CHAPTER I

INTRODUCTION

INTRODUCTION

Imagine working as a researcher at an independent research institute, a contract research organization with close connections to academia (www.chdr.nl). Recently, a new pharmaceutical company contacted your organization after they acquired a new compound with a mode of action possibly suitable for the treatment of asthma. Preclinical studies show promising results and expectations are high. Since the pharmaceutical company is small, it seeks external advice on how to perform a first in human study specifically, and on a drug development plan in general. As a clinical pharmacology researcher you are asked to advise the company how to proceed in demonstrating safety and unlock the blockbuster potential of the compound, under the constraint of limited time and budget.

This introduction describes several aspects that should be considered in the development of potential anti-asthmatic drugs as an example for drugs that may be developed for respiratory diseases. The same strategy could also be considered for other respiratory diseases such as cystic fibrosis, chronic obstructive pulmonary disease, but these disease entities and possible therapeutics are not discussed in this vignette case. The clinical features and pathophysiology of asthma, the added value of animal models, a general overview of asthma drug research and finally an overview of commonly uses biomarkers for asthma are described.

ASTHMA

EPIDEMIOLOGY ❦ Asthma is estimated to affect 300 million individuals worldwide [1;2]. The prevalence of asthma and associated allergic syndromes is increasing worldwide and the currently available drugs are not equally effective in all patients [3]. Hence there is still a demand for novel, targeted anti-asthma therapy.

CLINICAL CHARACTERISTICS ❦ Asthma is a chronic inflammatory disorder of the airways, often associated with atopy (allergy). Clinically, variability of symptoms and airway obstruction is the most striking phenomenon. The pathogenesis is still not fully elucidated, but chronic airway inflammation and airway hyper-responsiveness (AHR), an excessive response to triggers that have little effect in normal individuals, represent the key characteristics in the pathophysiology of asthma [4]. Along with these more or less dynamic features, structural changes of the airways (the so-called airway remodeling) occur early on in the disease, *Figure 1*.

All these asthma characteristics appear to be interrelated [5] and if the disease is persistently treated inadequately, this may result in the loss of asthma control and accelerated decline of lung function. Since none of these asthma features or underlying inflammatory mechanisms are present in healthy, non-asthmatic individuals, it is necessary to conduct a trial with a novel therapy on patients as soon as possible in early clinical proof of concept (POC) studies.

When a relevant allergen is inhaled by sensitized patients with allergic asthma, it can induce various airway responses [6]. An immediate or early asthmatic response (EAR) is characterized by a fall in the forced expiratory volume in one second (FEV1) of ≥ 15 % from baseline within 10 to 30 minutes following inhalation of the allergen. The EAR is an IgE-triggered phenomenon, mainly caused by mast cell release inducing acute bronchoconstriction, and usually resolves within one to three hours. In about 50 % of such patients an EAR is followed by a late asthmatic response (LAR), characterized by prolonged airway narrowing, often defined as a fall in FEV1 of ≥ 15 % occurring between three and eight hours post-allergen exposure in which TH2 lymphocytes, activated eosinophils, their pro-inflammatory and toxic products play a key role, *Figure 2*. These sequelae may last for several days or weeks and result in the development of the allergen-induced AHR.

PATHOPHYSIOLOGY OF ASTHMA ❦ Two main types of T helper lymphocytes have been characterized: TH1 and TH2. TH1 cells produce (amongst others) interleukin (IL)-2 and IFN-γ, which in general are critical in cellular defense mechanisms when responding to infection. TH2-cells generate a family of interleukins that can mediate allergic inflammation, *Figure 3* [7]. The TH2-cells produces IL-4 and IL-13 (causing B cell immunoglobulin (Ig) E production), IL-5 (causing eosinophilic inflammation) and IL-9 (promoting mast cells growth). Traditionally, it is thought that in atopic asthmatic patients, allergens tend to induce an unbalanced production of the TH2 type cytokines, but little of the TH1 cytokines.

NEW INSIGHTS ❦ For many years asthma was considered a straight forward disease driven by aberrant TH2 immunity. This hypothesis made it possible to understand classic allergic asthma, its association between atopy, eosinophilic lung inflammation, and the effect of corticosteroid treatments to reduce the TH2 type inflammation. However, it cannot explain why residual disease remains after optimized anti-inflammatory treatment. Also, numerous new targets for the treatment of asthma like key anti-interleukins 4 and 13 [8] and anti-interleukin 5 [9], have been based on this TH2 inflammation model but this approach has shown no or marginal

effect in the clinic. In addition, the TH2 inflammation model cannot explain some of the endophenotypes for asthma [10]. Asthma is nowadays recognized as a complex heterogeneous syndrome consisting of many different subtypes (E.G. intrinsic, extrinsic, seasonal, exercise induced, virally induced, aspirin sensitive, allergic, non-allergic, nocturnal and steroid resistant) each with different pathophysiology, expression of symptoms, response to treatment and prognosis. Lately, the role of the innate immune system with its Toll like receptors (TLR) and macrophages, originally thought of as a first defense against infections, has been more emphasized in relation to asthma [11;12].

TOLL LIKE RECEPTORS AND ASTHMA ❋ Before the immune system reacts, it must recognize the virus, bacterium or other infection. For the innate immune system, these recognitions are carried out by soluble elements (E.G. complement, binding proteins) or by pattern recognition receptors (PRRS) on macrophages, dendritic cells or polymorph nuclear leucocytes. The Toll like receptor is an example of a PRR recognizing lipopolysaccharides (LPS), a component of the outer membrane of Gram-negative bacteria. Provocation of the lung in asthma patients with LPS induces a predominantly neutrophilic type of inflammation, acute and chronic forms of airway obstruction and even airway remodeling [13]. More specific, bone marrow derived dendritic cells produce IL-12 in response to high doses of LPS, stimulating the TH1 response, *Figure 3*. This suggests that the role of other pathways, like the TH1 pathway, may play a more important role in the pathophysiology than originally acknowledged.

ADDED VALUE OF ANIMAL MODELS

The first β2 agonist for the treatment of asthma was introduced to the market in 1969 and corticosteroids were introduced since 1974. Since then, few new drugs have made it to the clinic. Multiple drugs performed well in preclinical animal models of asthma but did not live up to their promise in humans. Leukotriene antagonists (E.G. montelukast) and antibodies directed to immunoglobulin E (E.G. omalizumab) are an exception, even though both have restricted clinical indications.

There are several reasons for the limited utility of translating animal models to humans. It is suggested that asthma is a human disease only [14]. Several species and study designs have been used to try and mimic asthma. However, no animals, including those frequently used to study asthma, exhibit an asthma like syndrome that is similar to the disease in humans [15;16]. Moreover, many antigen challenge tests used in animals result in an acute inflammation phenotype and bypasses the etiology of asthma which develops over time through multiple step processes [17]. Even if longer duration models are used in animals with repeated exposure to allergens for many weeks, important clinical endpoints of human asthma do not develop such as chronic inflammation of the airway wall and airway remodeling [18;19].

THE DRUG DEVELOPMENT PROCESS FOR POTENTIAL NEW PULMONARY THERAPEUTICS

There are several possible classifications for the process of clinical drug development. Classically, drug development is considered to be a linear consecutive process in which drugs passes through four clinical phases of development [20], *Figure 4*. After each phase a Go/No Go decision is made where the drug progresses into later development, or the drug development program is discontinued. Generally, phase I trials, usually in healthy volunteers, are used to determine safety and sometimes also aim to investigate the pharmacokinetic profile of the drug (absorption, distribution, metabolization and excretion); phase II trials are used to get an initial understanding of efficacy and further explore safety in small numbers of patients; phase III trials are large, pivotal trials to determine safety and efficacy in sufficiently large numbers of patients and are often used to request market approval; and phase IV trials are post-approval trials and sometimes a requirement from the agencies for the evaluation of medicinal products.

The classification of drug development into proof of mechanism, proof of concept and proof of principle studies are related to the concept of these four phases. The underlying principle for these studies is use of biomarkers as surrogate endpoints [21]. In early development establishing the drug's effectiveness in the targeted disease population is not required, and instead surrogate endpoints are often used to guide decisions on further testing.

Proof of Mechanism (PoM). These studies often refers to early drug development in the preclinical phase (animal models), but sometimes this term is also used for testing pharmacodynamic effect of the drug in healthy volunteers. The intention is to show that the drug is available at the targeted site of action and that its interaction with the intended receptor results in a desired biological effect. It serves as an indication for the intended pharmacodynamic effect and is an important tool for

selection of appropriate dose for Proof of Concept studies. Proof of Concept (PoC) studies refers to clinical studies with a small group of patients. The aim of these studies is to show a useful clinical effect. Proof of Principle (PoP) studies are related to Phase III studies and are generally used for registration purposes. Some 15 years ago it was realized by the FDA and EMA that classification of the drug development process into four distinct phases provided an inadequate basis for classification of clinical trials as a single trial could occur in several phases. Furthermore, they concluded that the typical sequence is not applicable for each drug. Therefore they came up with the classification of studies using study objectives, summarized in *Table 1*.

Although the FDA and the EMA have abolished the use of the four-phase terminology for drug development, it is still widely applied amongst professional drug developers. The main reason for this is that this four-phase approach provides guidance for the planning of clinical development of any new drug. However, as this is likely to be a valid approach for drugs with low uncertainty regarding the development, it seems invalid for drugs with a high level of uncertainly. The uncertainty is based on the link between the molecular mechanism and its clinical effect, as well as its methodology to study it. It may be argued that a more useful model for drug development is the so called question based approach to drug development [22;23]. Question based drug developers make use of a logical progression of questions as shown in *Figure 5*. Does the drug get into the lungs? Does it reach the targeted receptors at its site of action? Will it affect the bronchodilation or its underlying inflammatory process in asthmatic patients? Does the drug have its pharmacological effect? For example, the mode of action of the new anti-asthmatic has to address the underlying endophenotype of asthma in the targeted patient population. What is the optimal dose with regards to meaningful clinical effects, and what is the therapeutic window? Other issues are whether the new anti-asthmatic drug has unwanted side effects, and how variations in the targeted asthmatic population, for instance genetic variations or lifestyle differences, affect the effectiveness of the drug. In addition, an investigation whether the applicable biomarkers and linkage markers are validated for this purpose can be deemed of value.

A schematic determination of objectives using this question based approach can be used to design a set of research questions to build the drug development plan. An integrated understanding of the fundamental principles of exposure at the site of action, target binding and expression of functional pharmacological activity determines the likelihood of the compound's potential survival in early phase trials, and improves the chance of progression to subsequent development phases. Questions that remain unanswered make up the development risk.

APPLICABLE BIOMARKERS IN ASTHMA DRUG DEVELOPMENT

A biological marker (biomarker) is a physical sign or laboratory measurement that can serve as an indicator of normal biological processes, pathophysiological processes or a pharmacological response to a therapeutic intervention. In asthma there are several validated pathophysiological and immunological biomarkers, and many others are being validated. In principle, all biological compounds of the asthma inflammatory cascade could serve as biomarkers. Ideally, a biomarker should have the following characteristics [24]:

- Clinical relevance: a clear relationship between the biomarker and the pathophysiological events leading to a well-defined clinical endpoint;
- Reliability and repeatability: the measurements of the biomarker should be precise and reproducible;
- Simplicity of sampling methodology, preferably via non- or semi-invasive sampling techniques, and measurement to promote widespread use; and,
- Sensitivity and specificity for treatment effects.
- Dose response relationship

Alongside safety data, it has become increasingly important to get as much information on the drug's potential efficacy as soon as possible in the early phases of drug development. Also, regulatory authorities have advocated the incorporation of validated biomarkers into early clinical studies to speed up drug development [25]. Therefore, in proof of pharmacology or proof of concept (POC) studies of asthma, biomarkers as read outs of pharmacological efficacy should be added. To obtain biomarkers containing tissue in asthma, bronchial biopsies are the 'gold standard'. However, this invasive technology requires trained staff and expensive equipment, and therefore it is increasingly being replaced by more patient-friendly, non- or semi-invasive sampling techniques, which enables evaluation of biomarkers for instance in blood, sputum or exhaled air.

In the last decade, several non-invasive airway sampling techniques have been validated, yielding several biomarkers in induced sputum and exhaled air to better characterize respiratory disease entities.

INDUCED SPUTUM ❋ During the standard procedure [26] sputum is induced by serial inhalations of hypertonic saline solutions. This semi-invasive method has been validated over the past 15 years and requires the combination of a patient's collaboration, investigator's expertise, a well-equipped lab and a certified cytopathologist. Obtained sputum samples are processed and can be divided into a solid phase

and a fluid phase containing soluble (bio)markers. The cell pellet is cyto-spinned, stained, and cell differentials are then counted [27]. Ample evidence exists that sputum eosinophil and neutrophil counts are reproducible biomarkers for allergic and non-allergic airway inflammation, allowing phenotyping and assessment of asthma severity [28;29]. Moreover, sputum eosinophil counts in patients with moderate to severe persistent asthma have been shown to be a superior guide of asthma control than traditional disease markers like symptom scores and lung function [30]. The fluid phase or the supernatant of the sputum can be analyzed by bioassays and many soluble markers can be quantified. This reflects on the disease severity and activity E.G. pro-inflammatory mediators, cytokines, chemokines, neuropeptides and growth factors [31]. The technique of sputum processing is currently being optimized to allow detection of a larger array of potential biomarkers in the supernatant. Novel methods including ultracentrifugation, sputum-dialysis and protease inhibition are being introduced and tested in combination with sensitive detection techniques, such as Luminex assays, proteomics and metabolomics.

EXHALED NITRIC OXIDE ❦ Exhaled nitric oxide (ENO) is a sensitive marker of acute airway inflammation and its measurement is increasingly applied to diagnose and monitor asthma [32]. Over the past decades various methods have been reported to measure ENO. The currently recommended ENO sampling method is performed by validated chemoluminescence analyzers during a single-breath exhalation against a fixed resistance, allowing reproducible (online) measurements [33]. This is a simple and patient-friendly procedure allowing serial measurements even in children. More recently, another device has been introduced for online ENO measurements: the handheld electromechanic analyzer Niox Mino® whose measurements are reproducible and in agreement with the chemoluminescence analyzers [34]. In patients with allergic asthma uncontrolled by inhaled corticosteroids (ICS), ENO has been found to correlate with sputum eosinophils, and hence may qualify as a biomarker of asthma control [35]. Indeed, ICS and other anti-eosinophil therapies, including leukotriene modifiers and anti-IgE, dose-dependently reduced ENO [36;37]. In the same way, several tapering studies have demonstrated that loss of asthma control is associated with an increase in ENO [38]. Hence, ENO is increasingly applied as a biomarker in the diagnosis, treatment monitoring and clinical trials of asthma.

EXACERBATION MODELS ❦ Exacerbation models are useful tools in clinical POC studies for asthma [39]. The crux of these models consists of a validated stimulus that is capable of inducing a reproducible, more or less specific, inflammatory response within the asthmatic airways (*Figure 2*). The endpoint measurements in such studies are the maximum early and late decrease in FEV1, and the areas under the curve for the early response (EAR; 0-2 H post challenge) and the late response (LAR, 3-8 hour post challenge).

The most commonly used exacerbation model for the investigation of potential anti-inflammatory therapy is the allergen inhalation challenge. The reproducibility in this model is high [40]. In a cross over designed study, less than 10 subjects are needed to have 95% power for a 50% reduction of percent of eosinophils [41]. Applying the allergen challenge is revealed as an overall good predictor regarding a drug's efficacy in asthma.

Methacholine has a direct effect on the airway smooth muscle via the muscarine-3 receptor. Its challenge test is the gold standard to quantify airway hyper responsiveness (AHR) by PC20. However, the test is less related to inflammation [42].

Another reproducible model inducing bronchoconstriction and airway hyper responsiveness (AHR) in healthy volunteers or asthmatic patients is the inhalation of lipopolysaccharide (LPS) [43], which causes neutrophilic inflammation [44;45]. Like allergen challenges, LPS challenges require close safety monitoring for airway and systemic effects. There are many more exacerbations models utilizing exposure to virus, ozone, adenosine 5 monophosphate, ETC. These models are not described in detail as in this thesis only the allergen and methacholine challenges were applied.

IN CONCLUSION ❦ For rapid and optimal evaluation of an investigative drug's clinical efficacy, the use of both clinical measures and biomarkers is advocated. For asthma research with potential anti-inflammatory compounds, this could indicate the combination of an exacerbation model (E.G. allergen or LPS challenge) with validated noninvasive airway sampling techniques (ENO, sputum induction). In addition, drug development programs should proceed in a much more adaptive manner, using a question based approach.

The aim of this thesis is to discuss and highlight several aspects that should be considered in the development of potential new respiratory therapeutics. In early clinical research the goal is to bridge preclinical development of potential new drugs into successful next phase studies. Therefore, early phase clinical studies are designed generate understanding of clinical characteristics of the drug, to determine its safety and to predict effectiveness in the targeted population. Information about this is often obtained by effective integration of modern technologies and tools like biomarkers into clinical development plans.

This thesis consists of two main parts; it covers biomarker development and evaluation in section 1 and the early clinical development of a new anti-asthmatic drug in section 2.

SECTION 1

BIOMARKER DEVELOPMENT AND EVALUATION

CHAPTER 2 In this chapter the development of a novel method to evaluate well-known and unknown biomarkers is described. By applying an allergen challenge in patients with clinical stable asthma, a large set of inflammatory TH2 derived markers obtained from induced sputum were evaluated. The ability to quantify changes in these cytokines in sputum after an allergen challenge could be useful to assess effects of anti-asthma therapeutics.

CHAPTER 3 Microarray assessment of gene expression in induced sputum obtained after allergen challenge was applied in the same study as described in chapter 2. Using this method, it was evaluated whether an RNA signature could be identified from induced sputum following an inhaled allergen challenge, whether a gene signature could be modulated by limited doses of inhaled fluticasone, and whether these genes would correlate with the clinical endpoints measured in this study.

CHAPTER 4 Fractional exhaled nitric oxide FeNO is a sensitive marker of acute airway inflammation. However, it has been shown that FeNO levels may be reduced after sputum induction by hypertonic saline in asthmatics and healthy controls. It is unknown if this phenomenon also occurs in asymptomatic chronic smokers, a population at risk to develop COPD. This was investigated and described in chapter 4.

CHAPTER 5 The reproducibility of the measurements of many soluble mediators in the supernatant of hypertonic saline-induced sputum and serum is still unknown. This hampers their use in clinical trials as possible read-out for treatment effect. Therefore we evaluated the reproducibility of a specific panel of soluble biomarkers in sputum and serum on healthy non-smokers and asymptomatic chronic smokers and this is described in chapter 5.

SECTION 2

CLINICAL STUDIES WITH A NEW ANTI-ASTHMATIC DRUG

CHAPTER 6 Chapter 6 describes the development process of a potential new anti-asthmatic drug, the combined PDE 3/4 inhibitor RPL554. The purpose of this first in man study was to identify a potentially effective dose and to assess in an early stage whether RPL554 possesses bronchodilative, bronchoprotective and anti-inflammatory properties in patients with allergic asthma and rhinitis. To this purpose a step by step adaptive design was used in which a safe dose of RPL554 was selected in healthy volunteers, a potentially effective dose was selected in patients with allergic asthma and finally, RPL554 effects were evaluated in more depth in both patients with allergic asthma and patients with allergic rhinitis. The use and evaluation of several technologies and biomarkers while using an adaptive study design are described in chapter 6.

CHAPTER 7 The next step in the development of RPL554 was to assess its effectiveness after repeated administrations. In this chapter the results on lung function are presented after repeated daily dosing of RPL554 for several consecutive days in order to evaluate the sustainability of effect on FEV1.

CHAPTER 8 Chapter 8 covers the discussion and conclusion section. It includes a critical evaluation of the biomarker selection and drug development program for RPL554.

Table 1 Classification of study objectives according to the EMA clinical guide guidance

Type of Study	Objective of Study	Study Examples
Human Pharmacology	Assess tolerance Define/describe PK and PD Explore drug metabolism and Drug interactions Estimate activity	Dose-tolerance studies Single and multiple dose PK and/or PD studies Drug interaction studies
Therapeutic Exploratory	Explore use for the targeted indication Estimate dosage for subsequent studies Provide basis for confirmatory study design, endpoints, methodologies	Earliest trials of relatively short duration in well- defined narrow patient populations, using surrogate or pharmacological endpoints or clinical measures. Dose-response exploration studies
Therapeutic Confirmatory	Demonstrate/confirm efficacy Establish safety profile Provide an adequate basis for assessing the benefit/risk relationship to support licensing Establish dose-response relationship	Adequate, and well controlled studies to establish efficacy Randomized parallel dose response studies Clinical safety studies Studies of mortality/ morbidity outcomes Large simple trials Comparative studies
Therapeutic Use	Refine understanding of benefit/ risk relationship in general or special populations and/or environments Identify less common adverse reactions Refine dosing recommendation	Comparative effectiveness studies Studies of mortality/morbidity outcomes Studies of additional endpoints Large simple trials Pharmaco-economic studies

Figure 1 Schematic relationship among asthma characteristics

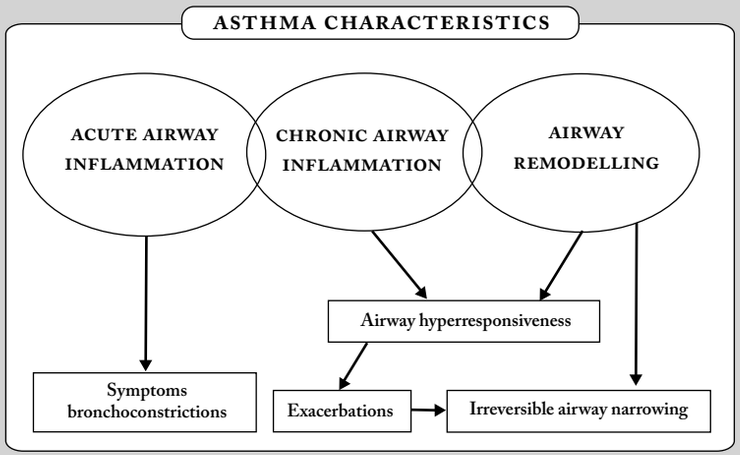


Figure 2 Early and late airway response after inhalation of grass pollen (this thesis)

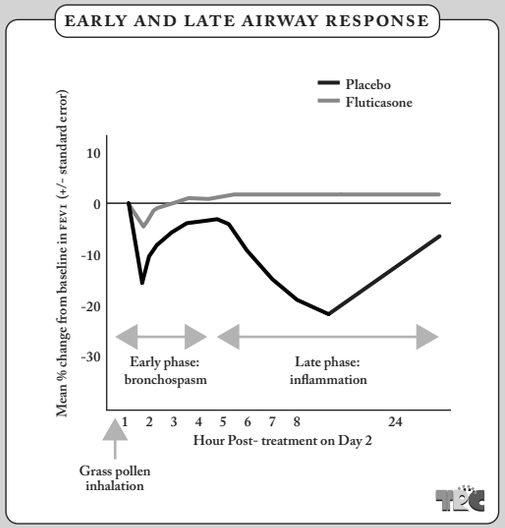


Figure 3 Pathogenesis of asthma

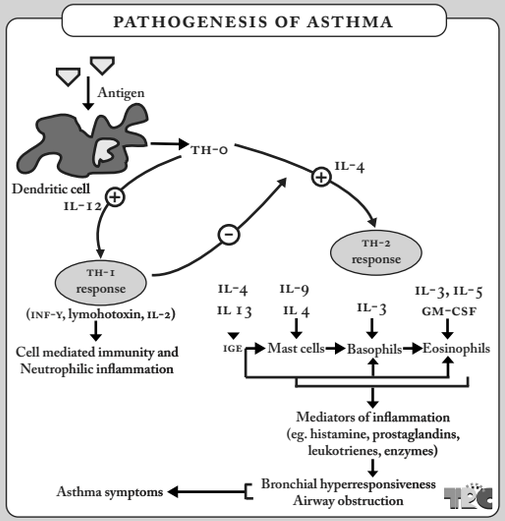


Figure 4 The classical drug development plan

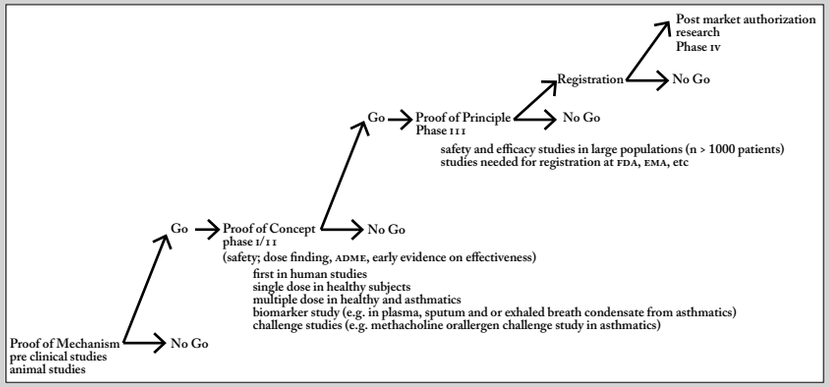
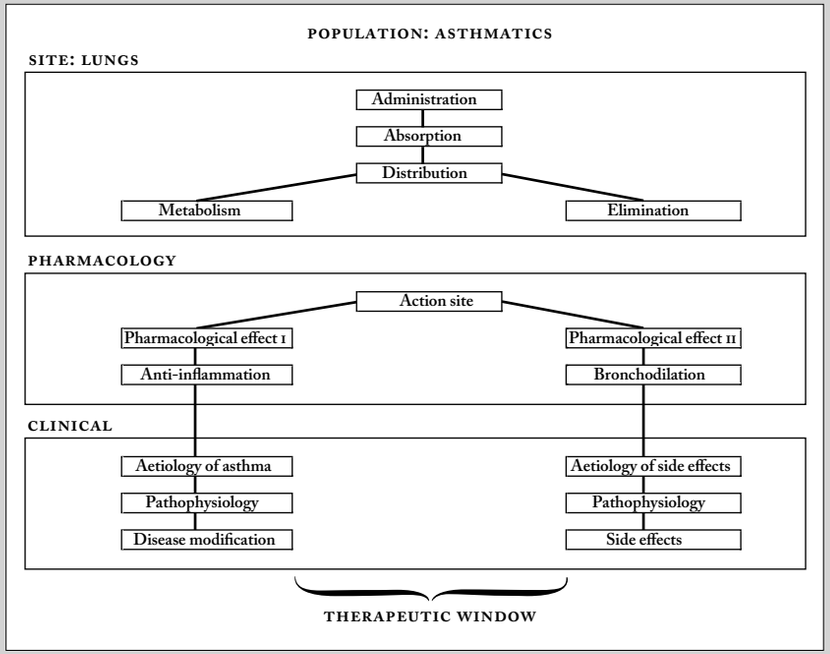


Figure 5 Schematic determination of objectives using the question based approach to drug development (Figure is reproduced and adapted with permission from reference [22])



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