

The Use of Mouse Asthma Models to Successfully Discover and Develop Novel Drugs

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Abstract

The past 20 years have seen a proliferation of scientific data on the pathophysiology of asthma. Most of these data were generated in mice using tool reagents, gene-deficient or transgenic animals. In contrast, studies on disease pathogenesis in patients are scarce. Previously, a good novel anti-asthma target for drug development was one that abrogated asthma in mice when it was knocked out, neutralized or induced asthma when it was overexpressed. This type of approach led to many drug candidates that worked in mice but unfortunately failed in patients, thereby demonstrating that the results of experiments in mice are not always predictive of clinical efficacy. Currently, there is active debate about the use of mouse models in drug discovery. In this review, we summarize the obstacles and challenges faced when using experimental mouse models of asthma in drug discovery. We propose that the initial selection of a novel drug target begins with defining the unmet medical need and specific patient population, followed by a thorough evaluation of available human data, and, only then, well-planned and ex-

ecuted mouse asthma experiments. Using this approach, we argue that mouse models lend support for the target when the models are tailored for the specific asthma patient population, and that targeted, reliable, and predictive mouse models can indeed improve and accelerate the drug discovery process.

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Introduction

Since the 1990s, there has been an explosion of scientific data on the pathophysiological mechanisms thought to play a role in asthma. Data have accumulated from experiments with congenic, gene-deficient, or transgenic animals and generated a solid knowledge base on the development of asthma as well as therapeutic interventions aimed at preventing disease onset and reducing disease progression [1]. Mechanistic insights gleaned from animal models are important because of the extrapolation potential for human disease [2–4], and mouse models, in particular, have led to the discovery of numerous novel targets for the treatment of asthma [2, 4, 5]. However, despite a myriad of potential drug targets, only anti-IgE monoclonal antibody (mAb) therapy (omalizumab) and,

recently, an anti-IL-5 mAb (mepolizumab [6]) have made it to the market [5, 7–9]. Additionally, anti-IL-4ra (dupilumab [10]), and anti-IL-13 (lebrikizumab) treatments have also been effective in phase II trials for selected patients with high eosinophilia and/or high periostin serum levels, respectively [4, 5, 7, 9, 11]. Unfortunately, many “proof of concept (POC)” targets derived from preclinical studies on mouse models failed when they were tested in patients, including anti-CCR3 antagonists, anti-IL-17, anti-IL-4, anti-OX40L, anti-TNF- α , anti-IL-1 β , PDE₄ inhibitors, and TLR agonists, amongst numerous others [5, 12, 13]. Notably, the failure of many drug candidates was not because they were ineffective, but because they were not any more effective than the current standard therapeutics.

The drug discovery strategy for asthma and other diseases is based on identifying the target, demonstrating in vivo POC in rodents, producing an effective, safe molecule to test for toxicity in vivo, and followed by clinical phase I studies [14]. Unfortunately, after progressing step-by-step through this tedious and expensive process, many promising candidates fail [13, 15], so this approach has become the bane of the major pharmaceutical companies and reduced the development of novel drugs to treat asthma. This is a dire situation for a sizeable population of patients who have severe disease and/or are resistant to the benefits of standard therapy [4, 8, 15–17].

In this review, we address whether targets derived from preclinical experimental mouse models of asthma POC studies are relevant, because they may not be adequately predictive of clinical efficacy. We address important issues such as the heterogeneous nature of asthma and the differences between mouse and human models, factors like gender, environment, and the experimental protocols that influence the results and predictive ability of experimental mouse models as well as which types of models are best to use, their role, and how they can be used effectively for drug discovery. We present a practical approach, which we consider will lead to more reliable and predictive mouse models for drug discovery.

Differences between Mice and Humans

Mice are vital in the field of immunological and pharmacological experimentation; however, their airway anatomy and pathophysiology and innate and adaptive immunity are different from in humans. For example, there is dichotomous tracheobronchial branching of the airway in humans and nonhuman primates with 17–21 airway generations, but mice only have monopodial

branching with 13–17 [18]. In mice, cartilage is found mainly in the trachea, but in humans, it extends from the trachea to the smallest bronchioles [18]. Human airways contain submucosal glands from the trachea to the proximal airways and many mucus cells within the epithelium [18], while mice have massive bronchus-associated lymphoid tissue and a higher number of Clara cells [19]. Other differences include a thinner epithelium and fewer basal cells throughout the mouse respiratory tract, and minor changes in smooth muscle hypertrophy during chronic allergen challenge with no mast cell infiltration of airway smooth muscle [18]. There is also a difference in the distribution of blood cells, with mice having 75–90% lymphocytes compared with human blood which contains 70% neutrophils [19]. Mice lack the IgA receptor Fc α RI (CD89), the IgG receptors Fc γ RIIA and Fc γ RIIC, and express the IgA, IgD, IgE, IgM, IgG1, IgG2a, IgG2b, and IgG3 isotypes [19], different from humans. Moreover, class switch in mice via IL-4 induces IgG1 and IgE versus IgG4 and IgE in humans [19]. The development of B cells and T cells and the receptor expression of many immune cells also differ, e.g., human T cells express MHC class II but murine T cells do not [19].

Is Asthma Heterogeneity Reflected in Mouse Asthma Models?

Mice do not spontaneously develop asthma. However, this statement is based mainly on the evaluation of congenic laboratory animal strains in controlled environments [2–4, 15, 20]. It is possible, however, that wild-type mice in their normal environment develop asthma, but this remains controversial [21, 22]. In the absence of spontaneous disease in laboratory mice, it is necessary to induce asthma by providing allergens that cause systemic and local lung allergic responses. In the 1990s, such models were developed in inbred, congenic mouse strains [22, 23], because it was not possible to use outbred strains due to the heterogeneous results (high variation). Congenic models enabled scientists to reduce experimental variation by managing mouse-to-mouse differences, although surprising variation remains despite identical genetics in congenic mice [23].

Congenic BALB/c mice are the strain of choice for models of allergic disease because of their propensity to develop T helper (Th)2 cell responses to antigen immunization [2, 4, 21, 23]. However, the use of only one congenic strain for all asthma studies is not ideal, for several reasons. The most important is that human asthma is a

Table 1. Asthma phenotypes in human patients

Asthma phenotype/endotype	Th cell high/ low	Characteristics	First author, year [ref.]
Early-onset allergic Th2 asthma (mild-to-severe disease)	Th2 high	atopic component: specific IgE; Th2 cytokines; often other allergic disease such as atopic dermatitis; sputum eosinophils	Wenzel, 2012 [45]
Early-onset non-Th2 asthma	Th2 low	lack of responsiveness to corticosteroids and lower concentrations of IgE	
Late-onset eosinophilic asthma (mild-to-severe disease)	Th2 high	eosinophilia; IL-5	
Late-onset aspirin-exacerbated respiratory disease (AERD) (often severe from onset)	Th2 high	adult-onset, eosinophilic asthma accompanied by sinusitis, nasal polyps; life-threatening, non-IgE-mediated responses	
Very-late-onset asthma (women)	Th2 low	low remission rate, faster decline in lung function	
Exercise-induced asthma (EIA) (often associated with mild asthma)	Th2 high	eosinophils in sputum and tissue; mast-cell activation; Th2 cytokines; cysteinyl leukotrienes	
	Th2 low	low-level eosinophilic inflammation	
Obesity-related asthma	Th2 low	no Th2 biomarkers	
Neutrophilic asthma (often associated with severe asthma or smoking)	Th2 low, Th17 high	sputum neutrophilia; Th17 pathways; IL-8	
Asthma-COPD overlap syndrome (ACOS) often in smoking asthmatics	Th2 high, Th1 high	sputum eosinophilia; serum IgE; nonfully reversible airflow obstructions with/without emphysema; lack of biomarkers; frequent exacerbations	Barrecheuren, 2015 [47]

heterogeneous disease that cannot be adequately addressed with only one mouse strain. For example, there are asthmatic subpopulations categorized by phenotype including (1) allergic, Th2 type only, (2) nonallergic, eosinophilic, (3) eosinophilic-neutrophilic combined with or without Th1 or Th17 cells, and (4) mild, moderate, or severe nonallergic, no eosinophilia (Table 1); such phenotypes are not sufficiently distinguished in mice (Tables 2, 3). There are mouse strains with distinct asthma phenotypes [2, 15, 24, 25], however. One example is the A/J strain that has intrinsic airway hyperresponsiveness (AHR), and reflects patients who exhibit AHR without the symptoms associated with airway inflammation [24]. Other examples of phenotypic differences in mouse strains include BALB/c and C57BL/6 mice. For instance, BALB/c mice have measurable strong allergen-induced AHR (K.J.E., unpubl. data) and high serum IgE titers, and disease occurs in the absence of mast cells [24, 25] whereas C57BL/6 mice have low measurable allergen-induced AHR [2] (K.J.E., unpubl. data) and low IgE titers [25], and mast cells are required for disease induction [24].

Tables 1–3 summarize the most important human and mouse asthma model phenotypes, respectively, with their

major hallmarks. We would like to point out that the information provided is not meant to be extensive and so refer the reader to other excellent review articles on these topics. Furthermore, we focused on the most commonly used murine asthma models, knowing well that many more are being used, e.g., with pollen, fungal antigens, cat antigens, adipose mice, etc. Our aim is to point out that human asthma phenotypes differ substantially from what can be mirrored in mice.

Are Gender, Environment, and Experimental Protocols Considered in Mouse Models?

The induction of allergen-induced asthma depends on genetic background, but is also influenced by gender and environment [26]. Female mice develop more severe allergic inflammation than males [22, 26] unless the males are castrated [26], and, in certain models, it is difficult to induce disease in males at all (M.M.E., unpubl. results). The environment undoubtedly influences data generated from mouse models, and such differences appear to underlie lab-to-lab differences [2, 27] which are attributed to

Table 2. Acute murine asthma model

Allergen administration (mouse model)	Th cell high/low	Characteristics	First author, year [ref.]
OVA/alum (i.p.): days 0, 2; OVA aerosol: days 18–23 (BALB/c, female)	Th2 high	AHR; eosinophils; remodelling and Th2 cytokines	Nials, 2008 [23]
OVA/alum (i.p.): days 0, 14; OVA aerosol: days 28–30 (BALB/c, female)	Th2 high	AHR; eosinophils; remodelling and Th2 cytokines	
OVA (i.p.): 7 alternate days; OVA aerosol: 8 consecutive days, starting on day 27 (BALB/c, male)	Th2 high	AHR; eosinophils; Th2 cytokines	
OVA/alum (i.p.): days 0, 14; OVA (i.n.): days 14, 25, 26, 27 (BALB/c, female)	Th2 high	AHR; eosinophils; remodelling	
OVA (i.p.): on 7 alternate days; OVA (i.t.): day 42 for 3 days, each 3 days apart (BALB/c, male)	Th2 high	eosinophils	
OVA/alum (i.p.): days 0, 7; allergen (CRA/HDM) (orotracheally): day 14 (A/J, male)	Th2 high	AHR (to CRA); eosinophils	
Der p 1/alum (i.p.) day 0; HDM (aerosol): day 14 for 7 consecutive days (C57BL/6, male)	Th2 high	AHR, inflammatory cell influx in BALF and tissue	
OVA/alum (i.p.): days 0, 14; OVA aerosol: days 28–30 and 72 days after last challenge (BALB/c, female)	Th2 high	AHR; eosinophils	
OVA/alum (i.p.): days 0, 5; 2 OVA inhalations, each 4 h apart on day 17 (BALB/c, male)	Th2 high	AHR, EAR, and LAR	
OVA/alum (i.p.): day 0; OVA (i.p.): day 10; OVA aerosol: days 17, 24 (BALB/c, male)	Th2 high	EAR and LAR; inflammatory cell influx in BALF und tissue	
OVA/alum (i.p.): days 0, 14, 21; OVA aerosol: days 26, 27 (BALB/c, male or female)	Th2 high	eosinophils; serum IgE; Th2 cytokines; AHR	Boehringer-Ingelheim inhouse data (K.J.E. unpubl. data)
OVA/alum (i.p.): days 0, 14; OVA (i.t.): day 24 (C57BL/6)	Th2 high	eosinophils; serum IgE; Th2 cytokines	
Infection with helminth larvae <i>Nippostrongylus brasiliensis</i> (i.p.): day 0; readout on day 14	Th2 high	eosinophils, serum IgE	
HDM/CFA (s.c.): day 0; HDM (i.t.): days 14, 15 (BALB/c, female)	Th17 high	eosinophils and neutrophils; Th17; AHR	
OVA (i.t.): day –1; Th17 cell transfer (i.v.): day 0; OVA i.t.: days 1, 2, 3 (BALB/c SCID mice)	Th17 high	increase in IL-17 and killer cells; neutrophil and lymphocyte recruitment; AHR	McKinley, 2008 [48]
Cat allergen Fel d 1/alum (s.c.): days –14, –7; Fel d 1 (i.n.): every 2nd day, 3-times per week for 6 weeks (BALB/c, female)	Th17 high	neutrophil and lymphocyte recruitment; AHR; Th2 cytokines, IL-17	Grundström, 2014 [29]
OVA/alum (i.p.): days 0, 7; OVA aerosol: days 14, 16, 18, 21, 24; cigarette smoke exposure: 4 times a day 5 days per week, starting on day 14	Th2 high	amplified allergic response; increased resistance; eosinophils and neutrophils	Moerloose, 2005 [49]

AHR, airway hyperresponsiveness; CFA, complete Freund's adjuvant; CRA, cockroach extract; EAR, early asthmatic response; HDM, house dust mite extract; OVA, ovalbumin; LAR, late asthmatic response; BALF, bronchoalveolar fluid.

the presence of pathogens in the mouse facility and effects on the mouse gut microbiome [28], water, food (a low-grade, or high-fiber diet which might influence gut microbiome [28]), and types of bedding and enrichments used [28]. Differences in experimental protocols are another important variable in disease models [2, 4]. The most commonly used allergen has been chicken egg ovalbumin (OVA) [2, 4, 20], which is usually contaminated with a

small amount of endotoxin (e.g., LPS) that is needed for dendritic cell activation and optimal Th2 responses [2]. Additionally, there are differences in sensitization and disease phenotype when using OVA compared with other antigens [2, 4] that contain different amounts and numbers of potential epitopes. An example is the comparison between disease induced by OVA and the house dust mite allergen. There are lower serum IgE titers and fewer mast

Table 3. Chronic murine asthma models

Allergen administration (mouse model)	Th cell high/low	Characteristics	First author, year [ref.]
OVA/alum (i.p.): days -7, -21; OVA: for 6/8 weeks (3 days/week) (BALB/c, female)	Th2 high	eosinophils; infiltration of lamina propria by inflammatory cells; remodeling; Th2 cytokine induction; AHR	Nials and Uddin, 2008 [23]
OVA/alum (i.p.): days 0, 14; OVA (i.n.): days 14, 27, 28, 47, 61, 73–75 (BALB/c, female)	Th2 high	eosinophils and mononuclear cells; goblet cell hyperplasia and mucus occlusion of airways; widespread deposition of subepithelial collagen	
OVA/aluminium potassium sulfate (i.p.): days 1, 11; OVA (i.n.): days 11, 19, 20, 33, 34, 47, 48, 61, 62, 75, 76, 89, 90 (BALB/c, female)	Th2 high	eosinophils; remodelling, Th2 cytokines; AHR	
OVA/alum (i.p.): days 0, 12; OVA aerosol: days 18–23 and then 3 days/week for 5/8 weeks, starting on day 26 (BALB/c, female)	Th2 high	inflammation; remodeling; Th2 cytokines; AHR; TGF- β induction	
HDM (i.n.): 5 days/week for up to 7 weeks (BALB/c, female)	Th2 high	eosinophils; remodeling; Th2 cytokines; AHR	
HDM (i.n.): 5 days/week for up to 5 weeks (BALB/c, female)	Th2 high	eosinophils; inflammatory gene expression	
OVA/alum: days 0, 5; OVA aerosol: 3 days/week for 6 weeks, starting on day 17, (BALB/c, male)	Th2 high	eosinophils; AHR, EAR, and LAR	
OVA/alum (s.c.): days 0, 7, 14, 21; OVA (i.n.): days 27, 29, 31, and then twice a week for 3 months (BALB/c, female)	Th2 high	eosinophilic inflammation, remodeling and TGF- β induction	
OVA/CRA/HDM/alum (i.p.): days 0, 14, 21; alternating allergen (i.t.) over 6–7 weeks, twice a week (BALB/c, female)	Th2 high	eosinophils; Th2 cytokines; allergen-specific serum IgE; collagen deposition; smooth muscle thickening; mucus hypersecretion; AHR; NO	Duechs, 2014 [36]

AHR, airway hyperresponsiveness; CRA, cockroach extract; EAR, early asthmatic response; HDM, house dust mite extract; OVA, ovalbumin; LAR, late asthmatic response; NO, nitric oxide.

cells in the airways in mice immunized with house dust mite compared to with OVA [4], but higher IL-17 levels and neutrophil cell counts with cat allergen (Fel d 1) compared to OVA [29]. Currently, there is no single asthma mouse model that fits all, and this is the likely reason for the conflicting reports in the literature, also possibly underlying the difficulties in the drug discovery process.

Do Mouse Models Have Predictive Value?

OVA-induced BALB/c mice are the most common model (Tables 2, 3) used for testing potential drug candidates for the treatment of asthma, and are generally useful for testing candidates aimed at inhibiting pulmonary Th2 responses (e.g., Th2-type cytokines) [4]. This is evident from the promising results in mice [4] and phase II and phase III trials with anti-IL-5, anti-IL-13, and anti-IL-4Ra

treatments for selected patients with eosinophilia [9, 10, 30]. However, for Th1, Th17, nonallergic phenotypes, and some Th2 phenotypes, the OVA-induced BALB/c model is not ideal. One ill-fated example of this is illustrated in the IL-5-based drug discovery process. Treatment with anti-IL-5 in OVA-induced BALB/c mice and experiments in IL-5-deficient mice revealed that blocking IL-5 reduced lung eosinophilia [31], but the overall effects were modest, because IL-13 and other Th2 pathways were not inhibited. This example demonstrated that the modest response in BALB/c mice was predictive, because it correlated with the initial disappointing results from clinical trials with a reduction in eosinophils but with a lack of efficacy in the overall population [12, 31]. However, when a targeted subpopulation of asthmatics with eosinophilia was treated with anti-IL-5, the treatment was promising [12], and the same occurred with anti-IL4ra and anti-IL-13 treatments which were efficacious in atop-

ic asthmatics [10, 12]. Another example is TSLP inhibition, which suppressed allergic Th2 responses in OVA-induced BALB/c mice and in clinical trials with a selected patient population [32, 33]. In contrast, there are many examples of effective treatment of pulmonary Th2 responses targeting IL-9, TNF- α , OX40L, IL-2, and others in OVA-induced BALB/c mice, but not in clinical trials [33]. Although treatment studies on OVA-induced BALB/c mice may be predictive, one must carefully consider the pathway that is inhibited and the mode of action when selecting a patient population for clinical trials.

What Are the Best Mouse Asthma Models to Use?

Although the immune system in mice and humans is similar, differences do exist, and it is essential to address these differences when interpreting the results obtained in mouse asthma models [1]. For example, while IgE causes mast cell degranulation in humans, both IgE and IgG1 may trigger mast cell activation in mice [19], which is why inhibiting IgE-mediated signaling in mice fails. Complicating matters further, mice have both anti-inflammatory and inflammatory IgG1. Therefore, measuring allergen-specific IgG1 does not suffice. After immunizing BALB/c mice with OVA (with a low LPS content) and the added adjuvant, alum, predominantly inflammatory IgG1 antibodies and mast cell degranulation are generated. In contrast, when proinflammatory stimuli such as TLRs, inflammasome activators, infectious agents, etc. are added, anti-inflammatory allergen-specific IgG1 may be generated, leading to a different outcome. One example of a pathophysiological difference was recognized during the development of montelukast. This drug was found to be effective in some patients in whom the leukotriene pathway is involved in disease pathogenesis. In contrast, in mice, montelukast, 5Lo, and other inhibitors of this pathway are not effective (K.J.E., unpubl. data), because these pathways do not strongly contribute to asthma in rodents [34]. Therefore, the common wild-type congenic mice are not the appropriate species for testing leukotriene inhibitors [2, 4]. Another important example that highlights pathophysiological differences between mice and humans is the promising TLR9 agonists, which were effective in preclinical mouse experiments and ineffective in clinical trials [35, 36].

Another use for mouse models in drug discovery is for the treatment of disease exacerbations of asthma with respiratory syncytial virus (RSV), rhinoviruses, and cigarette-smoking. In viral exacerbation experiments, the

challenge is that viruses that exacerbate asthma in humans neither replicate well in mice nor infect mouse epithelia (e.g., the rhinovirus major receptor group) [4, 37, 38]. Thus, RSV or rhinovirus exacerbation models are not useful for predicting the clinical outcome of a novel therapeutic. There is, however, a transgenic mouse that has a human receptor expressed for certain viruses which enables viral infection and replication (e.g., humanized ICAM-1 mice) [4], but viral replication in an immune system that has never adapted to the virus might be of limited use. Therefore, a more appropriate model to use would be a mouse-specific virus that causes lung infection such as the pneumonia virus which is an equivalent to human RSV infection [39]. An important concern in these studies is that viral and bacterial infections often protect against the allergic response in mice, e.g., influenza and RSV infection, which might cause either protection or exacerbation depending on the timing of the infection [39–43]. When addressing cigarette-smoking exacerbations, there are cigarette-smoking exacerbation mouse models designed to replicate the effects observed with smoking on the severity of lung inflammation and the worsening of symptoms in asthmatics [44]. However, the combination of cigarette smoke and allergen exposure in mice is a problem, because nicotine has anti-allergic activity and so establishing a model where cigarette smoke exacerbates the asthmatic phenotype is difficult to establish in mice [44].

Treatment of asthma in mice has often been done by administering the therapy during disease initiation rather than in mice with preexisting disease such as in subchronic [35], chronic [36], and relapsing-remitting models [27]. These more “clinically relevant” models are important because some compounds will inhibit the first wave of inflammatory cells from infiltrating the lungs, but once lung inflammation is established, they may be ineffective. Additionally, other features of disease, such as chronic inflammation and airway remodeling could be targeted, along with other advantages like new phenotype models. An example is the relapsing-disease model, in which disease relapses are controlled but not entirely suppressed with corticosteroid treatment, which may represent a model of steroid resistance [27].

How Can Mouse Models Be Used Effectively for Drug Discovery?

The most important aspect for drug discovery using mouse models of asthma is their predictive ability [1], which allows for the reliable extrapolation of preclinical

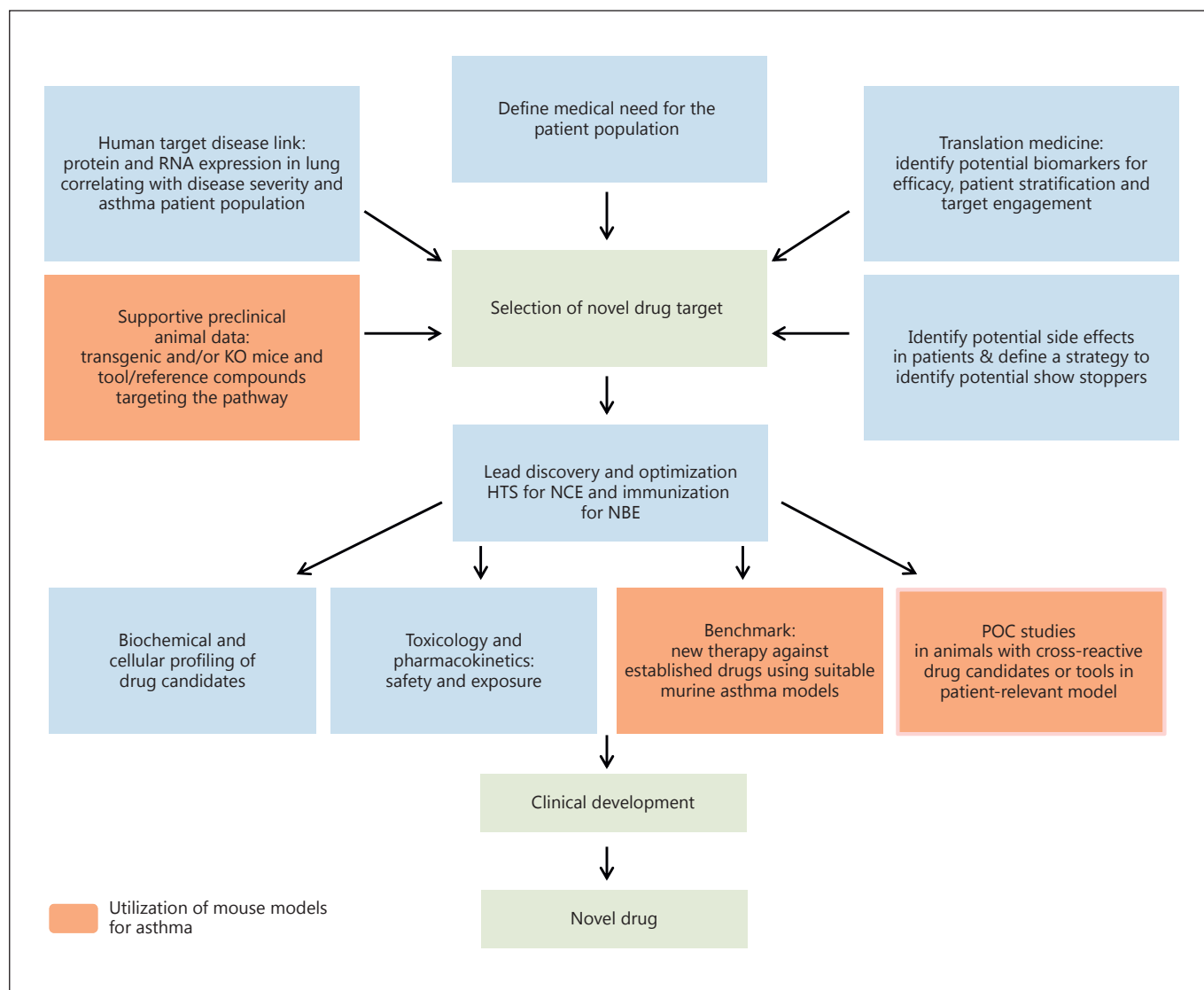


Fig. 1. The approach for using mouse asthma models in drug discovery and development. The initial selection of a novel drug target begins with defining the unmet medical need and the selection of the target patient population, e.g., asthma endotype/phenotype, in addition to patient stratification by efficacy and disease biomarkers, if possible. Drug target selection should be based on patient expression data that correlate with disease severity and are corroborated by mouse models that mimic the asthma phenotype in humans. These include knockout (KO) and transgenic mice, mice treated with substances that inhibit the target-specific pathway, and data derived from mechanistic mouse models in which the target-specific pathway is inhibited or overactivated. After generating first hits using high-throughput screens (HTS) for novel chemical entities (NCE), mAbs or other novel biological entities (NBE), a detailed biochemical and/or cellular profile identifies the first new drug candidates. Testing of the lead candidates follows

thorough toxicological, pharmacological, pharmacokinetic, and additional proof-of-concept studies (POC) in a mouse model resembling the patient phenotype. Selected biomarkers should be predictive for drug effect in patients and experimental mice. Ideally, the prospective drug cross-reacts with the mouse analog and can be directly tested in mice. If not, a mouse-reactive tool compound which closely resembles the antihuman counterpart, and with the same mode of action may be used. Finally, benchmarking new drug candidates or a suitable tool against drugs currently on the market/soon to be on the market in an appropriate mouse model is mandatory. This is necessary because novel drugs will only be successfully developed if they improve the symptoms and quality of life of those patients with a high unmet medical need, and if they significantly outperform, ideally both in efficacy and safety, the existing commercially available drugs.

treatment data from mouse models to clinical disease. The rule of thumb is “If it works in mice, it may work in humans. If it does not work in mice, it probably will not work in humans.” Therefore, the use of experimental mice for drug discovery remains promising. However, to improve mouse models for testing new asthma drug candidates, it is necessary to (1) develop models that correspond to asthmatic phenotypes, (2) identify the pathways involved for the particular drug, (3) if the mode of action is unknown, use more than one model to define the pathways involved, (4) use appropriate experimental controls, and (5) tailor the model to the type of treatment. An essential strategy is to address the pros and cons of each mouse model and carefully consider the primary end point and target. Figure 1 illustrates in which areas mouse models for asthma are important in the drug development process.

Because asthma is a heterogeneous disease, it is crucial to use appropriate models [45]. However, these models must be developed and validated. We encourage developing mouse models that, if possible, represent clearly identified and stratified patient populations [45], or else using various models to test specific aspects of the human disease. Currently, there are no models mimicking patients who (1) are not allergic, have eosinophilia without allergen-specific IgE, (2) are nonallergic, with non-Th2-mediated asthma with unknown mechanisms, and (3) show a mixed allergic response with Th1, Th2, and Th17 cells with innate and adaptive as well as viral or bacterial exacerbations. A potential approach would be to identify an asthmatic subpopulation based on biomarker signatures and to match the patient subset with the appropriate model. An example would be to compare human and mouse FeNO, FEV₁ measurements, blood sputum, or exhaled breath condensate biomarkers to select the optimal model.

The pathways targeted by a candidate drug must be considered. A Th2-driven mouse model using diverse allergens including OVA, house dust mite, or pollen, with or without adjuvants, and administered by the oral, respiratory, or systemic routes may be predictive for the development of allergic Th2-type asthma in humans. The quality of the model depends mostly on the activated pathways and contribution to the lung Th2 response, indicating that it is essential to test a drug candidate in a model in which the targeted pathways are similar in mice and in humans. Moreover, when the target is unknown, then it is essential to use more than 1 model for testing effectiveness and efficacy. This might include testing the candidate in more than one mouse strain, in chronic or

steroid-resistant models, in phenotypically distinct models, etc., in order to derive as much information as possible on the pathways targeted.

Another key issue in mouse experiments is the use of the appropriate experimental controls. Once the model is selected for the appropriate phenotype, pathway, and target, the experiments must be designed with appropriate control substances to ensure that there is a comparison to current therapeutics. For example, a new bronchodilating drug should be benchmarked against a combination of long-acting β agonist/muscarinic antagonist (LABA/LAMA; which will possibly become the new standard of care for asthmatics to control bronchoconstriction), and a new anti-inflammatory drug must be compared with an inhaled and/or oral steroid, the current standard anti-inflammatory therapy for asthmatics.

The mouse model should also be tailored to disease type. For example, a preclinical, 4-week, allergen-driven Th2 lung model might correlate with treating mild asthma. A model representing mild disease should be used to test new candidates and, as mentioned above, the experiments should include control substance currently in use. For example, most asthmatics are well controlled on LABA/ICS, and some are also well controlled on anti-IgE or montelukast [9, 46]. However, for severe, steroid-resistant asthma, a relapsing-remitting model that more closely mimics steroid-resistant disease could be utilized. In fact, when selecting the type of disease, it is also necessary to consider whether developing models to test drugs for mild asthmatics is necessary at all, considering that it costs companies billions to develop the new drugs and the health care system billions to reimburse patients for these new drugs, when the patients are actually already well controlled with standard care. Aiming for new treatments in mouse models that mimic the heterogeneous, inhaled steroid-insensitive patient population seems to be prudent [16, 45].

What Is the Role of Preclinical Mouse Models in Drug Discovery?

We argue that the initial selection of a novel drug target should begin with defining the unmet medical need and the patient population, e.g., severe, steroid-resistant asthma. Thus, a good target should be considered that is based on human data and then on supportive data in suitable animal models rather than the other way round. Figure 1 illustrates several roles for mouse models in drug development based on this notion. These include: (1) par-

allele expression profiling in patients and mice, (2) functional validation in a variety of mouse models (e.g., knockout and transgenic mice) as supportive data, (3) comparison of the same biomarkers used to follow drug effects in patients and in mice, and (4) final target validation in POC studies with cross-reactive drug candidates or suitable tools. Once the target and target population have been selected, tailored mouse models can be extremely useful for the process of drug discovery and validation. For example, a special patient population for severe asthma comprises obese women who are active smokers and suffer from asthma exacerbations. These patients often suffer from severe asthma and the prescribed drugs may be ineffective [16, 45]. This particular asthma phenotype is associated with obesity, smoking, allergic asthma, and infections. Therefore, using obese female mice exposed to cigarette smoke, in combination with allergen exposure and infection, could be a path forward. A useful animal model, therefore, should have a phenotype, biomarkers, and signs that correlate with the inflammatory phenotype and symptoms of patients. However, if it is not possible to fully match the mouse model with the patient subpopulation, then it may be more feasible to investigate specific pathways by using more than 1 model, with each model displaying different aspects of the human disease.

Conclusions

Although the use of asthma mouse models for drug discovery and development have been criticized for not accurately predicting drug effects in humans, we consider that mouse models should still be an integral part of the process of asthma target discovery and validation. These models should support human disease research and be tailored to the disease phenotype. The hope for the future is the development of new, predictive, tailored mouse models that better represent clinical diseases with the highest unmet medical needs such as severe, steroid-resistant disease.

Statement of Ethics

Animal models were performed according to local authorities and the law (the German Regierungspräsidium Tübingen and the Austrian Ministry of Science).

Disclosure Statement

The authors of the manuscript have read the journal's policy and the authors have the following conflict of interests: K.J. Erb and C. Tilp are employees of Boehringer-Ingelheim Pharma GmbH & Co. This does not alter our adherence to *International Archives of Allergy and Immunology* policies.

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