Current in vivo asthma models are poorly predictive of human disease. In vitro and human model approaches may fill remaining knowledge gaps and pharmaceutical company asthma drug pipelines, whilst reducing reliance on animal models.

Animal models of asthma: value, limitations and opportunities for alternative approaches

Anthony M. Holmes[1], Roberto Solari[2] and Stephen T. Holgate[3]

[1] National Centre for the Replacement, Refinement and Reduction of Animals in Research, 20 Park Crescent, London, W1B 1AL, UK
[2] GlaxoSmithKline, Allergic Inflammation DPU, Medicines Research Centre, Gunnels Wood Road, Stevenage, Hertfordshire, SG1 2NY, UK
[3] Infection, Inflammation and Immunity, Southampton General Hospital, Tremona Road, Southampton, SO16 6YD, UK

Asthma remains an area of considerable unmet medical need. Few new drugs have made it to the clinic during the past 50 years, with many that perform well in preclinical animal models of asthma, failing in humans owing to lack of safety and efficacy. The failure to translate promising drug candidates from animal models to humans has led to questions about the utility of *in vivo* studies and to demand for more predictive models and tools based on the latest technologies. Following a workshop with experts from academia and the pharmaceutical industry, we suggest here a disease modelling framework designed to better understand human asthma, and accelerate the development of safe and efficacious new asthma drugs that go beyond symptomatic relief.

Introduction

The Global Initiative in Asthma (2009) defines asthma as '. . .a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation is associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread, but variable, airflow obstruction within the lung that is often reversible either spontaneously or with treatment' [1]. It is considered one of the most common respiratory diseases worldwide, with almost 5.4 million people in the UK (http://www.asthma.org.uk) and 300 million people globally currently receiving treatment for the disease. The economic burden this represents is significant, costing the UK National Health Service £996 million per year, with global economic costs exceeding those of TB and HIV/AIDS combined (https://apps.who.int/inf-fs/en/fact206.html). With prevalence rates increasing globally by 50% every decade [2], these numbers and the global economic burden of asthma are set to keep rising.

The past 20 years have seen considerable advances in understanding of the pathological basis of asthma at the cellular, molecular and genetic levels; however, the fundamental causes of the disease

Corresponding author:. Holmes, A.M. (anthony.holmes@nc3rs.org.uk)

Dr Anthony Holmes is a Programme Manager at the UK NC3Rs with responsibility for the work of the Centre at the interface of academia and industry. He works with the scientific community to develop new research tools and approaches that minimise animal use and to translate these across sectors to address common problems associated with animal models. He obtained his PhD from Cambridge University in 2004 in molecular cell biology and went on to do post-doctoral research at the Babraham Institute in Cambridge, studying the role of calcium signalling in the development and death of hippocampal neurons.

Dr Roberto Solari is a cell biologist/immunologist and heads the Allergic Inflammation Discovery Performance Unit at GlaxoSmithKline. He obtained his PhD from Nottingham University in 1981 in Mucosal Immunology and went on to do post-doctoral research in Lausanne and Liverpool on the transepithelial transport of immunoglobulins. Since then, he has worked in the both pharmaceutical and biotechnology industries and was recently the CEO of Medical Research Council Technology (MRCT). His current role spans the discovery and validation of new drug targets through to proof-of-concept studies in humans.

Professor Stephen Holgate is Medical Research Council Clinical Professor of Immunopharmacology at the School of Medicine, Southampton, UK. After completing his medical training in London, he spent 2 years at Harvard Medical School to acquire skills in allergic disease mechanisms. On returning to Southampton in 1980, he established a research group focused on the mechanisms of asthma. He has used many approaches to study this disease, including epidemiology, genetics, pathology and immunology, pharmacology and experimental medicine. This research has informed guidelines on asthma management and has identified and validated novel therapeutic targets. His work has resulted in over 920 peer-reviewed publications.

1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2011.05.014

and the reasons for increased prevalence rates remain unclear. What was once considered a single disease is now recognised as a complex and heterogeneous syndrome made up of a collection of sub-phenotypes (e.g. viral-induced, allergic, non-allergic, intrinsic, extrinsic, occupational, persistent, seasonal, exercise-induced, nocturnal and steroid-resistant) with differing immunology, pathology, clinical expression, response to treatments and long-term outcomes [3]. The severity of the disease can also be affected by the patient's age, genetic background and environmental factors. More recently, cluster analysis applied to asthma of varying severity has led to further disease substratification [4–6].

Asthma has traditionally been considered an atopic disease, in which allergen sensitisation and continued exposure result in the clinical signs of the disease: airway inflammation, bronchial hyperresponsiveness and reversible airflow obstruction. However, atopy affects half the adult population, yet most do not develop asthma [7], thus signifying that other factors must have a role in the development and progression of the disease. Recent epidemiological data suggest that environmental and lifestyle factors (e.g. pollution, exposure to tobacco smoke, diet and infections) have a more prominent role in the aetiology of asthma than was previously thought [8–11]. This is supported by the increased prevalence of the disease observed in low and middle income countries as they become more westernised [12–14], suggesting that environmental factors related to 'modern lifestyle' are driving changes in disease prevalence. The realisation that factors other than atopy are implicit in asthma has significant implications for disease modelling and drug development.

Despite better understanding of the pathology and pathophysiology of asthma (reviewed in [15,16]), there are still considerable gaps in knowledge that have made it difficult to develop entirely new classes of therapeutic agent to treat the disease. The extent of the problem was summed up in a 2008 *Lancet* editorial: 'Progress in understanding asthma and its underlying mechanisms is slow; treatment can be difficult and response unpredictable; and prevention or cure is still a pipedream. Asthma, one of the most important chronic diseases, remains a genuine mystery' [17].

Most patients with asthma have mild to moderate forms of the disease, and are well controlled with the mainstay of available therapy: anti-inflammatory drugs especially inhaled glucocorticosteroids, leukotriene modifiers and bronchodilators, such as the short and long-acting β_2-adrenoceptor-selective agonists (β_2-agonists). Nevertheless, 5–10% of the asthma population experience more severe forms of the disease, which remain symptomatic despite high doses of conventional inhaled and oral anti-inflammatory drugs [18]. This represents a relatively small subset of the total population of asthma sufferers, but severe asthmatics account for almost half of the total healthcare costs associated with the disease, and the majority of asthma-related deaths [19,20]. Since the introduction of β_2-agonists (1969) and corticosteroids (1974) for the treatment of asthma, therapies directed to only two new asthma targets [cysteinyl leukotrienes and immunoglobulin E (IgE)] have been identified that have translated into clinical use, both of which have restricted indications. The removal from the market of xanthines (dose-limiting adverse effects) and chromones (efficacy) as asthma therapies during this time emphasises the need for new therapeutics that provide more than symptomatic relief for patients with asthma.

This poses considerable challenges for the asthma research community that need to be overcome if safe and effective new therapies that go beyond improving the profile of those drugs that already exist, are to be developed.

Unmet medical need

Despite substantial effort, asthma remains an area of considerable unmet medical need [21]. The UK research councils and research charities invest in excess of £64 million in respiratory research each year, of which a significant proportion is spent on asthma (http://www.ukti.gov.uk/download/108059_100608/Asthma%20and%20COPD%20research.html). This investment has resulted in greater insight into disease mechanisms and progression, but as yet has translated into only a few new effective therapies. Part of the reason for this is that asthma has traditionally been considered primarily as an inflammatory disease driven by allergy through T-helper (Th)2-type inflammatory reactions. This hypothesis provides a scheme for understanding the well-known associations of atopy and eosinophilic lung inflammation with asthma. Exposure of antigen-presenting cells (APCs) to allergen leads to the activation of Th2-cells and the production of IgE and a panel of cytokines, culminating in traits associated with classic allergic asthma.

The ability of corticosteroids to suppress Th2-type inflammation has been used as a strong argument for targeting this pathway. Corticosteroids have stood the test of time in controlling asthma symptoms and improving lung function for most patients with asthma. The development of more selective and longer acting β_2-agonists has provided additional support to the day-to-day management of asthma with good to moderate disease control. In general, the new generation of glucocorticoids and β_2-agonists are more selective and longer acting than their predecessors, resulting in improved safety profiles and allowing once or twice daily dosing regimens [22,23]. On this basis, some have suggested that global development programmes to improve access to these therapies might address some of world-wide burden associated with asthma [24]. However, real or perceived concerns about patient compliance and potential long-term adverse effects associated with continued corticosteroid and β_2-agonist use [25–29], and the need for disease-modifying drugs that go beyond symptomatic relief for all asthmatics, has driven the search for more specific drug targets high up in the inflammatory cascade.

Lost in translation

The identification of (i) key cytokines and their receptors that drive Th2-cell polarisation; (ii) pathways of leukocyte recruitment; and (iii) signalling pathways involved in cell activation and mediator secretion has provided new targets for drug development in asthma. As a result, a large number of potential small molecule therapies in addition to small chemical entities, recombinant proteins and monoclonal antibodies (mAbs), have been developed over the past 20 years. Of these, only two drug subtypes have made it to the clinic: leukotriene modifiers (e.g. montelukast, zafirlukast and pranlukast) and the IgG mAb directed to the allergic antibody IgE, omalizumab. According to asthma management guidelines, both of these therapeutic modalities have restricted indications. The lack of clinical translation of drugs developed on the back of promising preclinical data represents wasted expenditure of effort,

BOX 1

Targeting IL-13: a case study

In recent years, a great deal of interest has been directed towards the cytokine IL-13. The role that IL-13 has in asthma pathogenesis has been studied extensively (reviewed in [143]). In humans, IL-13 is upregulated in asthmatics both systemically and in the lungs during asthmatic attacks [144–146]; expression profiles that are not shared by similar diseases, such as eosinophilic bronchitis. This led researchers to conclude that IL-13 must be implicated in the pathogenesis of asthma; and has been supported by studies in mice, sheep and monkeys [147–152]. On the basis of these findings, the pharmaceutical and biotechnology industries have invested heavily in developing biotherapeutics that target IL-13, its pathways and co-factors (e.g. IL-4). However despite the positive results obtained in preclinical *in vivo* disease models, clinical trials conducted so far have been disappointing, although these are ongoing. For example, both IMA-638 (a humanised anti-IL-13 mAb) and AMG 317 (a fully human mAb to IL-4 receptor α, through which IL-13 complexes with IL-4 to form a functional IL-13 receptor) have not demonstrated clinical efficacy in phase II trials of patients with moderate and/or severe asthma [153,154]. Other targets where preclinical results have not translated into human efficacy include platelet-activating factor (PAF) antagonists [155–157], inductible nitric oxide synthase (iNOS) inhibitors [158], very late antigen-4 (VLA-4) antagonists [159,160], and anti-IL4 and -IL5 mAbs [105–107,153,161]. However, recent positive clinical trial results of an anti-IL5 mAb in well-selected patients with severe asthma and using exacerbations as an outcome parameter illustrate very well the difficulty of selecting appropriate outcome parameters for the study of possible new asthma therapies [162,163].

resources and animals; and indicates that a change in approach is necessary.

Drug attrition is a problem across all therapeutic areas. On average, only 11% of compounds entering first-in-human studies are successfully registered [30]. Lack of safety and efficacy are most commonly responsible for the high failure rate of new drugs, although other factors contribute to this. Current strategies rely on cell- and animal-based assays during preclinical and clinical stages of drug development. The predictive power of these assays plays a considerable part in the lack of efficacy and safety seen in human trials of new drugs [30,31], including those developed to treat asthma. The scientific literature includes many examples of potential asthma therapies that have been developed on the basis of positive preclinical data, only for them to fail on the grounds of safety and/or efficacy in clinical trials (Box 1). The lack of predictive preclinical models of asthma is contributing to the paucity of efficacious asthma drugs in pharmaceutical and biotechnology company pipelines, despite soaring discovery and development costs, making it increasingly difficult to sustain the current model of drug discovery [32]. The cost of taking a drug from discovery to market has been estimated to be as much as US$1.8 billion [31] so when a lack of efficacy is demonstrated in phase II, as was the case with AMG 317 and IMA-638 (Box 1), the majority of research and development costs (but not marketing costs) have already been incurred. The US Food and Drug Administration (FDA) [33] and European Innovative Medicines Initiative (IMI) [21] have acknowledged the limitations of animal models as a major bottleneck in the development of efficacious and safe medicines across many

BOX 2

Participants at the NC3Rs/MRC Asthma workshop*

The participating companies and academic research centres at the NC3Rs/MRC (National Centre for the Replacement, Refinement and Reduction of Animals in Research/Medical Research Council) asthma workshop included: GlaxoSmithKline, AstraZeneca, Novartis, Roche, Pfizer, Synairgen, Asthma UK, King's College London, University College London, Imperial College London and the Universities of Southampton, Cambridge, Manchester, Nottingham, Leicester and Edinburgh**.

*The workshop was held on 23 November 2009 at the Institute of Physics, London, UK

**This paper does not necessarily reflect the views of individual participants.

therapeutic areas, including asthma. Both the FDA and IMI recognise that current *in vivo* approaches might not be sophisticated enough to meet fully current complex demands for toxicity and efficacy testing, and that there is a need for new models and approaches to be incorporated into drug development strategies.

To identify opportunities to develop scientifically and clinically more relevant models for basic and applied asthma research, the National Centre for the Replacement, Refinement and Reduction of Animals in Research of the UK (NC3Rs; http://www.nc3rs.org.uk) held a joint workshop with the UK Medical Research Council (MRC). The workshop brought together more than 40 scientists from academia and industry (Box 2) to consider:

- the strengths and limitations of current models (animal and non-animal) used in asthma research,
- opportunities for the design and development of alternative approaches with improved scientific and clinical relevance, and reduced reliance on the use of animals.

Using the discussions at the workshop as a basis, here we consider general issues in using animals to model a uniquely human disease, and make the case for more widespread adoption of alternative approaches to support asthma research and drug development.

Modelling a uniquely human disease

Asthma is a uniquely human disease and, with the exception of perhaps cats (eosinophilic bronchitis) [34,35] and horses (heaves) [36], no animals, including those commonly used to study asthma (mice, rats, guinea pigs and rabbits) naturally exhibits an asthma-like syndrome that is similar to the disease in humans.

The utility of animal models of asthma to understand human disease and to develop therapeutics is the topic of considerable debate [37–46]. Several species and study designs have been used to try and model human asthma, suggesting that there is no generally accepted model of the human disease. Species and strain differences, variations in sensitisation and challenge schedules, and the method by which drugs are delivered to the lungs (e.g. intranasal or intratracheal instillation of solutions, nebulised compounds, or dry powders) are all parameters that can influence the outcome of a study. The extent to which these factors affect study outcomes, and the potential impact that they have on the relevance of these models to humans, is difficult to determine. Little information exists in the publically available literature, suggesting that further research to better characterise these parameters is required.

Nonetheless, some of these models have been instrumental in understanding some of the mechanistic basis of allergic asthma, providing considerable insight into pathways linked to various aspects of the disease (e.g. the role of Th2-type cytokines in airway inflammation). However overinterpretation of these models to the complex disease that occurs in humans means that considerable gaps in knowledge remain and no disease-modifying therapies exist.

The mouse has become the most widely used species because it is easy to breed, maintain and handle, a wide array of specific reagents are available for analysis of the cellular and mediator response, and genetically engineered transgenic or gene-knockout mice for modelling airway disease are available [47]. For these reasons, we focus here on the issues surrounding the use of mice as surrogates to model human asthma. Detailed reviews of the advantages and limitations of commonly used species in asthma, other than the mouse, have been published elsewhere [48].

Species differences

The reasons why positive preclinical data obtained in animals has been poorly predictive of human clinical study outcomes has been reviewed extensively by others, so will not be repeated in detail here. Briefly, these pertain mainly to species differences in airway physiology and how these relate to humans. There are well-documented disparities in the branching pattern, airway smooth muscle mass, and type and location of cells within human and mouse lungs that affect pulmonary responses [49,50]. Further variability is observed within species, with different strains of mouse exhibiting striking differences in the extent to which they develop allergic immunological responses to the same sensitiser [51,52]. Similar strain differences have also been observed in rats [53].

Methodological concerns and study design

There is widespread variation in the antigen sensitisation and challenge protocols adopted to induce Th2-type inflammation in the lung, the different endpoints used to assess this and other responses (especially lung function), and whether acute or chronic aspects of asthma are being modelled [54,55]. Traditional protocols using antigen sensitisation (commonly ovalbumin) with adjuvant (usually systemic e.g. intraperitoneal alum) followed by inhalation antigen challenge result in an acute asthma-like phenotype that focuses on inflammatory mechanisms linked to manifestations of established asthma in humans. This approach fails to model the aetiology and natural history of human asthma, which develops over time through multistep processes. As such, these antigen sensitisation and/or challenge models, although of some use in selecting bronchodilators and targets directly related to mast cell responses and relatively acute inflammation, are problematic regarding their ability to investigate disease processes associated with chronic asthma and to predict the impact of novel therapies on the underlying initiation of asthma or, once established, its evolution over time to become a chronic disease.

Efforts have been made to develop longer duration models that better simulate the chronic nature of human asthma and enable novel therapies to be evaluated in a 'therapeutic' rather than a prophylactic setting. These models attempt to use more physiologically relevant antigens (i.e. extracts of house dust mite, *Aspergillus*, cockroach or pollen) and sensitisation routes (i.e. via inhalation without adjuvant), and involve repeated exposure of the airways to low levels of allergen for periods of up to 12 weeks (reviewed in [56,57]). Chronic models have been shown to reproduce some of the hallmarks of human asthma not seen in acute models. However, their relevance is still open to interpretation; for example, the remodelling seen in chronic mouse models is due to fibrosis rather than smooth muscle thickening as known to occur in humans. In addition, there are important clinically relevant endpoints of human asthma that do not develop; for example, chronic inflammation of the airway wall and airway remodelling [45,57,58]. Initial reports using chronic models to profile drug therapy suggest they have some utility in predicting clinical efficacy [58]. However, these studies need to be substantiated with a wider range of compounds that have demonstrated negative and positive outcomes in the clinic before a real judgement can be made regarding the suitability of chronic models to predict human efficacy.

Modelling the influence of environment and lifestyle on asthma

A common feature of traditional acute and chronic animal models of asthma is that they use the concept that allergen-driven Th2-type inflammation is the underlying abnormality in asthma. This could shed light on why, despite helping elucidate some pathways that might relate to human asthma, animal models have failed to recapitulate important features of the disease. Recent birth cohort studies in asthma-susceptible families and epidemiological studies suggest that the Th2-type allergen concept is not the primary mechanism for disease development and progression [59,60]. Instead, infection (viral and bacterial), air pollution, diet, environmental tobacco smoke, drugs and other chemicals, and their interplay with genetic factors are being increasingly recognised as important risk factors for the development of asthma, its persistence and sudden exacerbations [61,62]. The action of environmental agents on the airway epithelium is translated to the underlying mesenchyme via the epithelial–mesenchyme trophic unit (EMTU). The EMTU is involved in foetal lung growth and branching during development, but appears to become reactivated in asthma, propagating and amplifying pathological inflammatory and remodelling responses in the sub-mucosa [63]. Many novel asthma susceptibility genes are expressed in the epithelium and mesenchyme [64,65], strengthening the link between environmental factors and genetics in the disease pathogenesis.

If animal models are to be predictive of human asthma, they should take account of not only relevant allergens but also those environmental factors associated with the disease. *In vivo* models to assess the role of pollution [66], obesity [67], environmental tobacco smoke (ETS) [68] and viral infections [69] in asthma are beginning to be reported in the scientific literature. These early models are limited by the same issues discussed above and might not be sophisticated enough to meet the current challenges of asthma research.

Innovation in model development

The lack of translation from preclinical to clinical studies of new asthma compounds and biologics is of particular importance to the severe and/or therapy-resistant asthma group, where new effective therapies for improved asthma control are crucial. The challenge is to develop models that more accurately recapitulate

the asthmatic airway for mechanistic studies, target identification and validation, and efficacy and safety testing. Addressing this will require a multidisciplinary, collaborative and innovative approach, as highlighted by the 2006 Asthma UK 'Basic Asthma Research Strategy': 'Imagination is to be encouraged as traditional approaches using standard models have not produced much in the way of new therapies' (http://www.asthma.org.uk/document.rm?id5282). Here, we highlight' how this challenge might be met.

Exploiting new technologies

Advances in new technologies, including tissue engineering, imaging and *in silico* modelling, can provide researchers with new opportunities for innovative model development. Integrating these technologies in existing preclinical testing programmes is crucial to accelerating the development of more predictive but fewer animal models and a fundamental shift in the way that asthma research and drug development is carried out.

The best model to use to study human asthma are patients, a view that is shared by the wider research community [70]. Technical and ethical difficulties associated with *in vivo* human studies limit research aimed at elucidating the interrelationship between the main cell types involved in pathology of the airway disease and their role in disease development [71]. As a result, human-based test systems focusing on the assessment of tissue biopsies or cells obtained by bronchoalveolar lavage (BAL) have been developed [61]. Although these provide valuable insight into inflammatory cell and mediator changes in the disease context, they only present a snapshot of the disease.

As highlighted above, beyond allergen exposure, a range of environmental factors (e.g. infection, pollution, environmental tobacco smoke, chemicals, drugs and diet and/or obesity) are increasingly recognised as contributing to asthma pathogenesis. The airway epithelium serves as the primary barrier between these environmental insults and the lungs, reinforcing its central position in the pathogenesis of asthma. Culturing epithelial cells from BAL has provided a tool to assess normal and diseased airway epithelial structure and function, and changes in response to exposure to environmental insults [72–75]. However, alone, these models are not amenable to studies investigating communication between the epithelia and underlying immune, inflammatory and mesenchymal cells in response to environmental insults, which is a key step in translating mucosal gene–environmental interactions that are crucial for the development of asthma. Tissue engineering technology provides a mechanism to study interactions between human cells in a complex three-dimensional disease-specific context, which could overcome the limitations of current two-dimensional cell culture studies. Such an approach might facilitate the search for novel therapeutics that go beyond current strategies focussing on the Th2 paradigm suggested by mouse immunology research.

Biomimetic models

The opportunities afforded by tissue engineering, coupled with advances in bioreactor and scaffold design, have resulted in several tissue-engineered human airway equivalents becoming available. Although these have added valuable insight into the pathophysiology of the disease (reviewed in [76]), they are still too simplistic to mimic important *in vivo* features, such as fully functioning immune and/or circulatory systems.

Microfluidics: Tissue engineering is a rapidly evolving science and tissue engineers have embraced the challenge of building complexity into their models. Although there might still be some way to go in recapitulating *in vitro* entire immune or circulatory systems, steps are being made. Advances in the emerging field of microfluidic lab-on-a-chip technologies, combined with tissue engineering, offers great opportunity in this regard. Microfluidics provide many advantages over current macroscopic techniques (summarised in Box 3) and have been used to microfabricate successfully blood vessels [77,78], muscles [79,80], brain [81], kidney [82] and liver [83–85] for basic research and drug discovery [86–88].

Importantly, there have also been attempts to develop airways models based on microfluidic approaches. These first steps in applying microfluidics to modelling airways have established important proof-of-concept information on the utility of these systems to reproduce complex, integrated organ-level physiological and pathological responses [89–91]. The immediate application of these models is likely to be in toxicity testing and chemical safety assessment [92,93], where they would offer an alternative screening platform to reduce animal use in inhalation toxicology studies, for example. However, the potential utility of these models for improving understanding of complex disease processes has been established. Recently, Huh *et al.* [89] used a 'lung-on-a-chip' device to demonstrate that the respiratory 'crackling' sounds picked up by stethoscopes in patients with lung diseases (including cystic fibrosis, pneumonia and, in some instances, asthma), are not symptoms, but are the audible

BOX 3

Advantages of microfluidic systems

- Require reaction volumes that are typically found in biological systems, offering conditions as close to the physiological context as possible. This is an important advantage of these systems because microscale behaviour can differ from macroscale behaviour.
- Amenable to high-throughput processing and analysis.
- Development and application can be automated more easily.
- Can be fabricated to incorporate multiple, interconnected tissue culture chambers for the simultaneous culture of different tissues to recapitulate interactions between different organs of the body and enable more realistic *in vitro* assays of the response of the whole body to various stimuli. This might be especially useful in drug development.
- Multicompartments can be connected by recirculating tissue culture medium that acts as a blood surrogate, providing a flow system (haemodynamic shear stress) that is known to modify cell morphology and biological activity.
- Can be fabricated to incorporate real-time sensor technology to monitor numerous parameters continually, including pH level, oxygen saturation, barrier integrity, temperature, and optics, for example.
- Can be fabricated using flexible membranes [e.g. polydimethylsiloxane (PDMS)] for the inclusion of mechanical forces to simulate breathing, for example.
- Relatively inexpensive when compared with existing macroscale methods.

manifestation of events that might be contributing to the lung damage associated with disease (e.g. airway remodelling). The 'crackling' represents the rupture of liquid plugs formed across the airways lumen as a result of surfactant dysfunction, which accompanies a variety of pulmonary diseases [89]. Second-generation models based on this system might enhance understanding of cellular responses to complex pulmonary mechanical forces, potentially improving strategies for treating lung disease.

The authors of these studies [89–91] recognise their proof-of-concept models have limitations. However, microfluidic approaches have real potential to improve understanding of the pathophysiology of asthma, and the development of novel efficacious therapies. For this to be realised, next-generation models need to bring together several features. First, they must demonstrate the correct architecture and dynamic interaction between different cell types that is vital for integrated tissue responses. Second, they must take into account the effects of haemodynamic and mechanical forces present in the lungs *in vivo*. Third, they must include the dynamic influx and/or efflux of immune and inflammatory cells from the circulation that normally occurs in the airways *in vivo*. Achieving this will take a synergistic effort between biologists and engineers, and willingness on the part of those that fund research to invest in some potentially risky, but high-return blue skies science.

Precision cut lung slices: *Ex vivo* precision cut lung slices (PCLS) from human lung also offer exciting opportunities for increasing understanding of asthma development and progression, and bridge the gap between cell culture and isolated tissue preparations and animal studies. Despite this, however, PCLS approaches are rarely adopted. Originally developed for toxicological purposes, PCLS have been successfully adapted to study airways disease and have several advantages over current *in vivo* approaches [92,93]. The main focus of their use has been to assess airway smooth muscle responses, including hyperresponsiveness, remodelling and bronchioconstriction in response to several stimuli, such as allergens, pharmacological challenge and infection [92,94–96]. Recently, PCLS has been used to investigate the response of airways to drugs used in the treatment of asthma, where these models are already identifying alternative treatment strategies as potential future asthma therapies [97]; however, these are yet to be tested clinically.

The role of airway smooth muscle (ASM) in asthma is important, after all asthma is defined in terms of reversible airway narrowing ('bronchospasm'). Widely recognised as the key determinant of airway narrowing in the disease, ASM is also emerging as an effector of airway inflammation and remodelling [98,99]. There is, however, a lack of mechanistic information on human asthma, especially of airways hyperresponsiveness (AHR), despite intensive research in numerous species [40]. New research paradigms with greater use of human three-dimensional biomimetic models and PCLS to identify pathological pathways, mediators and neurotransmitters that might alter ASM involved in AHR, inflammation and remodelling could address this. Knowledge gained from these studies could be reverse translated to develop fewer, but more predictive animal models to study these pathways and to identify similar pathways that might be predictive of human disease and that might facilitate target identification.

In silico *modelling*

In silico and mathematical modelling approaches might also provide insight into the underlying mechanisms of asthma. Integrating genomic, proteomic, physiological, environmental and behavioural data generated by traditional approaches into mathematical models that represent the mechanistic underpinnings of disease pathophysiology provides researchers with another approach to explore the relationship between cellular and whole-organ responses. The adoption of *in silico* modelling by the biomedical research community has been relatively slow in comparison to other businesses, such as the aerospace or automotive industries. However, this might change following recent reports from the IMI [21] and the FDA [33] that recognise the potential of *in silico* technologies to alleviate bottlenecks in the drug discovery process and reduce the overall cost of drug development.

In silico modelling can be applied at every stage of the drug discovery and development process. Some companies are making major investments in *in silico* modelling, changing the way they develop drugs, and accelerating the drug discovery process [100]. These approaches are forming the newest wave of modelling tools in asthma research and drug development. Numerous models have been developed to study basic events, such as molecular interactions (ligand–receptor), whole-organ functions (e.g. bronchioconstriction and aerosolised drug deposition in the lung), and even virtual patients (reviewed in [101,102]).

In collaboration with life scientists from academia and industry, companies are developing virtual patients for a range of pathologies, including cardiovascular, metabolic and respiratory diseases. Technology platforms are able to simulate the complexity of human biology in the context of a disease or therapeutic area; focussing on determining the clinical response to potential treatment and understanding the biological mechanisms, pathways and feedback loops underlying disease progression [103]. A respiratory module is reported to recapitulate the biology behind asthma and has the capacity to reproduce the disease in different types of patient (e.g. established chronic disease in adults; therapeutic responders and reduced responders; mild, moderate or severe asthmatics) to understand patient heterogeneity, something not possible in current *in vivo* approaches. Several pharmaceutical companies, including Pfizer, Merck, Sanofi Aventis and Bayer, are already using this technology platform to streamline their asthma drug discovery processes. It has been estimated that the use of this approach saved Aventis (now Sanofi Aventis) millions of dollars by predicting that, despite promising preclinical data in animals, interleukin (IL)-5 was not a viable target in human asthma; consequently, Aventis stopped developing therapies targeting this cytokine [104]. This result has subsequently been confirmed by other companies conducting clinical trials of patients with asthma [105–107]. However, recent studies have demonstrated that a blocking mAb targeting IL-5 (mepolizumab) might be useful in a very small subset of the total asthma population (<0.5%). Mepolizimab has proved to be highly efficacious in some hypereosinophilic syndromes [108,109] as well as in exacerbations of asthma in those with severe disease who had persistent sputum eosinophilia in the presence of high dose inhaled as well as oral corticosteroids [110,111]. Exacerbations have not been modelled in *in silico* models and, therefore, it is not surprising that

efficacy against this outcome measure of asthma was not predicted. As confidence in the technology grows with continued use, it is likely that these *in silico* models will be used earlier in the drug development process to screen out unsuitable compounds before preclinical studies.

These systems biology approaches are being applied widely across the pharmaceutical industry as adjuncts to traditional preclinical *in vitro* and *in vivo* models. It is unlikely at this stage that they will replace animal use, but as in the IL-5 example above, it is feasible that animal use on candidate compounds that will be dropped later in development will be reduced with more effective early screening tools. The IMI regards the development of *in silico* methods as one of two 'Individual Research Projects of Priority' to be dealt with immediately by an as yet to be established European Centre of Drug Safety Research [21], thus highlighting the potential importance of this technology to support the faster discovery and development of better medicines with reduced animal use.

Although the use of *in silico* modelling is becoming more common, there are several factors slowing its adoption. These include a lack of scientists with the necessary skills and expertise specific to the field (mathematicians, engineers and informaticians), and the need for computers and software with the required power to be able to deal with the increasing volume of data required to create these models. An additional barrier to adopting these approaches is that *in silico* methods rely on the accuracy and extent of the existing knowledge base to populate them. Any gaps in knowledge of the mechanisms of the disease will be reflected in the models, and this is why some have suggested that the technology will never replace traditional drug discovery and development methods.

Patient cohort studies

Asthma consists of multiple and co-existent disease mechanisms. Individual patients are clinically very different and, at present, the efficacy of new drugs cannot be predicted from current models or from currently defined patient characteristics. A common limitation in all models used to study asthma is a lack of knowledge regarding the pathophysiology of the human disease. Detailed phenotype studies combining molecular, histological, clinical and patient-reported data from large patient (adult and paediatric) cohorts could provide a solution. Such an approach would allow greater stratification of disease and mechanisms as well as supporting stratification of experimental interventions and studies [112,113]. UBIOPRED (Unbiased BIOmarkers in PREDiction of Respiratory Disease Outcomes), an European Union consortium of academia, major pharmaceutical companies, SMEs (small to medium enterprises), charities and patient organisations (http://www.ubiopred.european-lung-foundation.org) was established by the IMI to take this approach to better understand the heterogeneity of severe asthma and enable significantly better prediction of therapeutic efficacy of new medicines. The data generated from this work will be used iteratively to improve current models so that they better reflect the human disease, and to better understand the opportunities and limitations of these models in respect of predictive power for human clinical studies.

An important aspect of UBIOPRED and other similar approaches (e.g. GA^{2}LEN; http://www.ga2len.net) is the collection and storage of human tissues, primary cells and biological fluids (e.g. sputum, lavage, exhaled breath condensate and blood) from well-phenotyped, representative patients and controls. Access to well-characterised human tissue samples is essential to validate animal and *in vitro* test methods to enable a more informed choice as to their use, and will greatly enhance the quality of resulting clinical go/no go decision making. Several repositories for human biological samples already exist so it might not be necessary to create new biobanks. What is required, however, is a mechanism to ensure the contents of these biobanks are mapped and publicised and that they are easily accessible to the research community.

Improving in vivo *models*

The data generated from patient cohort, biomimetic, and *in silico* studies will provide the key tools necessary to develop more predictive models of human asthma, which could ultimately lead to new therapies for all asthma groups that go beyond symptomatic relief. Until more predictive approaches are developed, the current animal models, however poor, continue to be used for target validation and to provide regulators with some evidence of efficacy before clinical trials. The risk is that potentially new targets identified from a better understanding of human asthma are tested in models whose relevance is debatable. There is a pressing need in the short term to critique and prioritise the current animal models that will be used for the next 5–10 years for these purposes. Collaborative projects where this is an objective have been initiated; for example, the MRC/ABPI public-private Immunity and Inflammation Initiative (http://www.mrc.ac.uk/Fundingopportunities/Calls/MRC-ABPI-Initiative/MRC006733) and the pharmaceutical industry-hosted Cross-Company Animal Models Symposium series. However, more could be done with current knowledge to improve the utility of traditional *in vivo* models.

The utility of animal models of asthma has been the subject of extensive review [37,41–44,46,49,57,58,114–116]. However, these reviews only provide a high-level qualitative analysis of some of the models used to assess the gross pathological changes observed in human asthma. They do not provide the level of detail and interrogation required to enable others to develop more predictive research tools. Critical appraisals of animal models, including meta-analyses of data derived from these models, should be included as a standard step in model development processes, potentially leading to better animal models with improved relevance to clinical human disease [117,118].

Allied to this, a more selective approach is required when deciding which animal models to use in preclinical testing programmes. The current approach is to use 'off-the-shelf', well-characterised historical models, rather than those that directly measure what the candidate molecule is targeting. For example, there is no point in using an allergen challenge model to test a new drug that does not work through immune modulation. A new preclinical testing paradigm to develop targeted models before *in vivo* screening should be adopted to ensure suitable candidate molecules enter into human clinical trials as quickly and safely as possible. Similar suggestions have been made for other therapeutic areas, including oncology [119].

As discussed above, efforts to improve the predictiveness of *in vivo* asthma models are ongoing, and include developing longer duration chronic models, models to better understand asthma exacerbations, and sensitising animals with more physiologically

relevant antigens. It is too early to say how predictive these new models are of clinical asthma, but recent data are promising, at least for chronic models (reviewed in [58]). However, further validation against a wider range of molecules, with more clinically relevant endpoints is required before it can be judged whether these approaches really are an improvement on traditional acute models.

Transgenic approaches: Genetically modified animals, especially mice, are considered important models in basic research to define specific pathways for drug discovery, mechanistic studies and target identification and/or validation. They have been extensively used in asthma, with numerous 'off-the-shelf' transgenic mice and species-specific probes and reagents commercially available, which enable researchers to suppress, switch off or upregulate a single molecular signalling pathway specifically. However, it is questionable how useful these models are for studying a disease that is associated with several molecular and cellular pathways that function synergistically or independently of each other.

Transgenic mice have highlighted the importance of numerous cytokines in Th2-driven inflammatory responses in pulmonary inflammation, including IL-4, IL-5, IL-9, IL-13, eotaxin and regulated upon activation, normal T cell expressed and secreted (RANTES) [120]. These studies have helped analyse the pathophysiology of allergic asthma, but have yet to prove their usefulness in understanding asthma induced by environment and lifestyle factors. This might be attributable to the fact that asthma is the result of many factors, so suppressing or upregulating a single molecular pathway is unlikely to impact on the disease, as proved for IL-4, IL-5 and IL-13 [107,121–123]. However, whether this is the consequence of inadequate evidence for the involvement of these cytokines in human asthma (as opposed to animal models of allergic-type inflammation), or because of poor clinical trial study design (i.e. participants not reflecting asthma heterogeneity), is the subject of debate [113,122,124]. What is clear is that findings from any of these genetically modified models cannot be viewed in isolation but must be interpreted in the context of the complexity of human asthma. Double and triple transgenic mice are being developed to try and model this complexity. These provide researchers with models to unravel multiple signals and components that make up specific pathways by selectively turning gene expression on or off at any time [125–127], allowing the reversibility of phenotypic responses to be defined. However, factors that limit traditional animal models (e.g. strain and species differences, and sensitisation protocols) still apply when developing transgenic models, making it even more important that animal experiments are reported accurately [128] so that results can be interpreted appropriately. There is little doubt that transgenic models will shed light on the pathobiology of disease, but it is still too early to assess whether they are useful predictors of efficacy in humans, although initial results suggest the data should be treated with some caution.

Lung function endpoints: The reassessment of lung function endpoints used in current preclinical testing strategies is an important consideration in the development of more predictive models of human asthma. Major anatomical and size differences exist between human and animal airways, making comparable lung function measurements difficult [129–132]. Despite this, numerous approaches to measure lung function have been developed, including both invasive and non-invasive methods. Which of these a researcher chooses often requires a compromise between precision and invasiveness, with the most precise techniques also being the most invasive. The outcome endpoints used in *in vivo* lung function models, including pulmonary resistance, dynamic compliance and enhanced pause, are not those used by regulators in clinical trials of potential new therapeutics, further complicating translation of these results to humans. Furthermore, serious concerns exist regarding the validity of some of these endpoints as a predictive measure of human lung function [129,133–135]. Problems arise because of the different way that lung function is monitored in animals and humans, which produces different results [113]. Developing *in vivo* lung function endpoints that better align with those used in clinical studies [e.g. change in FEV_1 (forced expiratory flow rate in one second)] is essential. Doing so is likely to improve translation of lung function data, which are so important in providing diagnostic information about the disease process.

Do preclinical animal models of efficacy add value to drug development?

Given the current limitations of preclinical asthma models for efficacy studies, it is pertinent to explore whether the fresh perspective needed in this area is to reduce the use of current *in vivo* efficacy models. Such a paradigm shift would see animals used for mechanistic studies and for safety and dose finding before human studies. This concept, called 'experimental medicine', is not new and could traditionally be considered as pharmacology, both preclinical and clinical. The approach uses human data to demonstrate the mechanistic activity of new drugs at safe doses that link that activity to efficacy in humans [136]. Taking this approach could increase confidence in the relevance of novel drug targets and provide better information than the current animal efficacy models. For these reasons, many pharmaceutical and biotechnology companies have created experimental medicine groups to demonstrate that new compounds can be administered safely in humans, and that the pharmacological activity provides sufficient efficacy information in the disease indication. The key to the success of experimental medicine approaches is the identification, development and validation of appropriate biomarkers. This has been facilitated during the past decade by rapid advances in key enabling technologies, such as polyomics [137,138] and molecular imaging [139].

The lack of efficacious new asthma drugs suggests that this is an area where greater adoption of experimental medicine approaches is appropriate. Data from large-scale patient cohort studies, including the discovery of new genetic risk factors [140], will help to identify novel biomarkers to predict efficacy and trigger *in vitro* experiments that better mimic the human disease. For these studies to be meaningful, they need to go beyond the conventional approach of determining the concentration of free drug in the plasma that is associated with the therapeutic effect in an animal. Probably of greater importance is the demonstration that the molecule being tested hits its intended target and pathway in the patient, ensuring only those compounds that are most likely to be efficacious in humans continue to be developed. When combined with microdosing and pharmacologically active does studies

(phase zero) [141,142], these human-based testing strategies provide powerful tools that can enhance many aspects of the pharmaceutical business and reduce reliance on animal models. This will be further enhanced by encouraging greater imagination in the development of better human test procedures to test and demonstrate the efficacy of novel disease-modifying agents; however, it is beyond the scope of this review to comment on this in more detail.

Conclusions

Asthma is a complex, heterogeneous disease, much of the pathophysiology of which remains unknown despite considerable investigation over the past century. This is reflected in the number of new therapies that have reached the market during the past 50 years, and the complete lack of any drug that can provide long-lasting remission from symptoms or a cure. Many novel compounds directed at new and existing targets have entered human trials on the basis of positive preclinical animal data and failed because of a lack of safety and/or efficacy.

It is valid to question whether the current asthma research paradigm is appropriate, or if a substantial shift in approach is necessary to meet the needs of the pharmaceutical industry, funders and patients. The current paradigm where animal models of allergen sensitisation and challenge are considered the gold standard falls some way short of human asthma. New models need to be developed that take into account emerging knowledge underpinning the mechanistic basis of the disease and that reflect: (i) the chronic nature of asthma; (ii) environmental factors that drive asthma development and exacerbations; (iii) the importance of smooth muscle and other aspects of airway wall remodelling in asthma pathophysiology; and (iv) the role of airway epithelium in translating immunological and inflammatory reactions.

No single method is sufficient to model a syndrome as complex as asthma accurately. To address current knowledge gaps and drive a fundamental shift in the way asthma research and drug development is carried out requires a tool-kit of clinically relevant approaches addressing specific questions that, when combined, build a holistic picture of the disease. Developing this tool-kit is reliant on large and comprehensive patient cohort studies, access to human tissue, a human phenotyping programme, bioinformatics to interrogate the human models and integration of new technologies and multidisciplinary expertise to exploit this new information (Box 4). This is an iterative process that builds on the foundations of existing knowledge and, perhaps more importantly, new insights gained from well-characterised human cohort studies of asthmatic patients and healthy controls. The European GABRIEL Consortium recently demonstrated the importance of human data in identifying possible new disease mechanisms and therapeutic targets. In a genome-wide association study on nearly 27 000 asthmatics and healthy volunteers, Moffatt and co-workers discovered genes that support a role for communication of epithelial damage to the adaptive immune system, thus strengthening the case for the importance of airway epithelium and impaired wound-repair responses in the development and progression of asthma [140].

Here, we have highlighted the role that *in vitro* and *in silico* technologies can have in improving knowledge of the mechanistic basis of asthma if exploited to their full potential. Clearly, there is scope for further development in these models. Greater levels of complexity, based on information from patient cohort studies need to be built into the next generation of *in vitro* models to recapitulate important aspects of the disease, such as chronicity, and incorporating its formed as well as inflammatory aspects.

To encourage the asthma research community to rise to this challenge, the NC3Rs has recently invested £1 million in two new research projects to develop tissue engineering approaches to model the asthmatic airway using cells taken directly from well-characterised patients with asthma and healthy volunteers. By combining microfluidics into new bioreactors, the models will incorporate a rudimentary circulatory system to better understand the dynamic interplay between static and mobile cells in asthmatic airways; and to examine how environmental insults interact with asthma susceptibility genes (http://www.nc3rs.org.uk/asthmaaward). Both awards will bring together multidisciplinary teams comprising tissue engineers, cell biologists, biomaterials specialists, immunologists and clinicians. This broad expertise is crucial to drive the development of models that will have the most utility in predicting human asthma.

To move these aspirations on and link them to experimental medicine requires greater collaboration between industry, academia, clinicians and regulators. These groups need to adopt a framework for continuous integration of basic and disease research, and drug development knowledge to ensure the latest advances are being used to improve the power of asthma modelling. Regulatory conservatism might be considered a barrier to the adoption of model system approaches with which to generate safety and efficacy data. However, this view might no longer hold sway as regulatory authorities begin to accept the mounting evidence base supporting the utility of these approaches for drug development [32,138]. To maintain momentum in this area, it is imperative to continue engaging regulators early in these discussions. Adopting a more collaborative rather than competitive model should be viewed as a crucial driver for the successful

BOX 4

A framework for improved asthma modelling

- More focus on studying asthma in humans and sub-phenotyping (biobanks, experimental medicine and biomarkers).
- Application of technology platforms to well-phenotyped patients ('omics, e-science and in-depth analyses of clinical trials).
- Consideration of asthma over the life course (aspire to prevent or cure, not just suppress disease).
- Embed experimental medicine studies in longitudinal cohort and epidemiological studies.
- Develop disease-related *in vitro* human models to test novel mechanisms and therapeutics.
- Integrate data from human and *in vitro* studies to accelerate the development of fewer, but more predictive animal models of human asthma.
- Adopt 'dry lab' and systems approaches to understanding disease complexity.

development of safe and efficacious new therapies to treat asthma based on human data and knowledge of the human disease. This will both benefit patients and help reduce reliance on *in vivo* models.

Conflicts of interest

AMH is employed by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs). The authors state no other conflicts of interest.

Acknowledgements

The National Centre for the Replacement, Refinement and Reduction of Animals in Research expresses its sincere thanks to all speakers and delegates at the workshop. Particular thanks to Stephen Holgate (University of Southampton) for chairing the workshop and to Donna Davies (University of Southampton) for her assistance in putting the workshop together. The authors thank Vicky Robinson and Kathryn Chapman (NC3Rs) for help developing the concepts in this review.

References

1 GINA (2009) Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma
2 Masoli, M. *et al.* (2004) The global burden of asthma: executive summary of the GINA Dissemination Committee report. *Allergy* 59, 469–478
3 Anderson, G.P. (2008) Endotyping asthma: new insights into key pathogenic mechanisms in a complex, heterogeneous disease. *Lancet* 372, 1107–1119
4 Bousquet, J. *et al.* (2010) Uniform definition of asthma severity, control, and exacerbations: document presented for the World Health Organization Consultation on Severe Asthma. *J. Allergy Clin. Immunol.* 126, 926–938
5 Haldar, P. *et al.* (2008) Cluster analysis and clinical asthma phenotypes. *Am. J. Respir. Crit. Care Med.* 178, 218–224
6 Hastie, A.T. *et al.* (2010) Analyses of asthma severity phenotypes and inflammatory proteins in subjects stratified by sputum granulocytes. *J. Allergy Clin. Immunol.* 125, 1028–1036
7 Pearce, N. *et al.* (1999) How much asthma is really attributable to atopy? *Thorax* 54, 268–272
8 Kauffmann, F. *et al.* (1999) EGEA (Epidemiological study on the Genetics and Environment of Asthma, bronchial hyperresponsiveness and atopy): descriptive characteristics. *Clin. Exp. Allergy* 29 (Suppl. 4), 17–21
9 Kauffmann, F. *et al.* (2002) Epidemiologic study of the genetics and environment of asthma, bronchial hyperresponsiveness, and atopy. *Chest* 121 (Suppl. 3), 27S
10 Kauffmann, F. *et al.* (2002) Epidemiological study on the genetics and environment of asthma, bronchial hyperresponsiveness and atopy (EGEA)—first results of a multi-disciplinary study. *Rev. Mal. Respir.* 19, 63–72
11 Custovic, A. and Simpson, A. (2006) What are we learning from genetic cohort studies? *Paediatr. Respir. Rev.* 7 (Suppl. 1), S90–S92
12 Chen, Y.Z. (2004) Comparative analysis of the state of asthma prevalence in children from two nation-wide surveys in 1990 and 2000 year. *Zhonghua Jie He He Hu Xi Za Zhi* 27, 112–116
13 Paramesh, H. (2002) Epidemiology of asthma in India. *Indian J. Pediatr.* 69, 309–312
14 Addo-Yobo, E.O. *et al.* (2007) Exercise-induced bronchospasm and atopy in Ghana: two surveys ten years apart. *PLoS Med.* 4, e70
15 McFadden, E.R., Jr (2004) A century of asthma. *Am. J. Respir. Crit. Care Med.* 170, 215–221
16 Walter, M.J. and Holtzman, M.J. (2005) A centennial history of research on asthma pathogenesis. *Am. J. Respir. Cell. Mol. Biol.* 32, 483–489
17 Editorial, (2008) Asthma: still more questions than answers. *Lancet* 372, 1009
18 Sullivan, S.D. *et al.* (2007) Association of control and risk of severe asthma-related events in severe or difficult-to-treat asthma patients. *Allergy* 62, 655–660
19 Smith, K. *et al.* (2009) Evaluation of risk factors and health outcomes among persons with asthma. *J. Asthma* 46, 234–237
20 Watson, L. *et al.* (2007) Factors associated with mortality after an asthma admission: a national United Kingdom database analysis. *Respir. Med.* 101, 1659–1664
21 Anon, (2006) *The Innovative Medicines Initiative (IMI) Strategic Research Agenda*. European Federation of Pharmaceutical Industries and Associations
22 Adcock, I.M. *et al.* (2008) New targets for drug development in asthma. *Lancet* 372, 1073–1087
23 Holtzman, M.J. (2003) Drug development for asthma. *Am. J. Respir. Cell. Mol. Biol.* 29, 163–171
24 Bousquet, J. *et al.* (2010) Prioritised research agenda for prevention and control of chronic respiratory diseases. *Eur. Respir. J.* 36, 995–1001
25 Beasley, R. *et al.* (1999) Beta-agonists: what is the evidence that their use increases the risk of asthma morbidity and mortality? *J. Allergy Clin. Immunol.* 104, S18–S30
26 Beasley, R. *et al.* (2009) Safety of long-acting beta-agonists: urgent need to clear the air remains. *Eur. Respir. J.* 33, 3–5
27 Martinez, F.D. (2005) Safety of long-acting beta-agonists: an urgent need to clear the air. *N. Engl. J. Med.* 353, 2637–2639
28 Nelson, H.S. *et al.* (2006) The Salmeterol Multicenter Asthma Research Trial: a comparison of usual pharmacotherapy for asthma or usual pharmacotherapy plus salmeterol. *Chest* 129, 15–26
29 Severi, G. *et al.* (2010) Asthma, asthma medications, and prostate cancer risk. *Cancer Epidemiol. Biomarkers Prev.* 19, 2318–2324
30 Kola, I. (2008) The state of innovation in drug development. *Clin. Pharmacol. Ther.* 83, 227–230
31 Paul, S.M. *et al.* (2010) How to improve R&D productivity: the pharmaceutical industry's grand challenge. *Nat. Rev. Drug Discov.* 9, 203–214
32 Fitzgerald, G.A. (2005) Opinion: anticipating change in drug development: the emerging era of translational medicine and therapeutics. *Nat. Rev. Drug Discov.* 4, 815–818
33 FDA, (2004) Innovation/stagnation: challenge and opportunity on the critical path to new medical products. *FDA*
34 Reinero, C.R. (2010) Advances in the understanding of pathogenesis, and diagnostics and therapeutics for feline allergic asthma. *Vet. J.* doi:10.1016/j.tvjl.2010.09.022
35 Venema, C.M. and Patterson, C.C. (2010) Feline asthma: what's new and where might clinical practice be heading? *J. Feline Med. Surg.* 12, 681–692
36 Herszberg, B. *et al.* (2006) Heaves, an asthma-like equine disease, involves airway smooth muscle remodeling. *J. Allergy Clin. Immunol.* 118, 382–388
37 Krug, N. and Rabe, K.F. (2008) Animal models for human asthma: the perspective of a clinician. *Curr. Drug Targets* 9, 438–442
38 Coleman, R.A. (1999) Current animal models are not predictive for clinical asthma. *Pulm. Pharmacol. Ther.* 12, 87–89
39 Corry, D.B. and Irvin, C.G. (2006) Promise and pitfalls in animal-based asthma research: building a better mousetrap. *Immunol. Res.* 35, 279–294
40 Wanner, A. (1990) Utility of animal models in the study of human airway disease. *Chest* 98, 211–217
41 Pabst, R. (2003) Animal models of asthma: controversial aspects and unsolved problems. *Pathobiology* 70, 252–254
42 Gelfand, E.W. (2002) Pro: mice are a good model of human airway disease. *Am. J. Respir. Crit. Care Med.* 166, 5–6 discussion 7-8
43 Persson, C.G. (2002) Con: mice are not a good model of human airway disease. *Am. J. Respir. Crit. Care Med.* 166, 6–7 discussion 8
44 Shapiro, S.D. (2006) Animal models of asthma: Pro: allergic avoidance of animal (model[s]) is not an option. *Am. J. Respir. Crit. Care Med.* 174, 1171–1173
45 Wenzel, S. and Holgate, S.T. (2006) The mouse trap: it still yields few answers in asthma. *Am. J. Respir. Crit. Care Med.* 174, 1173–1176 discussion 1176-1178
46 Persson, C.G. *et al.* (1997) The mouse trap. *Trends Pharmacol. Sci.* 18, 465–467
47 Shapiro, S.D. (2008) The use of transgenic mice for modeling airways disease. *Pulm. Pharmacol. Ther.* 21, 699–701
48 Canning, B.J. and Wright, J.L. (2008) Special section: animal models of asthma and chronic obstructive pulmonary disease. *Pulm. Pharmacol. Ther.* 21, 695–832
49 Hyde, D.M. *et al.* (2006) Asthma: a comparison of animal models using stereological methods. *Eur. Respir. J.* 15, 122–135
50 Martin, J.G. and Ramos-Barbon, D. (2003) Airway smooth muscle growth from the perspective of animal models. *Respir. Physiol. Neurobiol.* 137, 251–261
51 Brewer, J.P. *et al.* (1999) Genetic variability in pulmonary physiological, cellular, and antibody responses to antigen in mice. *Am. J. Respir. Crit. Care Med.* 160, 1150–1156
52 Shinagawa, K. and Kojima, M. (2003) Mouse model of airway remodeling: strain differences. *Am. J. Respir. Crit. Care Med.* 168, 959–967
53 Schneider, T. *et al.* (1997) Kinetics and quantitation of eosinophil and neutrophil recruitment to allergic lung inflammation in a brown Norway rat model. *Am. J. Respir. Cell. Mol. Biol.* 17, 702–712

54 Pauluhn, J. and Mohr, U. (2005) Experimental approaches to evaluate respiratory allergy in animal models. *Exp. Toxicol. Pathol.* 56, 203–234
55 Endpoints in asthma drug trials: what do they mean? *Drug Ther. Bull.* 44, 21–24
56 Kumar, R.K. *et al.* (2008) The 'classical' ovalbumin challenge model of asthma in mice. *Curr. Drug Targets* 9, 485–494
57 Lloyd, C.M. (2007) Building better mouse models of asthma. *Curr. Allergy Asthma Rep.* 7, 231–236
58 Nials, A.T. and Uddin, S. (2008) Mouse models of allergic asthma: acute and chronic allergen challenge. *Dis. Model. Mech.* 1, 213–220
59 Bosco, A. *et al.* (2010) Decreased activation of inflammatory networks during acute asthma exacerbations is associated with chronic airflow obstruction. *Mucosal Immunol.* 3, 399–409
60 Jackson, D.J. *et al.* (2008) Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. *Am. J. Respir. Crit. Care Med.* 178, 667–672
61 Holgate, S.T. (2010) A look at the pathogenesis of asthma: the need for a change in direction. *Discov. Med.* 9, 439–447
62 Holgate, S.T. *et al.* (2010) A new look at the pathogenesis of asthma. *Clin. Sci.* 118, 439–450
63 Holgate, S.T. (2002) Asthma: more than an inflammatory disease. *Curr. Opin. Allergy Clin. Immunol.* 2, 27–29
64 Cookson, W. (2004) The immunogenetics of asthma and eczema: a new focus on the epithelium. *Nat. Rev. Immunol.* 4, 978–988
65 Holgate, S.T. *et al.* (2007) Local genetic and environmental factors in asthma disease pathogenesis: chronicity and persistence mechanisms. *Eur. Respir. J.* 29, 793–803
66 Alberg, T. *et al.* (2009) Fine ambient particles from various sites in Europe exerted a greater IgE adjuvant effect than coarse ambient particles in a mouse model. *J. Toxicol. Environ. Health A* 72, 1–13
67 Calixto, M.C. *et al.* (2010) Obesity enhances eosinophilic inflammation in a murine model of allergic asthma. *Br. J. Pharmacol.* 159, 617–625
68 Konga, D.B. *et al.* (2009) Oxidative stress and antioxidant defenses in asthmatic murine model exposed to printer emissions and environmental tobacco smoke. *J. Environ. Pathol. Toxicol. Oncol.* 28, 325–340
69 Bartlett, N.W. *et al.* (2008) Mouse models of rhinovirus-induced disease and exacerbation of allergic airway inflammation. *Nat. Med.* 14, 199–204
70 Perlmutter, R.M. (2005) Roger Perlmutter on shaping Amgen's R&D strategy. Interview by Christopher Watson. *Drug Discov. Today* 10, 745–748
71 O'Byrne, P.M. *et al.* (2009) Provoked models of asthma: what have we learnt? *Clin. Exp. Allergy* 39, 181–192
72 Bucchieri, F. *et al.* (2002) Asthmatic bronchial epithelium is more susceptible to oxidant-induced apoptosis.. *Am. J. Respir. Cell. Mol. Biol.* 27, 179–185
73 Contoli, M. *et al.* (2006) Role of deficient type III interferon-lambda production in asthma exacerbations. *Nat. Med.* 12, 1023–1026
74 Wark, P.A. *et al.* (2005) Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. *J. Exp. Med.* 201, 937–947
75 Holgate, S.T. (2007) Epithelium dysfunction in asthma. *J. Allergy Clin. Immunol.* 120, 1233–1244 quiz 1245-1236
76 Nichols, J.E. and Cortiella, J. (2008) Engineering of a complex organ: progress toward development of a tissue-engineered lung. *Proc. Am. Thorac. Soc.* 5, 723–730
77 Song, J.W. *et al.* (2009) Microfluidic endothelium for studying the intravascular adhesion of metastatic breast cancer cells. *PLoS ONE* 4, e5756
78 Song, J.W. and Munn, L.L. (2010) Microfluidic platform for reproducing blood vessel microenvironment. *FASEB J.* 24 1031.1
79 Tourovskaia, A. *et al.* (2005) Differentiation-on-a-chip: a microfluidic platform for long-term cell culture studies. *Lab. Chip.* 5, 14–19
80 Tourovskaia, A. *et al.* (2006) Local induction of acetylcholine receptor clustering in myotube cultures using microfluidic application of agrin. *Biophys. J.* 90, 2192–2198
81 Ma, S.H. *et al.* (2005) An endothelial and astrocyte co-culture model of the blood–brain barrier utilizing an ultra-thin, nanofabricated silicon nitride membrane. *Lab. Chip.* 5, 74–85
82 Jang, K.J. and Suh, K.Y. (2010) A multi-layer microfluidic device for efficient culture and analysis of renal tubular cells. *Lab. Chip.* 10, 36–42
83 Chao, P. *et al.* (2009) Evaluation of a microfluidic based cell culture platform with primary human hepatocytes for the prediction of hepatic clearance in human. *Biochem. Pharmacol.* 78, 625–632
84 Lee, K.H. *et al.* (2010) Microfluidic synthesis of pure chitosan microfibers for bio-artificial liver chip. *Lab. Chip.* 10, 1328–1334
85 Domansky, K. *et al.* (2010) Perfused multiwell plate for 3D liver tissue engineering. *Lab. Chip.* 10, 51–58
86 Maguire, T.J. *et al.* (2009) Design and application of microfluidic systems for *in vitro* pharmacokinetic evaluation of drug candidates. *Curr. Drug Metab.* 10, 1192–1199
87 Kang, L. *et al.* (2008) Microfluidics for drug discovery and development: from target selection to product lifecycle management. *Drug Discov. Today* 13, 1–13
88 Tatosian, D.A. and Shuler, M.L. (2009) A novel system for evaluation of drug mixtures for potential efficacy in treating multidrug resistant cancers. *Biotechnol. Bioeng.* 103, 187–198
89 Huh, D. *et al.* (2007) Acoustically detectable cellular-level lung injury induced by fluid mechanical stresses in microfluidic airway systems. *Proc. Natl. Acad. Sci. U. S. A.* 104, 18886–18891
90 Huh, D. *et al.* (2010) Reconstituting organ-level lung functions on a chip. *Science* 328, 1662–1668
91 Sun, T. *et al.* (2010) On-chip epithelial barrier function assays using electrical impedance spectroscopy. *Lab. Chip.* 10, 1611–1617
92 Ressmeyer, A.R. *et al.* (2006) Characterisation of guinea pig precision-cut lung slices: comparison with human tissues. *Eur. Respir. J.* 28, 603–611
93 Cooper, P.R. *et al.* (2009) Airway mechanics and methods used to visualize smooth muscle dynamics *in vitro*. *Pulm. Pharmacol. Ther.* 22, 398–406
94 Chew, A.D. *et al.* (2008) Effects of allergen on airway narrowing dynamics as assessed by lung-slice technique. *Eur. Respir. J.* 31, 532–538
95 Delmotte, P. *et al.* (2010) Mechanisms of airway smooth muscle relaxation induced by beta2-adrenergic agonists. *Front. Biosci.* 15, 750–764
96 Booth, J.L. *et al.* (2004) Adenovirus type 7 induces interleukin-8 in a lung slice model and requires activation of Erk. *J. Virol.* 78, 4156–4164
97 Wohlsen, A. *et al.* (2003) The early allergic response in small airways of human precision-cut lung slices. *Eur. Respir. J.* 21, 1024–1032
98 Tliba, O. and Panettieri, R.A., Jr (2008) Regulation of inflammation by airway smooth muscle. *Curr. Allergy Asthma Rep.* 8, 262–268
99 Tliba, O. *et al.* (2008) Is airway smooth muscle the 'missing link' modulating airway inflammation in asthma? *Chest* 133, 236–242
100 van de Waterbeemd, H. and Gifford, E. (2003) ADMET *in silico* modelling: towards prediction paradise? *Nat. Rev. Drug Discov.* 2, 192–204
101 Epstein, M.M. (2004) Modeling allergic asthma: from *in vitro* assays to virtual patients. *Drug Discov Today: Dis. Models* 1, 387–394
102 Huang, S. *et al.* (2009) *In vitro* organ culture models of asthma. *Drug Discov Today: Dis. Models* 6, 137–144
103 Musante, C.J. *et al.* (2002) Small- and large-scale biosimulation applied to drug discovery and development. *Drug Discov. Today* 7 (Suppl. 20), S192–S196
104 Lewis, A.K. *et al.* (2001) The roles of cells and mediators in a computer model of chronic asthma. *Int. Arch. Allergy Immunol.* 124, 282–286
105 Flood-Page, P. *et al.* (2007) A study to evaluate safety and efficacy of mepolizumab in patients with moderate persistent asthma. *Am. J. Respir. Crit. Care Med.* 176, 1062–1071
106 Kips, J.C. *et al.* (2003) Effect of SCH55700, a humanized anti-human interleukin-5 antibody, in severe persistent asthma: a pilot study. *Am. J. Respir. Crit. Care Med.* 167, 1655–1659
107 Leckie, M.J. *et al.* (2000) Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 356, 2144–2148
108 Schwartz, L.B. *et al.* (2010) Current strategies in the management of hypereosinophilic syndrome, including mepolizumab. *Curr. Med. Res. Opin.* 26, 1933–1946
109 Kim, S. *et al.* (2010) Mepolizumab as a steroid-sparing treatment option in patients with Churg–Strauss syndrome. *J. Allergy Clin. Immunol.* 125, 1336–1343
110 Gleich, G.J. (2009) Anti-interleukin-5 therapy and severe asthma. *N. Engl. J. Med.* 360, 2577 author reply 2578
111 Pavord, I.D. *et al.* (2010) Mepolizumab in refractory eosinophilic asthma. *Thorax* 65, 370
112 Auffray, C. *et al.* (2010) An integrative systems biology approach to understanding pulmonary disease. *Chest* 137, 1410–1416
113 Trusheim, M.R. *et al.* (2007) Stratified medicine: strategic and economic implications of combining drugs and clinical biomarkers. *Nat. Rev. Drug Discov.* 6, 287–293
114 Canning, B.J. (2003) Modelling asthma and COPD in animals: a pointless exercise? *Curr. Opin. Pharmacol.* 3, 244–250
115 Epstein, M.M. (2006) Are mouse models of allergic asthma useful for testing novel therapeutics? *Exp. Toxicol. Pathol.* 57 (Suppl. 2), 41–44
116 Zosky, G.R. and Sly, P.D. (2007) Animal models of asthma. *Clin. Exp. Allergy* 37, 973–988
117 Perel, P. *et al.* (2007) Comparison of treatment effects between animal experiments and clinical trials: systematic review. *Br. Med. J.* 334, 197
118 Sibbald, W.J. (2000) An alternative pathway for preclinical research in fluid management. *Crit. Care* 4 (Suppl. 2), S8–S15
119 Ellis, L.M. and Fidler, I.J. (2010) Finding the tumor copycat. Therapy fails, patients don't. *Nat. Med.* 16, 974–975

120 Kaiko, G.E. and Foster, P.S. (2011) New insights into the generation of Th2 immunity and potential therapeutic targets for the treatment of asthma. *Curr. Opin. Allergy Clin. Immunol.* 11, 39–45

121 Borish, L.C. *et al.* (2001) Efficacy of soluble IL-4 receptor for the treatment of adults with asthma. *J. Allergy Clin. Immunol.* 107, 963–970

122 Corren, J. *et al.* (2010) A randomized, controlled, phase 2 study of AMG 317, an IL-4Ralpha antagonist, in patients with asthma. *Am. J. Respir. Crit. Care Med.* 181, 788–796

123 Oh, C.K. *et al.* (2010) Investigational therapeutics targeting the IL-4/IL-13/STAT-6 pathway for the treatment of asthma. *Eur. Respir. Rev.* 19, 46–54

124 Borish, L. (2010) IL-4 and IL-13 dual antagonism: a promising approach to the dilemma of generating effective asthma biotherapeutics. *Am. J. Respir. Crit. Care Med.* 181, 769–770

125 Elias, J.A. *et al.* (2003) Transgenic modeling of interleukin-13 in the lung. *Chest* 123 (Suppl. 3), S339–S345

126 Lee, C.G. *et al.* (2006) Transgenic modeling of transforming growth factor-beta(1): role of apoptosis in fibrosis and alveolar remodeling. *Proc. Am. Thorac. Soc.* 3, 418–423

127 Ochkur, S.I. *et al.* (2007) Coexpression of IL-5 and eotaxin-2 in mice creates an eosinophil-dependent model of respiratory inflammation with characteristics of severe asthma. *J. Immunol.* 178, 7879–7889

128 Kilkenny, C. *et al.* (2010) Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *PLoS Biol.* 8, e1000412

129 Bates, J.H. and Irvin, C.G. (2003) Measuring lung function in mice: the phenotyping uncertainty principle. *J. Appl. Physiol.* 94, 1297–1306

130 Glaab, T. *et al.* (2007) Invasive and noninvasive methods for studying pulmonary function in mice. *Respir. Res.* 8, 63

131 Irvin, C.G. and Bates, J.H. (2003) Measuring the lung function in the mouse: the challenge of size. *Respir. Res.* 4, 4

132 Sly, P.D. *et al.* (2004) Measuring lung function in murine models of pulmonary disease. *Drug Discov Today: Dis. Models* 1, 337–343

133 Bates, J. *et al.* (2004) The use and misuse of Penh in animal models of lung disease. *Am. J. Respir. Cell. Mol. Biol.* 31, 373–374

134 Lundblad, L.K. *et al.* (2002) A reevaluation of the validity of unrestrained plethysmography in mice. *J. Appl. Physiol.* 93, 1198–1207

135 Lundblad, L.K. *et al.* (2007) Penh is not a measure of airway resistance. *Eur. Respir. J.* 30, 805

136 Littman, B.H. and Williams, S.A. (2005) The ultimate model organism: progress in experimental medicine. *Nat. Rev. Drug Discov.* 4, 631–638

137 O'Connell, D. and Roblin, D. (2006) Translational research in the pharmaceutical industry: from bench to bedside. *Drug Discov. Today* 11, 833–838

138 Goodsaid, F.M. *et al.* (2010) Voluntary exploratory data submissions to the US FDA and the EMA: experience and impact. *Nat. Rev. Drug Discov.* 9, 435–445

139 Willmann, J.K. *et al.* (2008) Molecular imaging in drug development. *Nat. Rev. Drug Discov.* 7, 591–607

140 Moffatt, M.F. *et al.* (2010) A large-scale, consortium-based genomewide association study of asthma. *N. Engl. J. Med.* 363, 1211–1221

141 Combes, R.D. *et al.* (2003) Early microdose drug studies in human volunteers can minimise animal testing: proceedings of a workshop organised by Volunteers in Research and Testing. *Eur. J. Pharm. Sci.* 19, 1–11

142 LoRusso, P.M. (2009) Phase 0 clinical trials: an answer to drug development stagnation? *J. Clin. Oncol.* 27, 2586–2588

143 Wills-Karp, M. (2004) Interleukin-13 in asthma pathogenesis. *Immunol. Rev.* 202, 175–190

144 Berry, M.A. *et al.* (2004) Sputum and bronchial submucosal IL-13 expression in asthma and eosinophilic bronchitis. *J. Allergy Clin. Immunol.* 114, 1106–1109

145 Brightling, C.E. *et al.* (2003) Interleukin-4 and -13 expression is co-localized to mast cells within the airway smooth muscle in asthma. *Clin. Exp. Allergy* 33, 1711–1716

146 Saha, S.K. *et al.* (2008) Increased sputum and bronchial biopsy IL-13 expression in severe asthma. *J. Allergy Clin. Immunol.* 121, 685–691

147 Blease, K. *et al.* (2001) Therapeutic effect of IL-13 immunoneutralization during chronic experimental fungal asthma. *J. Immunol.* 166, 5219–5224

148 Bree, A. *et al.* (2007) IL-13 blockade reduces lung inflammation after Ascaris suum challenge in cynomolgus monkeys. *J. Allergy Clin. Immunol.* 119, 1251–1257

149 Grunig, G. *et al.* (1998) Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* 282, 2261–2263

150 Wills-Karp, M. *et al.* (1998) Interleukin-13: central mediator of allergic asthma. *Science* 282, 2258–2261

151 Zhu, Z. *et al.* (1999) Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. *J. Clin. Invest.* 103, 779–788

152 Kasaian, M.T. *et al.* (2007) Efficacy of IL-13 neutralization in a sheep model of experimental asthma. *Am. J. Respir. Cell. Mol. Biol.* 36, 368–376

153 Corren, J. *et al.* (2010) A randomized, controlled, phase 2 study of AMG 317, an IL-4Rα antagonist, in patients with asthma. *Am. J. Respir. Crit. Care Med.* 181, 788–796

154 Gauvreau, G.M. *et al.* (2008) The effects of IMA-638 on allergen induced airway responses in subjects with mild atopic asthma. *Eur. Respir. J.* 32 (Suppl. 52), 827s

155 Gomez, F.P. *et al.* (1999) Gas exchange response to a PAF receptor antagonist. SR 27417A, in acute asthma: a pilot study. *Eur. Respir. J.* 14, 622–626

156 Kasperska-Zajac, A. *et al.* (2008) Platelet-activating factor (PAF): a review of its role in asthma and clinical efficacy of PAF antagonists in the disease therapy. *Recent Pat. Inflamm. Allergy Drug Discov.* 2, 72–76

157 Hozawa, S. *et al.* (1995) Effects of a PAF antagonist, Y-24180, on bronchial hyperresponsiveness in patients with asthma. *Am. J. Respir. Crit. Care Med.* 152, 1198–1202

158 Singh, D. *et al.* (2007) Selective inducible nitric oxide synthase inhibition has no effect on allergen challenge in asthma. *Am. J. Respir. Crit. Care Med.* 176, 988–993

159 Woodside, D.G. and Vanderslice, P. (2008) Cell adhesion antagonists: therapeutic potential in asthma and chronic obstructive pulmonary disease. *BioDrugs* 22, 85–100

160 Norris, V. *et al.* (2005) Effect of IVL745, a VLA-4 antagonist, on allergen-induced bronchoconstriction in patients with asthma. *J. Allergy Clin. Immunol.* 116, 761–767

161 Riffo-Vasquez, Y. and Spina, D. (2002) Role of cytokines and chemokines in bronchial hyperresponsiveness and airway inflammation. *Pharmacol. Ther.* 94, 185–211

162 Haldar, P. *et al.* (2009) Mepolizumab and exacerbations of refractory eosinophilic asthma. *N. Engl. J. Med.* 360, 973–984

163 Nair, P. *et al.* (2009) Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. *N. Engl. J. Med.* 360, 985–993