was based on reducing the incidence of exacerbations in moderate to severe asthmatics inadequately controlled with ICS. New biologics in clinical development, such as mepolizumab and reslizumab, use exacerbation rate as the primary endpoint for clinical evaluation (see [3]).

While exacerbations might be appropriate for a group of severe asthmatics at high risk for a future exacerbation, for the bulk of patients an exacerbation is a relatively rare event, and thus not an appropriate endpoint for study. Disease-specific questionnaires have been developed and validated in an attempt to address multiple components of the disorder, including symptoms, need for rescue medication, impact on daily life and sleep, as well as lung function. Two such questionnaires have been adopted—one directed at asthma control (ACQ) [95] and one at asthma-related quality of life (AQLQ) [96]. To date no drug has been approved based on positive outcomes from these questionnaires alone, and they are frequently used as co-primary endpoints with other measures with a recognized history for FDA approvals, such as lung function or exacerbations. Thus the disconnect between different features of asthma – lung function, symptoms, exacerbations – is increasingly recognized and clinical trial design has moved to incorporate new endpoints to assess the value of a drug. It remains to be determined if any earlier therapeutics, discontinued for a lack of effect on airflow, will be resurrected because they impact other measures of the disorder.

7.3. Psychological features: potential confounding factor in clinical trial outcomes

Sir William Osler, regarded as the "father of modern medicine", considered asthma "all in the mind" and "a slight ailment that promotes longevity" (see [97]). The idea that asthma was largely psychosomatic was popular in the 1940s and 50s, where one widely regarded theory developed by Alexander and French was that childhood asthma developed from an unresolved dependence on the mother, where fear of separation could provoke an asthmatic attack that represented a suppressed cry for the mother [97]. While today asthma is recognized as a complex pathophysiologic insult, with genetic and environmental components, it does wax and wane, and stress and emotion are known contributory factors to precipitating an attack. In one instance, asthma patients were asked to describe a stressful environmental situation that would provoke their asthma. Six of twelve subjects did develop respiratory symptoms when the situation they described – riding in an elevator, knitting, or seeing a goldfish in a bowl for example – was reproduced experimentally [98]. In another study, 10 of 25 asthmatics developed an ipratropium-sensitive bronchoconstriction, with a mean 17.8% fall in FEV1, when inhaling saline and shown a placard that stated they were receiving a solution that would cause "breathlessness, tightness or wheeze" [99].

The objective in depicting these mind-body links in asthma is to highlight that such factors could contribute to the significant placebo effect that is frequently encountered in clinical studies of asthma [100]. Even randomized, double-blind studies are not immune to placebo effects. In one such study methacholine sensitivity could be modified by placebo when the physician indicated expectations, resulting in 18% patients being designated as responders [100]. Poorly controlled asthmatics were treated with either montelukast or a placebo and received either an optimistic (called "enhanced") message about their treatment or a neutral one. Asthma control, but not measures of lung function, improved in placebo-treated patients receiving the enhanced messaging, but not those given montelukast and enhanced messaging [101]. Consequently, a patient's expectations, particularly when given a placebo, potentially can impact the outcome of clinical trials—a factor that should be considered in the trial design.

8. Implications and future directions

From the wealth of research on asthma over the last 30 years only two new targets have been broached successfully resulting in new asthma therapeutics—the leukotrienes and IgE. One reason for this limited success, the poor translational history of preclinical models of the disorder to the clinical condition, is addressed in an accompanying article [3]. But this issue is more than just an analysis of what has worked and what has not, but a fundamental disconnect between basic research and clinical science. Basic research studies tend towards developing a standardized, unifying concept of the disorder, relying exclusively on models of antigen provocation. These studies attempt to pinpoint key "master switches" as therapeutic targets appropriate to all asthmatics. In contrast, clinical research is heading in the opposite direction to recognize the diversity of the condition, and the varying susceptibility to different treatments. Defining the heterogeneity and various phenotypes is still in its infancy, but is the foundation upon which can be built the identification of the genes underlying asthma vulnerability, which together with the epigenetic changes (an area hardly touched yet in asthma) result in the particular phenotype. Recognition of this heterogeneity, its implications and its consequences, has to be factored into the drug discovery process.

Along with defining the heterogeneity of the disorder has come the recognition that other features of asthma, for example how symptoms or exacerbations impact the quality of life, are of themselves important endpoints that are not captured or predicted by measures of lung function. Such an association had been assumed earlier, when lung function was classically regarded as the most appropriate endpoint for clinical studies. This separation of the clinical features of asthma has opened up opportunities for a broader array of treatments to provide individualized asthma control. Again, a mismatch between this clinical recognition and research models that rely on surrogate markers for efficacy, such as airway inflammation and AHR, since most animals do not develop asthma per se, is another hindrance to capitalizing on developments in the field (Fig. 4). While by definition animal models must follow on from defined clinical conditions, and the relative lack of definition in asthma has been a handicap, nonetheless, basic science and clinical research appear to be heading down almost divergent paths, and need better integration for drug discovery to be successful.

8.1. Significant progress has been made

It is the goal of this article to highlight some of the impediments to asthma R&D that have contributed to the paucity of new drugs making it to the market, thereby pinpointing areas for further development. However, it would be a misperception to conclude that all is "doom and gloom" in the asthma field. To the contrary, remarkable advances have been made. What was labeled as one disease is now recognized as a disorder made up of many phenotypes, whose identification will help unravel the genes involved in asthma susceptibility, and, together with cytokine and inflammatory cell profiling, will help identify relevant patients for future clinical studies. Significant advances in pharmacogenomics have contributed to our understanding of why drugs work in some patients and not others, and now needs to be integrated with the

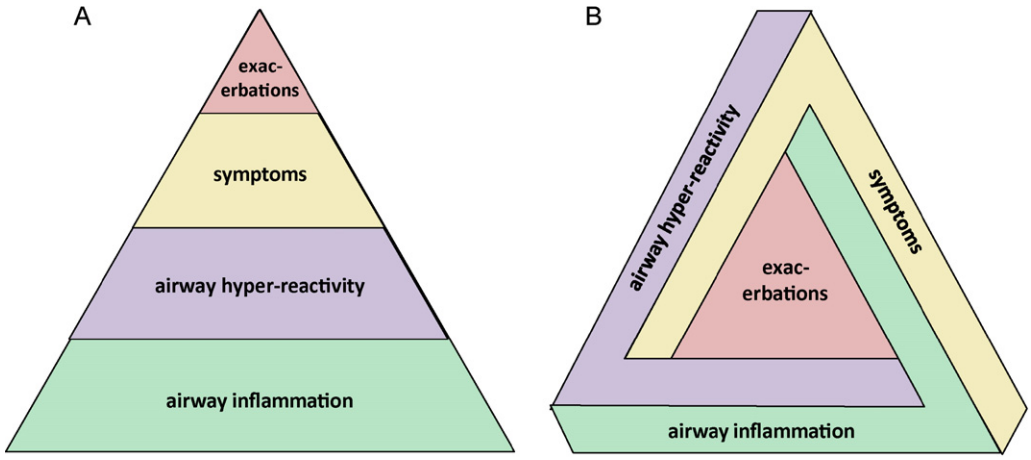

Fig. 4. Pictorial representation of the differing views on the relationship between the cardinal features of asthma. (A) Basic research view with airway inflammation as the underlying feature that causes airway hyper-reactivity (AHR), which, in turn, is responsible for all other symptoms and the propensity for exacerbations. This is due, in part, to inflammation and AHR being the only features measurable in most research models. (B) Clinical research view where the cardinal features are inter-related, but not in a direct and linear manner, depicted here as an Escher-like association. Exacerbations sit in the middle as a quasi-dependent product of the other features.

phenotypic analyses. Recognition of these phenotypes also enables studies to track if the increasing asthma prevalence is in one particular phenotype, or across the board. On the therapeutic front there have also been significant achievements. Acceptance and utilization of different clinical trial endpoints than those used historically, coupled with an appreciation that the cardinal features of asthma might be differentially regulated, has opened up new opportunities in both treatment paradigms and clinical development options. The identification of viruses as an important trigger to sensitize the airways and promote asthma exacerbations has identified new cells and mediators for consideration. Biologics such as CYT003-QbG10, which, acting through toll-like receptor (TLR) 9 has demonstrated efficacy in a key Phase 2 study [102], potentially point to new pathways for treatment, while other TLRs and pattern recognition receptors have also been implicated in atopy and asthma.

Fetal development of the airways involves complex intercommunications between the epithelium and the mesenchyme, resulting in the formation of an intricate network of different epithelial cell-types, the vasculature and neural networks in a supporting stroma. Investigators such as Holgate have proposed this "epithelial mesenchymal trophic unit" as the link between the environment and the lungs, that becomes chronically reactivated in asthma to support the local inflammation and associated functional derangements characteristic of the disorder [64]. Observed SNPs in asthma susceptibility genes associated with cytokines produced by the epithelium [74] and identification of two asthma phenotypes based, in part, on epithelial gene expression regulated by IL-13 [68], provide support for this concept. Changes in GPCR signaling and cross-talk between transduction pathways is leading to new approaches to modulating bronchomotor tone and AHR [89,90,103]. Other potentially fruitful areas of research coming to the fore include the importance of cells and mediators involved in the resolution of inflammation [3,104], since the chronic nature of asthma indicates a need to focus on reversing the response rather than trying to prevent the early steps in its development. These represent a mere handful of the exciting developments in the field, while the constraints of this article do not allow them full justice. However, the question is whether the ultimate success of these opportunities will merely benefit from, or prove to be dependent on, resolution of the issues identified in this article. Ramping up clinical research and improving integration with basic science, which itself needs to realign with the clinical advances, is required urgently.

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