Bridging the gap between science and clinical efficacy:

Physiology, imaging and modeling of aerosols in the lung

Chantal Darquenne, PhD[1*], John S. Fleming, PhD[2,3], Ira Katz, PhD[4,5], Andrew R. Martin, PhD[6], Jeffry Schroeter,PhD[7],Omar S. Usmani, MD[8], Jose Venegas, PhD[9] and Otmar Schmid, PhD[10,11*]

[1]Department of Medicine, University of California, San Diego, La Jolla, CA, [2]National Institute of Health Research Biomedical Research Unit in Respiratory Disease, Southampton, UK, [3]Department of Medical Physics and Bioengineering, University Hospital Southampton NHS Foundation Trust, Southampton, UK, [4]Medical R&D, Air Liquide Santé International, Centre de Recherche Paris-Saclay, Jouy-en-Josas, France. [5]Department of Mechanical Engineering, Lafayette College, Easton, PA, USA, [6]Department of Mechanical Engineering, University of Alberta, Edmonton, AB, Canada, [7]Applied Research Associates, Inc., Raleigh, NC USA, [8]Airway Disease Section, National Heart and Lung Institute, Imperial College London & Royal Brompton Hospital, London, United Kingdom, [9]Department of Anesthesia (Bioengineering), MGH/Harvard, Boston, Massachusetts, [10]Comprehensive Pneumology Center (CPC), Member of the German Center for Lung Research, Munich, Germany [11]Institute of Lung Biology and Disease, Helmholtz Zentrum München – German Research Center for Environmental Health, Neuherberg, Germany.

*****Corresponding authors

Running title: From aerosol science to clinical efficacy

Contact information: Chantal Darquenne, Ph.D.

Department of Medicine, University of California, San Diego

9500 Gilman Dr. mail code 0623A

La Jolla, CA 92093-0623

USA

Phone 858-534-9171, Fax: 858-534-4812

Email: cdarquenne@ucsd.edu

ABSTRACT

Development of a new drug for the treatment of lung disease is a complex and time consuming process involving numerous disciplines of basic and applied sciences. During the 2015 Congress of the International Society for Aerosols in Medicine, a group of experts including aerosol scientists, physiologists, modelers, imagers and clinicians participated in a workshop aiming at bridging the gap between basic research and clinical efficacy of inhaled drugs. This paper summarizes the current consensus on the topic. It begins with a short description of basic concepts of aerosol transport and a discussion on targeting strategies of inhaled aerosols to the lungs. It is followed by a description of both computational and biological lung models, and the use of imaging techniques to determine aerosol deposition distribution (ADD) in the lung. Finally, the importance of ADD to clinical efficacy is discussed. Several gaps were identified between basic science and clinical efficacy. One gap between scientific research aimed at predicting, controlling, and measuring ADD and the clinical use of inhaled aerosols is the considerable challenge of obtaining, in a single study, accurate information describing the optimal lung regions to be targeted, the effectiveness of targeting determined from ADD (and potentially subsequent dispersion), and some measure of the drug's effectiveness. Other identified gaps were the language and methodology barriers that exist among disciplines along with the significant regulatory hurdles that need to be overcome for novel drugs and/or therapies to reach the marketplace and benefit the patient. Despite these gaps, much progress has been made in recent years to improve clinical efficacy of inhaled drugs. Also, the recent efforts by many funding agencies and industry to support multidisciplinary networks including basic science researchers, R&D scientists and clinicians will go a long way to further reduce the gap between science and clinical efficacy.

Keywords: Aerosol deposition distribution, *in-silico* lung modeling, biological lung models, PET, SPECT

List of abbreviations:

ADD: aerosol deposition distribution (regional distribution of the deposited aerosol in the lung)

AR: anatomical region

CFD: computational fluid dynamics

CT: Computed Tomography

DPI: dry powder inhaler

ET: extra-thoracic

FDA: federal drug administration

FEV_1: forced expired volume in 1 second

FRC: functional residual capacity

GI: gastro-intestinal

HRCT: High Resolution Computed Tomography

ICS: inhaled corticosteroids

ISAM: International Society for Aerosols in Medicine

LABA: long-acting β2-agonist

LAM: long acting muscarinic antagonist

^{13}N: nitrogen-13

PET: Positron Emission Tomography

pMDI: pressurized metered dose inhaler

P: pulmonary

ROI: region of interest

SPECT: Single Photon Emission Computed Tomography

TB: tracheobronchial

^{99m}Tc: Technetium-99m

VIM: voxel influence matrix

1. INTRODUCTION

Aerosol inhalation is a well-established means of delivering drugs to the lungs of patients. For treatment of lung diseases it requires a smaller dose than oral or intravenous administration; it minimizes systemic effects and has a more rapid onset of action than through other delivery routes. Aerosol inhalation has therefore the potential to maximize therapeutic effects and minimize side effects. However, there are several factors to consider for optimal treatment of the lungs. These include aerosol characteristics, breathing patterns, geometrical factors (i.e lung morphology), disease state, pharmacokinetics and drug-cell interactions (pharmacodynamics) to name a few. The aerosol characteristics are mainly determined by the drug formulation and the inhalation device. All other aspects are – at least to some degree - patient-specific and may depend on age, sex, type of disease and/or severity of disease[1,2]. This has stimulated recent efforts towards more personalized therapeutic approaches aiming at optimized pulmonary drug delivery and selection of the most effective type of drug for any given patient[3].

Delivering inhaled drugs preferentially to the diseased site of the lung is the prime objective of the science of pulmonary aerosol deposition. While the treatment of asthmatic patients requires drug delivery to the bronchial and bronchiolar airways, emphysema patients may also benefit from drugs delivered to the alveolar region. Hence, not only the total pulmonary drug dose, but also the regional distribution, serial (proximal vs peripheral) and parallel (among anatomical regions), of the lung-deposited aerosol, henceforth referred to as aerosol deposition distribution (ADD), is a key factor for the clinical success of an inhalation therapy. Optimized ADD is expected to increase drug effectiveness and reduce drug cost, side effects and treatment times.

During the 2015 Congress of the International Society for Aerosols in Medicine (ISAM), a group of experts including aerosol scientists, physiologists, modelers, imagers and clinicians participated in a workshop aiming at bridging the gap between basic research and clinical

efficacy of inhaled drugs. This paper summarizes the current consensus on the topic as it was discussed at the workshop.

2. BASICS OF AEROSOL TRANSPORT AND DEPOSITION IN THE LUNGS

In the human lung, the airways form a dichotomous tree where each airway gives rise to two daughter branches. Because the airway tree needs to fill a space matching the shape of the chest cavity, the number of generations needed to reach the alveolar sacs from the trachea is not the same throughout the lungs but varies between 18 and 30[4]. Even though the airways become gradually shorter and narrower with each generation, the increasing number of airways with each generation number leads to a large increase in total airway cross-section towards the lung periphery. An important consequence in terms of aerosol transport is that this cross-sectional increase results in a large decrease in airflow velocity from the first few airway generations down to the lung periphery. Accordingly, even with the decrease in airway length from proximal to peripheral airways, the residence time in each generation increases with each generation (Figure 1).

INSERT FIGURE 1 HERE [5]

The deposition of inhaled particles occurs mainly by the mechanisms of inertial impaction, gravitational sedimentation and Brownian diffusion and to a lesser extent by interception, turbulent mixing and electrostatic precipitation. A detailed description of these mechanisms can be found elsewhere[1,6]. Briefly, inertial impaction results from the inability of particles to follow sudden changes in gas flow direction and is a primary mechanism for particles larger than 5 µm; gravitational sedimentation results from the settling of the particles under the action of gravity and significantly affects the deposition of 1 to 8 µm-diameter particles; and Brownian diffusion is the dominant mechanism of deposition for particles less than 0.5 µm in diameter. As inertial

impaction is a velocity-dependent mechanism, deposition by this mechanism occurs preferentially in the first few generations of airways where gas and particle velocity are high. Inversely, deposition by gravitational sedimentation and Brownian diffusion, which are time-dependent mechanisms, is most efficient in the lung periphery where airspace size is small and residence time high (Figure 1).

Although often neglected in aerosol deposition analyses, turbulent flows, electrical charges and particle shape can also significantly affect overall deposition. Turbulent mixing refers to the irregular fluctuations or mixing undergone by the fluid in a turbulent regime. Turbulent flows can be described in terms of their mean values over which are superimposed the fluctuations. Such flows generally occur at the glottic constriction and persist over several airway generations. Deposition due to turbulent mixing results from the flow fluctuations as opposed to deposition by inertial impaction that is affected by the mean flow. Space charge forces (the repulsion between charged particles in an aerosol cloud) and/or image charge forces (the attraction between a charged particle and its image charge on an electrically conducting surface) lead to deposition by electrostatic precipitation. Finally, interception refers to particles coming onto contact with the airway wall because of their shape and size. While negligible for spherical particles such as liquid droplets created by nebulizers, this mechanism becomes important for elongated particles such as fibers.

3. TARGETING APPROACHES

An impressive body of work, now spanning back several decades, has been conducted to describe and predict regional deposition patterns (or aerosol deposition distribution) for inhaled aerosols. These empirical, analytical, and computational models build on our fundamental knowledge describing various mechanisms through which aerosol particles come to deposit within upper, central, and/or peripheral airways of the respiratory tract. Naturally, such

fundamental understanding of deposition mechanisms has been explored extensively for the purpose of optimizing delivery of pharmaceutical aerosols to the lungs, and to targeted sub-regions within the lungs, most often the central or peripheral airways. Many improved aerosol formulations and delivery devices currently in development, or recently arrived on the market, offer much greater efficiency of delivery to the lungs than do traditional pressurized metered dose inhalers (pMDIs), dry powder inhalers (DPIs), and nebulizers. These improvements are the result of significant investment in research and development programs enabling the application of complex and interdisciplinary science, including aerosol mechanics underlying regional deposition patterns. However, as will be discussed below, there exist some important caveats that have combined to limit the extent to which targeting approaches that bear considerable promise have found their way into commercial aerosol drug delivery systems and into clinical practice.

The accurate description of aerosol deposition processes through mechanistic *in silico* and experimental models permits the influence of various parameters on regional deposition patterns to be explored. Two parameters for which effects on regional deposition pattern are particularly well established are the particle aerodynamic diameter and inhalation maneuver. Manipulation of these two parameters for the purpose of regional targeting is discussed below along with the potential use of aerosol bolus and of heliox (helium-oxygen gas mixture) for targeting peripheral and poorly-ventilated lung regions. Various additional targeting approaches, including use of intracorporeal nebulizing catheters[7,8], non-spherical particles[9], and application of external magnetic fields[10-12] are not discussed herein, but have been recently reviewed elsewhere[13].

3.1. Total lung deposition

Administering therapeutic agents directly to the lungs through inhalation is in itself an example

of targeted drug delivery. Improved lung targeting may be achieved by reducing upper airways deposition, such that a greater fraction of the emitted dose reaches the lungs. Reducing upper airways deposition also reduces intersubject and intrasubject variability in lung dosing[14]. Conventional pMDIs and DPIs typically deposit drug in high fraction in the mouth-throat airways, resulting in lung doses well below 50% of the nominal dose, with considerable variability. In contrast, introduction of new formulation and delivery technologies over the past decade has enabled more efficient targeting of the lungs from single-breath inhalers[15-17]. Existing empirical correlations to predict mouth-throat deposition (as described below) are not applicable to pMDI aerosols that enter the oral cavity as a plume of rapidly decelerating and evaporating propellant droplets. For pMDIs, the influence of inspiratory flow rate on mouth-throat and lung deposition has been shown to vary from one device/formulation to another[18]. Empirical correlations must also be applied carefully for predicting mouth-throat deposition for DPIs, owing to flow rate dependence of fluidization and deagglomeration of many DPI aerosols, as well as to effects of unsteady inhalation flow rate on mouth-throat deposition[19]. The complex processes of aerosol formation and behavior within and immediately following emission from pMDIs and DPIs also render numerical simulation difficult and computationally demanding. For both pMDIs and DPIs, it is preferable to perform *in vitro* experiments in realistic or idealized mouth-throat geometries[20] in order to estimate *in vivo* lung doses for a given device/formulation combination. For nebulizers, where fine droplets are well entrained in the inhaled airflow, deposition correlations and models tend to be more reliable[21], provided hygroscopic behavior of the nebulized aerosol is included in the model, or may be safely neglected[22].

3.2. Regional lung deposition

Many inhaled therapies are developed under the assumption that, in addition to the total dose delivered to the lungs, the regional deposition pattern within the lungs is an important determinant of efficacy. For example, both asthma and COPD have been characterized by

inflammation affecting the small, peripheral airways; therefore, targeting deposition of inhaled corticosteroids to small airways may improve treatment. In other circumstances, where the inhalation route is used for systemic delivery, targeting deposition to the alveolar lung region may be used to enhance drug absorption efficiency. As discussed below, regional targeting that can be achieved using aerodynamic particle size alone is limited, with significant overlap in deposition efficiencies between lung regions. Furthermore, for any given particle size, regional deposition will additionally be influenced by an individual patient's lung volume, airway geometry, disease state, and breathing pattern (or inhalation maneuver). Accordingly, strategies to target central or peripheral lung regions with precision will generally need to incorporate some form of control over patient breathing in addition to particle size.

3.3. Parameters affecting ADD

3.3.1. Aerodynamic Size

Inertial impaction is the primary mechanism of aerosol deposition in the upper and central airways (Figure 1). Accordingly, those factors that influence impaction affect the fraction of aerosol that deposits in the upper and central airways versus that which penetrates to the peripheral lung regions (typically to deposit by sedimentation). The aerodynamic particle size is a key determinant of inertial impaction efficiency; targeting deposition to the central or peripheral airways using larger or smaller aerodynamic particle sizes, respectively, is well entrenched in aerosol medicine. This said, the refinement in regional targeting that can be achieved through variation of aerodynamic size alone is coarse, with considerable overlap in deposition between upper and central airways, and/or central and peripheral airways for a given aerodynamic size[(23)]. In addition, pharmaceutical aerosols are generally polydisperse, spanning a range of aerodynamic sizes, which tends to further spread deposition between upper, central, and peripheral airways. Variability in regional deposition efficiencies between patients with different underlying airway geometries and varying manifestation of disease, potentially inhaling at

different flow rates, will also influence regional deposition patterns at a given aerodynamic particle size. Accordingly, while aerodynamic size is clearly a critical factor in determining regional deposition, targeting deposition by aerodynamic size alone has limitations, and may jeopardize the clinical success of inhaled therapies with steep dose-response relationship or narrow therapeutic index.

3.3.2. Controlled ventilation and bolus aerosol delivery.

Along with particle size, the inhalation flow rate has long been identified as a primary determinant of inertial impaction in the upper and central airways. Instructing patients to inhale at low flow rates has been shown to shift deposition from more proximal to more distal airways[24-26]. Unfortunately, for DPIs and pMDIs use of low inhalation flow rates to reduce inertial impaction is typically compromised by the requirement of reasonably high flow rates needed to fluidize and deaggregate dry powders[27,28], or by complex interaction between high-speed droplet clouds emitted from pMDIs with enveloping air flow[18,29,30]. Independent of the inhalation flow rate, patients are commonly instructed to perform a breath-hold for several seconds after inhaling, so as to allow aerosols additional time to settle under the influence of gravity in peripheral airways[31].

For nebulizers used to deliver aerosols during controlled tidal breathing, there exists greater opportunity to manipulate regional deposition patterns by varying the breathing pattern and employing the aerosol bolus technique. Commercial devices have been developed to guide or control patient breathing through nebulizers[32,33]. It should be understood that both central and peripheral deposition fractions, as well as their uniformity among parallel lung regions, will depend on an individual subject's lung volume and airway geometry, thus the effectiveness of any prescribed inhalation pattern (or particle size) in achieving regional targeting will be variable between subjects. Accordingly, definition of a single set of targeting parameters suitable for all

subjects within a large population is not possible, at least with current methods[34]. However, enhanced delivered drug dose, reduced treatment times and reduced variability in lung-delivered dose amongst patients have been observed for inhalation devices with controlled breathing[32]. A reduction in dose variability combined with enhanced pulmonary deposition may also decrease the number of patients required to assess efficacy of new pharmaceutical compounds in clinical trials.

To further improve upon regional targeting that can be achieved by varying aerodynamic particle size and inhalation pattern, bolus delivery of aerosol during only a portion of the total inhalation time has been employed[32,35]. Limiting aerosol administration to the early portion of inhalation allows inhaled fine particles time to transit through the conducting upper and central airways to reach peripheral lung regions, but under severely constricted patchy conditions inhaled particles may preferentially reach well-ventilated regions. Indeed, poorly-ventilated regions tend to receive a greater fraction of ventilation late in inhalation, as the driving pressure gradient between the airway opening and other, better-ventilated regions decreases during filling. Conversely, administering a bolus of larger particles later in the breath may be used to limit their penetration into the peripheral airways, thereby improving targeting to central airways and also preferentially reach airways leading to poorly ventilated regions.

3.3.3. Hygroscopicity.

Unlike stable aerosols commonly selected for use in laboratory deposition experiments, aerosol particles or droplets inhaled from drug delivery devices can undergo transient size changes that arise due to evaporation of propellants or solvents, and/or to condensation of water vapor from the warm, humid environment of the respiratory tract[22,36]. Longest, Hindle, and colleagues[37,38] have proposed to take advantage of such effects so as to allow aerosol sizes to adjust in transit through the respiratory tract, thereby promoting deposition in desired lung regions. Approaches

to advantageously spur transient hygroscopic size changes have also been analyzed by Javaheri and Finlay[39]. In brief, inhaled submicrometer particles that grow by condensation as they travel from upper to central to peripheral airways will tend to deposit by sedimentation in distal regions while avoiding upstream filtering due to impaction in more proximal regions. Without condensational growth, these submicrometer particles would be exhaled in high fraction. Promoting condensational growth therefore has potential to improve the precision of targeting to peripheral lung regions over more conventional approaches relying on stable aerosols combined with controlled ventilation.

3.3.4. Carrier gases

Helium-oxygen gas mixtures (hereafter referred to as heliox) have been used as a respiratory therapy for decades, often including aerosol inhalation[40]. The differences in the physical properties of heliox compared to air (lower density, higher viscosity and higher mean free path) have a direct effect on the mechanics of particle motion and deposition, but also on the fluid mechanics of the gas that can indirectly change the aerosol deposition distribution in the respiratory tract[41].

When breathing heliox rather than air, particle deposition tends to occur deeper in the respiratory tract[42,43] and ADD tends to be more homogeneous in particular in obstructed lungs[41]. On one hand, the lower density of heliox compared to air reduces the extent of turbulent flow, and as such reduced extra-thoracic (ET) deposition due to turbulent mixing[42]. On the other hand, the higher viscosity of heliox compared to air tends to reduce impaction of >1µm particles in the tracheobronchial (TB) region by keeping particle paths aligned with flow streamlines. For <1 µm particles, the larger mean free path of heliox results in a lower effective viscosity for the particles, i.e particles are less likely to follow flow streamlines. As a result, deposition of these small particles might be increased, especially in the lung periphery where

sedimentation and diffusion dominate. In terms of flow, the lower gas density results in lower inertial flow losses (as these are proportional to gas density) that can lead to a more homogenous flow in diseased lungs suggesting more uniform ADD[41].

The use of heliox has shown promising clinical results in some studies even though physiological studies conducted to measure the effect of carrier gas (air versus heliox) on aerosol deposition have lead to conflicting results[44-46]. For example, Kim and colleagues have shown that nebulized racemic epinephrine delivered with helium-oxygen inhalation therapy via high-flow nasal cannula was associated with a greater degree of clinical improvement compared with that delivered by oxygen among infants with bronchiolitis[47]. Kress et al. showed that during acute asthma exacerbations, albuterol nebulized with heliox provided a larger improvement in lung function as measured by FEV_1 when compared to albuterol nebulized with oxygen[48]. However, to this date, clinical trials on the use of heliox in inhalation therapy have not been definitive[49].

While heliox-based inhalation therapies can modify regional aerosol deposition patterns, as noted above, consistent improvement in clinical outcomes has not been established. The inconsistent clinical results reported suggest that the use of heliox for inhalation therapy may lead to improvements for only selected medications that have dose responses sensitive to regional deposition patterns. Furthermore, the existence of non-responders (see Figure 2) calls for the development of techniques (such as SPECT and other imaging technologies; see section 5 below) for easy and quick determination of patients, which will respond positively to treatment. These steps would tend to optimize the cost-benefit ratio, making helium-oxygen a more viable and widely used option for improving the effectiveness of inhalation therapies.

INSERT FIGURE 2 HERE

3.4. Limitations, perspectives and relevance for bridging the gap between science and clinics

Extensive efforts have been made in research and development to improve lung targeting and regional targeting within the lungs. Generally speaking, formulations and devices become increasingly complex (and costly) as the level of refinement in targeting increases. Therefore, delivery technologies must be carefully selected and matched to a given active ingredient and intended use. Devices that guide or control patient breathing during inhalation drug delivery offer potential to target deposition to central or peripheral airways beyond what is possible by tuning aerodynamic particle size alone. However, they come with a considerably higher price tag as compared with conventional inhalers, and require significant patient training and adherence monitoring. Further, intersubject variability in lung volume and airway anatomy cannot be controlled, such that any single prescribed inhalation pattern and aerodynamic size range will result in variable effectiveness of targeting between individual subjects. Perhaps a primary reason that a perceived gap exists between scientific research aimed at predicting, controlling, and measuring regional deposition and clinical use of inhaled aerosols lies in the considerable challenge of obtaining, in a single study, accurate information describing the optimal lung regions to be targeted, the effectiveness of targeting determined from regional distribution of deposited drug (and potentially subsequent dispersion), and some measure of the drug's effectiveness. Such scientific studies, with the potential to establish links between regional targeting and therapeutic effects, are necessary in order to further motivate development of commercially viable products that target inhaled drug to specific lung regions.

4. THE USE OF MODELS

4.1. Cell-free models for predicting ADD

4.1.1 In-vitro models

ADD depends on numerous parameters including aerosol size distribution (median diameter and standard deviation), inhalation maneuver and lung morphology. While *in vivo* imaging of ADD is possible (see section 5), *in vitro* models for predicting ADD have been established for rigorous testing and quality control of inhalation devices and aerosol formulations. Considerable effort has been made over the past decade to improve *in vivo-in vitro* correlations[20]. These *in vitro* models typically consist of an aerosol inlet, which is designed to mimic extrathoracic aerosol deposition followed by an aerosol-sizing device such as a multi-stage aerosol impactor. While these models are especially adapted to the standardized and reproducible physical testing of aerosol therapy devices (e.g., nebulizers, pMDIs and DPIs), they allow only approximate assessment of the total and regional aerosol dose delivered to the lung under clinical conditions. It is well known that not only aerosol size, but also the inhalation maneuver and handling of the inhaler device may have a strong impact on the pulmonary aerosol dose. As these parameters can be highly variable depending on the patient, the ADD and hence the therapeutic outcome of an inhalation therapy may be highly variable[50,51]. Numerous studies have focused on intersubject variability due to lung morphology (in adults and children) and spontaneous breathing patterns[52-56]. Results of *in vitro* modeling have also been used to validate models or to derive correlations that can be used in lieu of models especially for extrathoracic deposition[52,53,55,57], though such correlations must be applied with caution in predicting ADD or total aerosol deposition from single-breath inhalers, as discussed above in section 3.1.

4.1.2 In-silico models

In contrast to *in vitro* models, *in silico* models can provide information on the specific location of

deposition (e.g., lung generation) and allow for fundamental understanding of the main parameters governing ADD. Repeated numerical experiments can be performed by changing only a single variable that may be extremely difficult or impossible to achieve with *in vivo* experiments. Thus *in silico* models have been used to assess the hazards associated with environmental aerosol toxicology and for the assessment and optimization of aerosolized drug delivery covering a wide range of conditions[58]. In particular, ADD optimization through modeling could increase efficacy, and reduce drug cost, side effects and treatment time if more drug reached the intended lung target.

There are two broad categories of *in silico* models[58,59], analytical and numerical models derived from fundamental analysis of mechanisms (see Section 2) and those derived from empirical data fits of *in vivo*[23,60] *or in vitro* experiments. While empirical models are based on actual data, they may not be applicable outside of the experimental conditions of the data upon which they were derived. *In silico* models based on fundamental mechanisms can simulate a wider range of applications, but must be validated with experimental data where appropriate.

Several scientific gaps may lead to difficulties in comparison between *in silico* models and *in vivo* measurements of lung ADD, including imaging experiments[61,62]. Models typically calculate deposition for a single breath, or equivalently several identical breaths, of a perfectly characterized monodisperse or lognormally dispersed aerosol. For models based on the application of physical deposition mechanisms (i.e., as opposed to empirical models fitted to deposition data), a morphological lung model of some kind must be employed. Even for the empirical models, some coarse correlations are needed to reflect intersubject variability of the respiratory tract. Imaged data is spatially resolved, but not anatomically specific in terms of generation-specific ADD. Thus, direct comparison of spatially resolved aerosol distribution and lung morphology requires co-registration of the imaged deposition data and of anatomical

images, a task that itself requires significant effort to reliably locate deposition to specific airways. This is likely why the deposition data sets most widely used for model validation are those that provide only total and/or regional (i.e., extrathoracic (ET), tracheobronchial (TB), and pulmonary (P)) deposition, where the differentiation between lung regions is accomplished by attributing the aerosol dose cleared rapidly from the lung (typically within 24 hours) to the TB region and the remainder to the P region[60].

4.1.3. Computational Fluid Dynamics (CFD) models

Predictive mathematical models of aerosol deposition are useful tools for the assessment and optimization of ADD in the lung. While whole lung models, empirical models, and other macroscale models have the advantage of being able to predict deposition throughout the entire lung, they lack the ability to describe site-specific deposition within individual airways or specific locations of the lung. In addition, they may not be robust enough to simulate the complex particle dynamics emanating from inhalation delivery devices, as discussed in Section 3.1. Computational fluid dynamics (CFD) models, on the other hand, allow for the prediction of microdosimetry patterns that can be useful for assessing ADD in specific regions of interest throughout the lung. However, models based on first principles such as CFD models that capture the underlying physics of airflow and aerosol transport are more difficult to implement, require extensive computing resources, and can presently only capture a small subset of the respiratory tract. Recent advances in imaging, computing power, and commercial software have made CFD modeling more available. Even with these advances, CFD models are typically limited to the upper respiratory tract and upper lung airways, but it is these regions of the respiratory tract where accurate predictions of airflow and particle dynamics are most needed due to the complex surface geometries, laminar/transitional/turbulent airflow profiles, and inertial effects.

CFD modeling involves the numerical solution of the governing airflow and transport equations to simulate the aerodynamics and deposition behavior of inhaled particles in the respiratory tract. The first step in the development of a CFD model of the respiratory system is to develop the surface geometry, which defines the extent of the model and forms the model domain. Earlier models of the oral airways and upper lung airways used approximate surface descriptions based on idealized shapes, such as connected cylinders for each airway bifurcation[63-68]. More recently, CFD applications have used surface models derived from imaging data from individual subjects[69-72]. These models typically comprise the upper respiratory tract consisting of the oral airways, pharynx, and larynx, and upper lung airways from the trachea extending down through several generations of the lung. The limiting factor in determining how far down into the lung patient-based CFD models can go is driven by the resolution of the scanning procedure. Currently, CFD models extend to about 7-8 airway generations where airway diameters are about 1-2 mm, and can encompass on the order of 200-300 individual airways.

CFD models integrate aerosol characteristics, breathing patterns, and geometric features to predict ADD throughout the upper lung airways. Parameters such as particle sizes and flow rates can be easily varied to study deposition trends and sensitivity. CFD predictions in oral airways and upper lung airways can be compared with experimental data from replica casts for model validation. Ultimately we want to be assured that the CFD predictions can accurately simulate conditions found *in vivo*. One important aspect of CFD modeling is the selection of boundary conditions. When considering CFD models of the upper respiratory tract, boundary conditions are fairly straightforward because there is only one outlet, although inlet conditions can get complex when simulating drug delivery from a device such as an MDI. However, CFD models of the lung contain many outlets if the model spans several airway generations. In this

case, the simplest choice is to impart a uniform pressure condition on all model outlets. This is typically done in the absence of other physiological information concerning the lung airways. During inhalation, the lobes of the lung are known to expand at different rates, leading to non-uniform airflow profiles in each lobe. Uniform pressure conditions do not capture this phenomenon and will lead to erroneous airflow distribution. Another option is to consider a weighted outflow boundary condition where the fraction of airflow at each outlet is apportioned according to the distal volume of the lung. This should lead to more accurate results, but it is not always possible to have anatomical information for distal lung volumes. Another avenue that is receiving recent interest is the integration of distal lung mechanics through coupling of the 3D CFD model with 1D or 0D models at each outlet[69,73,74]. This should allow for a more realistic definition of airflow distribution at model outlets. DeBacker et al.[75] showed good agreement between CFD predictions of airflow distribution and those derived from SPECT/CT by accounting for airway resistance. It should however be noted that the airway resistance from the airway tree measurable by CT is only a very small fraction of the total pathway resistance, particularly in bronchoconstricted lungs[76]. Therefore to be quantitatively realistic in non-uniform diseased lung, either the distribution of regional ventilation should be determined experimentally, or the resistance of the peripheral airways needs to be accounted for.

4.1.4. Limitations, perspectives and relevance for bridging the gap between science and clinics

In a recent study, preliminary comparisons of *in-silico* predictions from existing ET and lung deposition models with individualized 2D and 3D imaging measurements of aerosol deposition in healthy lungs [61] showed that predicted deposition in the ET models did not correspond well with each individual's experimental data [77]. However, there was rather good agreement between simulated and experimental results for regional lung deposition comparable to those previously found in the literature. Comparison of the model predictions to lung generational distributions was also relatively poor. These preliminary results suggest not only the need for

further developments in deposition modeling such as individual lung morphology models, but also the need for better methods for analyzing experimentally determined 3-D deposition distributions for comparison to simulated results (see Section 5). Furthermore, developments are also needed to account for ET variability including movement of the glottis [78] and to determine deposition patterns for individuals, especially heterogeneous 3D hotspots caused by disease. If progress is made in these areas modeling might make become a tool to improve an individual patient's aerosol therapy.

While CFD models are very useful at providing site-specific deposition at distinct locations within the respiratory tract, two other important considerations are 1) what happens to the drug particle after deposition and 2) how we can obtain ADD predictions in distal lung airways beyond the CFD model. Comparing model predictions with experimental data from *in vitro* and *in vivo* studies is important, but ultimately we would like to use CFD predictions in lieu of experiments to assess the distribution of drug throughout the entire lung. One option is to utilize the strengths of both CFD and whole-lung deposition models to predict site-specific deposition behavior using the CFD approach in upper lung airways, and have the model outlets connected to 1D models of airflow and deposition to predict deposition in remaining lung airways. The fate of the drug particle after deposition is an important gap to overcome to relate deposition predictions to clinical efficacy. To bridge this gap, CFD models can be linked with pharmacokinetic models that incorporate mucociliary clearance, dissolution and absorption of the drug in lung airways and with biological models discussed in the next section.

4.2. Biological models of the lung

Once the aerosolized drug has been deposited onto the lung epithelium, the fate of the drug is determined by the interaction of the drug with biological entities with the lining fluid, mucus and the pulmonary cell barrier at the air-liquid interface. The understanding of pathomechanisms

and the development of novel therapeutic intervention strategies are largely driven by preclinical studies with biological models mimicking the physiology and pathology of the lung. However, translation of preclinical therapeutic successes into clinical outcome has proven difficult resulting in considerable waste of financial resources by performing clinical trials with false positive preclinical substances[79]. On the other hand, false negative preclinical substances may never reach the clinics depriving the patients of potentially powerful new therapeutic options. Hence, the availability of highly predictive physiological models of the human lung/organism is crucial for translation of preclinical studies into clinically successful therapies[80].

4.2.1. Top-down approach

Figure 3 presents an overview of preclinical biological models of the lung. These models can be stratified according to their level of complexity and the way of how different grades of complexity are accomplished (*top-down* or *bottom-up* approach). In general, advanced biological complexity is associated with a higher degree of similarity with clinical conditions. Four main levels of complexity can be distinguished, namely the levels of organism, organ (here the lung), tissue and cell. Animal models such as rodents, pigs and monkeys offer a means of studying biology in the complex context of higher organisms both under healthy and diseased conditions. It is well-known that animal models are limited in terms of their relevance for human disease due to species-specific differences in physiology, pathogenesis and disease progression[81]. As an alternative a whole suite of *ex vivo* and *in vitro* models of the lung are available for medical science studies. However, it is important to note that all of these models (*in vivo, ex vivo, in vitro*) have their strengths and weaknesses. Scientists have to select the most suitable models depending on numerous aspects including relevance for the scientific issue to be addressed as well as model availability and cost of the study[82]. Reduced biological complexity of a model is typically associated with mitigated physiologic relevance, easier handling and reduced economic burden. On the organ level, studies with perfused excised animal lungs can be

performed for up to hours and days, the time period these models maintain many physiological functions such as cellular signaling, tissue homeostasis, and integrity of the air-blood barrier[83]. Direct access to both the epithelial (via tracheal) and the endothelial side (via perfusion liquid) of the air-blood barrier makes the perfused lung an excellent *ex vivo* model for pulmonary pharmacokinetics studies. On the next lower level of biological complexity, slices of the lung may closely mimic the 3D microenvironment of the lung and capture organ-specific mechanisms of disease for days to weeks. A unique feature of organ slices is the combination of biomimetic tissue characteristics and direct tissue access for four-dimensional confocal live tissue imaging techniques allowing for simple assessment of molecular and functional characteristics of the sample[84]. As lung slices can be obtained from both animals and humans, they may serve as a direct link between animal studies (science) and clinics[84]. In spite of the increasing interest in advanced lung models such as perfused lungs and lung slices, most of the medical science research has been performed with biologically and technically more simple lung models, namely human-/animal-derived cell culture models (mainly single cell type models). An overview of the available single- and multi-cell models of the lung is given elsewhere[85-87]. Recently, these cell culture models have matured into biomimetic models of the lung by incorporating previously neglected but crucial physiological aspects of the lung such as 1) cultivation at the air-liquid interface with air on the apical side and liquid on the basal side of the epithelial barrier[88,89], 2) growing the cells on biocompatible 3D (or 2D) matrices mimicking the elasticity of the pulmonary extracellular matrix, 3) exerting cyclic stretch on the cell layer simulating the mechanical strain profile experienced by the alveolar tissue during breathing activity[90] and 4) combinations thereof[91]. Moreover, the recently introduced ALICE-Cloud technology (licensed by VitroCell Systems, Germany), for rapid (<5 min) and efficient (>15% substance efficiency) aerosol-to-cell delivery of drugs, may pave the way for dose-controlled delivery of aerosolized drugs to air-liquid interface tissue cultures mimicking the physiologic conditions encountered during inhalation therapy[92,93].In addition to the vast body of knowledge on biological endpoints and

mechanisms obtained with the *in vivo*, *ex vivo* and *in vitro* models listed, to a limited extent these models have also been utilized for aerosol deposition distribution measurements[68,94].

INSERT FIGURE 3 HERE

4.2.2. Bottom-up approach

For decades the *top-down* approach dominated the development of lung models with different complexity levels, i.e. models of lower complexity are obtained by sectioning models with higher complexity. Animal models represented the gold standard for drug testing and served as source for excised organs, tissue sections and primary cells. Only the latter two of the suite of *top-down* lung models (see Figure 3) can also be obtained from patient-derived material, but tissue availability is severely limited by the number of donors. As an alternative route towards the design of human lung models with varying complexity, bioengineering has embraced the *bottom-up* approach to reconstitute biomimetic tissue, organ and organism models from primary human cells[86,95]. The *bottom-up* approach is still in its infancy, but tremendous progress has been made in the past decade and tissue engineering holds great promise for substantially improving the availability and clinical relevance of future human lung models[96]. There are already several commercial providers for bioengineered biomimetic bronchial lung tissue reconstituted from primary human cells (e.g. Epithelix, Switzerland; MatTek Corp., USA), which closely resembles the human epithelial tissue of the respiratory tract. This tissue remains fully differentiated and functional for several months[97,98], it is available from both healthy and diseased donors and it can be modified to incorporate disease-relevant features (e.g. lung cancer lesions)[99]. All of these characteristics facilitate the clinical relevance of medical research performed with these primary tissue models. While bottom-up approaches are still in their infancy, several proof-of-concept studies have already been performed on the organ-level. For instance, repopulation of a decellularized extracellular lung matrix (from rats) with

endothelial and epithelial cells with subsequent incubation in a bioreactor resulted in an engineered lung with similar mechanical and functional properties to those of native lungs[100]. This *in vitro* lung was able to contribute to gas exchange when implanted into rats *in vivo* for a short period of time (up to 2h). While fully functional bioengineered human lungs are not available, they appear to be within reach. Moreover, miniaturization of advanced 3D multi-cell culture models is under way leveraging microfabrication and microfluidics technologies to create cell culture microenvironments conducive for differentiation and self-organization of human tissue[96]. These 'organs-on-chips' are expected to facilitate *in vitro* studies of human physiology in an organ-specific context. Ultimately, networks of these 'organs-on-chips' may permit the development of biomimetic *in vitro* organisms ('organisms-on-chips') resembling the hallmarks of healthy and diseased subjects offering an alternative and possibly superior (more predictive) methodology than the currently used animal experiments[96].

4.2.3. Limitations, perspectives and relevance for bridging the gap between science and clinics.

All lung models have their advantages and limitations. Scientists have to select the most suitable models depending on the physiological relevance for the scientific issue to be addressed and constraints due to availability and budget. In general biologically less complex models (e.g. cell culture models) are more readily available, easier to handle and more cost effective than models with higher biological complexity, but of course their clinical relevance is mitigated compared to more complex models.

In the field of pulmonary toxicology there has already been a paradigm shift towards an evidence-based research discipline which can be supported by advanced *in vitro* and *ex vivo* models instead of animal models[95]. These *in vitro* and *ex vivo* models should be able to mimic the complexity of an organ or tissue as much as possible, while maintaining the capability for standardization, high throughput and reproducibility. Validation of the *in vivo* relevance of these

models by comparison with animal models and clinical studies is currently under way with a focus on toxicological endpoints. The preclinical and clinical communities should embrace these efforts with emphasis on pathological and therapeutic endpoints to lead the way into modern animal-free, yet clinically more relevant and hence more reliable, preclinical drug testing.

It is evident that validated *in vitro* models of human organisms will not be available in the near future. Hence, animal experiments will continue to play an important role in preclinical drug testing for some time to come. In light of species-specific differences in physiology, pathogenesis and disease progression the predictive power of animal models for clinical outcome has been under scrutiny. Thus complex biological models of the lung such as lung tissue slices, which are available from both animal and human tissue, are expected to play a pivotal role in closing the gap between (animal-based) biological science and clinics. Moreover, advances in tissue and organ engineering hold the promise for overcoming many of the shortcomings associated with therapeutic strategies developed from currently available biological models of the lung. Novel lung models combining innovative technologies for aerosolized drug delivery and tissue engineering *(bottom-up* approach) are expected to further reduce the gap between medical science and clinical relevance.

5. IMAGING TECHNIQUES FOR THE CHARACTERIZATION OF ADD

5.1. Why do we want to image ADD?

In disease, the distribution of aerosol deposition within the lungs may be highly heterogeneous. As discussed above, since aerosol particles are carried along by gas, their local distribution and deposition are affected not only by the particle properties and local gas flow characteristics but also by the distribution of the inhaled gas into different regions of the lung. Depending on the subject's breathing pattern, aerosol and carrier gas characteristics and pathophysiological

differences within the lung, the local dose of a medication delivered by the aerosol could vary substantially in different parts of the lung.

Imaging provides a non-invasive way to measure *in vivo* the regional deposition of inhaled drugs along the airway tree and between different anatomical regions of the lung parenchyma. Hence, imaging provides a bridge between science and the clinic, by enabling the study of the relationship between aerosol characteristics, their mode of delivery, and the concentration and location of the medication in the lungs (ADD), which in turn affects its clinical efficacy and/or side effects[1]. Quantifying ADD variability among anatomical regions of the lung is the first step needed to study and understand the factors affecting such variability. For example, in asthmatic subjects the pattern of ventilation is patchy with large and contiguous regions receiving very low ventilation and others receiving greater than normal levels[101]. This and the remodeling of airway dimensions results in a highly heterogeneous ADD that includes both serial differences in the fraction of aerosol retained by the central airways feeding each lobe as well as parallel differences in the aerosol that deposited distal to these airways[102]. Positron Emission Tomography (PET) imaging of ADD combined with quantification of regional ventilation and detailed imaging of lung structure with computed tomography (CT) can be used to understand how serial differences in airway deposition couple with other factors to achieve the parallel heterogeneity in deposition of aerosol among lobes. Those detailed 3D data sets can also be used for advancing CFD models of ADD by providing patient-specific realistic boundary conditions and allowing their quantitative validation (see Section 4.1.3).

Estimation of local dosing in terms of the pharmacologically relevant parameters is necessary to bridge in vitro and animal model experiments to the human scale. Evaluation of local dose is also important to understand the regional and global effectiveness of an inhaled drug. Accurate assessment of aerosol deposition along the airway tree and the concentration of deposition on

the airway surface may also be useful to evaluate airway functional features, such as mucocilliary clearance rate, a hallmark of some clinical conditions (e.g. cystic fibrosis).

5.2. Imaging Fundamentals

Clinical aerosol imaging relies almost entirely on radionuclide imaging, which uses radioactively labeled aerosols[103]. When inhaled, these allow the fate of the inhaled drug in the body to be monitored using an appropriate imaging device. Two types of radionuclide labels are used, gamma emitters and positron emitters[104]. Imaging with gamma emitters is focused around technetium-99m, because of its near ideal physical properties. It uses a gamma camera, which can be used to obtain either planar 2D images (scintigraphy) or 3D images (Single Photon Emission Computed Tomography, SPECT). The principal positron emitters used are fluorine-18, carbon-11[105] and nitrogen-13[102]. These are imaged using a PET scanner, which also provides 3D images with somewhat higher spatial detail than SPECT.

Radionuclide imaging is well suited to assessing the fate of aerosol in the body, as it provides accurate quantitative assessment of the amount and location of aerosol deposition. However quantitative imaging requires careful attention to detail in the methodology and good quality control. A document providing a comprehensive description of recommended techniques for radionuclide aerosol imaging is available[106]. This covers methods of labeling and delivering the aerosols, image acquisition procedures and image analysis.

State of the art imaging uses scanners that combine SPECT or PET imaging with x-ray computed tomography (CT) (Figure 2). The aligned scans improve the interpretation of the aerosol distribution images and also provide detailed information on airway structure.

Magnetic Resonance (MR) imaging has also been used to image aerosol deposition, but this technology is still very much in the development phase, as practical difficulties of using the technique are being tackled[107,108].

5.3. Applications and relative merits of techniques

All forms of radionuclide imaging provide measures of the percentage of inhaled aerosol deposited in the lung and of its regional distribution (ADD). Most work to date has used planar imaging. This provides reasonably accurate measurement of total lung deposition [109,110] and has been very useful in monitoring the increase of lung deposition efficiency in inhalation therapy, as inhaler design has improved over the past 20 years. It has also contributed to elucidating the relationship between delivery of dose to the lung and clinical outcome allowing assessment of the efficacy of the inhaled drug [111].

Planar imaging also provides information on the regional distribution of aerosol. This is usually determined by dividing the lung into inner and outer zones and comparing the amount of aerosol in these zones by calculating a penetration index [112]. This analysis is limited by the two dimensional nature of the images meaning that the inner and outer zones only correspond very approximately to central and peripheral airways. However it has been useful in demonstrating the influence of ADD on clinical efficacy [113].

3D imaging (SPECT and PET) is more complex to use than planar imaging, but provides better information on regional deposition [114,115], and has application in studies where this is the endpoint. Therefore it has a role in assessing the influence of regional deposition pattern on the clinical effectiveness of inhaled therapy. When combined with high resolution computed tomography (HRCT) 3D imaging provides excellent information on aerosol deposition pattern in

relation to lung anatomy, and is useful in validating computer models of aerosol deposition as described above in section 4.1.4 [77].

SPECT is easier to use and more widely available than PET but has some disadvantages, particularly in the inability to directly label aerosols from portable inhalers with technetium-99m [112]. PET radiotracers by contrast can be directly incorporated into drug molecules[105] and also provide better image resolution.

HRCT imaging provides useful information on lung anatomy and is of value in providing individualized models of the airway tree useful in computer modeling of deposition. Radionuclide imaging can also provide information on lung ventilation, which is valuable in understanding the link between the aerosol characteristics and location of deposition in the airway tree [116].

5.4. Challenges

The standard approach to evaluate local concentration of a radio labeled substance is to measure the average activity per unit volume within defined Regions of Interest [ROIs]. ROIs are binary masks that serve to group voxels corresponding to specific anatomical regions. These ROIs are either defined based on generalizations of the anatomy[105,112,117,118], or are segmented from detailed HRCT[116,119] scans, when available. In the lungs, it is helpful to segment the lungs into anatomical regions that encompass portions of the central airway tree and the lung periphery. However, the number, size and location of airways that can be accurately segmented from HRCT images can vary substantially among subjects and within lobes, and may depend (among other factors) on the lung volume at which the CT images are acquired. Therefore a method to segment the lungs into consistent anatomical regions is required to compare aerosol deposition across subjects and among lobes.

Even after defining consistent anatomical regions, other challenges need to be confronted before aerosol deposition can be accurately evaluated. Regional deposition quantification with binary ROIs may be inaccurate due to partial volume and spill-over effects[120-122]. These inaccuracies are caused by image blurring due to the limited spatial resolution of the nuclear imaging methods (~6 and ~15 mm, for PET and SPECT respectively) and by the breathing motion of the lung during imaging. Also, although PET (or SPECT) images may be automatically co-registered with the CT images in combined imaging instruments (i.e. PET-CT or SPECT-CT), shifts in patient position, or differences in average lung volume between the PET and CT images, often require additional co-registration. The co-registration process includes errors that need to be considered when estimating regional activity.

Methods to evaluate aerosol deposition that accounts for the effects described above by expanding the concept of binary ROIs into the Grayscale domain have been described[102]. This is accomplished by defining a Voxel Influence Matrix [VIM] to describe how activity originating from any anatomical region is sampled in each voxel of the 3D ADD image (Figure 4). VIMs can include the combined effect of sources of blurring such as breathing motion, limited spatial resolution, registration and model uncertainties, and allows accurate estimation of activity originating from airways within anatomical regions with dimensions that may be smaller than the spatial resolution of the nuclear medicine method. Applied together, these methods leverage the exquisite anatomical detail provided by HRCT to consistently and accurately estimate the distribution of aerosol among specified anatomical regions, and to describe such a distribution in pharmacologically relevant terms such as airway inner surface concentration, and peripheral tissue dosing[102].

5.5 Limitations, perspectives and relevance for bridging the gap between science and clinics

5.5.1. Limitations of imaging techniques

Producing adequate radiolabels is not easy. Ideally they need to robustly label the aerosol and remain attached to the therapeutic molecule during the imaging period, which may be several minutes. Many ^{99m}Tc labeled aerosols do not fulfill this requirements and further work to produce more robust radiolabels is required. PET radionuclides can directly label the drugs and the short half-lives of the radionuclides may reduce the radiation exposure to the subject, but developing and validating radiolabels is expensive and requires an in-house cyclotron facility to generate the short half-live isotopes.

Radionuclide imaging provides an estimate of the spatial distribution of the aerosol, whereas what is more clinically relevant is the anatomical distribution in the different airways. This is complicated by the relatively poor resolution of the images (0.6 - 1.5 cm) and the complex structure of the lung airway, with nearly all the airways being much smaller than the image resolution. Methods have been described for correcting for the limited image resolution and for transforming the spatial distribution to an anatomical distribution by airway generation[102,123], but most work to date has relied on simplified models of the lung airway. Nevertheless 3D models combined with CT does provide a wealth of data on aerosol deposition related to anatomy, which would benefit from improved analysis techniques.

Radionuclide imaging has the disadvantage of exposing subjects to some health risk, due to the use of ionizing radiation. In the longer term magnetic resonance imaging may develop as the method of choice for studying aerosol deposition, as it provides improved resolution images and without ionizing radiation dose. However the practical difficulties in assessing aerosol deposition

with MRI mentioned above, suggest that radionuclide imaging will continue to have a role for some time to come.

Despite its limitations radionuclide imaging provides very valuable and unique information on aerosol deposition and should continue to provide useful data in bridging the gap between science and the clinic.

5.5.2. Bridging the gap with a theoretical framework to describe the sources of ADD variability.

Based on what is known about the physics of aerosol deposition, it is likely that most of the variability in peripheral aerosol deposition among lobes, sub-lobes, or any set of peripheral lung regions, can be attributed to one of four distinct factors: 1) differences in regional ventilation[1,124,125], 2) differences in how the aerosol and air distribute between branches in the series of bifurcations along the pathway feeding the region [125], 3) variability in the amount of the aerosol that escapes the series of airways along that pathway[126-128], and 4) variability in the amount of aerosol that reaches the periphery and is not exhaled[129].

Using the concept of aerosol concentration as the average mass in suspension crossing any point of the bronchial tree, a theoretical framework can be defined to quantify each of the factors that lead to heterogeneous aerosol deposition among lobes[130]: differences in lobar ventilation per unit volume (specific ventilation), uneven splitting of aerosol and air at bifurcations (bifurcation factor), differences in the fraction of aerosol deposited along the feeding airways (escape factor), and differences in the fraction of aerosol that reaches the periphery but escapes via exhalation (retention factor).

Such a framework was used to quantify the contribution of these factors from ADD images in a group of bronchoconstricted subjects with asthma. That analysis gave the following results: 1)

Differences in lobar specific ventilation (measured from the turnover rate of ^{13}N washout) and in net branching factors each accounted for more than a third of the variability in deposition among lobes and subjects. The remaining variability was due to differences in deposition along the feeding airways as characterized by their net escape fractions. 2) Subjects breathing slowly (< 9BPM) during nebulization had a strong relationship between regional lobar deposition and the respective ventilation measured with PET, while the relationship weakened in subjects breathing more rapidly. Also in subjects breathing faster, the more expanded lobes showed lower deposition per unit ventilation than less expanded lobes, while the opposite was true for subjects breathing slowly. 3) Differences in lobe expansion between HRCTs at two lung volumes, used by others as a surrogate of regional ventilation[75], failed to explain the variability in regional deposition across subjects.

6. IMPORTANCE OF AEROSOL DEPOSITION DISTRIBUTION TO CLINICAL EFFICACY

Currently there is much debate on whether it is advantageous to direct inhaled therapeutic aerosols to deeper lung regions than is currently achieved by conventional medical aerosols used in clinical practice[131,132]. In order to achieve this very coarse differentiation in regional inhaled aerosol deposition within the lungs, many factors can be influential of which particle size and aerosol velocity are key aerosol attributes[2] (see section 2).

Indeed, empirical correlations show that particle size influences not only the total but also the regional site of airway drug deposition[60]. Many investigators have attempted to determine the relationship between β2-agonist particle size and clinical effect using polydisperse and also monodisperse aerosols, but have reached differing conclusions on the optimal particle size and this probably relates to differences in experimental aerosol methodology and delivery as well as the variable clinical endpoints used and patient characteristics. In a series of well validated experiments utilizing monodisperse albuterol aerosols, Usmani and colleagues initially showed

that larger 6 µm albuterol particles achieved better bronchodilation in asthmatic subjects than smaller 3 and 1.5 µm particles, when the clinical endpoint of FEV_1 was employed to assess efficacy[133]. The investigators hypothesized that as a result of the physical nature of aerosol monodispersity, there was relative selectivity in the airway tree that the different sized particles were being distributed to in that, larger 6 µm particles were preferentially targeting the proximal airways whereas smaller particle were being directed to the deeper lungs. This group subsequently undertook a novel imaging study using a validated radioaerosol generation system[134], to assess the effect of different β2-agonist particle sizes and their influence on the aerosol deposition-clinical efficacy relationship[113]. Indeed, it was observed that larger particles visually deposited more proximally, whereas smaller particles distributed deeper into the lungs and had a greater penetration index[112,135]. The paradox of overall less total lung deposition with the 6 µm particles but greater efficacy in FEV_1 was explained by better matching of the inhaled therapeutic to its receptor distribution. However, the authors discussed the fact that spirometry was unable to differentiate between deep (smaller particle) and proximal (larger particle) airway effects and that possibly the larger particles were showing better bronchodilation because the FEV_1 was assessing the proximal lung region and that the smaller particles may also have 'clinical efficacy', but the appropriate clinical endpoint to assess deep airway effects had not been used. In this context over the last decade, technological advances have been made in imaging and physiological measures to probe the deep lung and are progressing the field to assess the so-called 'quiet' deep lung region[136] (see section 5).

Inhaled corticosteroids (ICS) remain the most important anti-inflammatory treatment for patients with asthma and advances in drug formulations and device design have generated a variety of differing ICS particle sizes. These commercial inhalers have been investigated using scintigraphy to assess whether differences in aerosol deposition and regional lung distribution are observed due to differences in ICS particle size. Indeed, data show that small particle

achieve higher total lung deposition than larger particles, and that smaller particles penetrate deeper into the lungs[137,138]. With this fundamental difference observed in aerosol deposition and distribution, investigators have utilized different ICS particle sizes in patient studies to determine if there is a difference in clinical efficacy[132]. Hoshino[139] showed that smaller ICS particles (~1.1 µm) significantly improved distal airway resistance compared to larger ICS particles (~5.4 µm), but also showed that only the smaller particles significantly improved asthma control. Importantly it was observed that there were no significant changes in spirometry in either group, but that distal airway physiological measures were able to pick up changes in smaller particle therapy that proximal airway measures such as spirometry were unable to. Similar data have been shown using combination ICS and long-acting β2-agonist (LABA) inhalers where smaller ICS-LABA particles (~1.5 µm) achieved greater asthma control than separate standard larger (~3 µm) inhalers[140]. Data is now accumulating that treating the deeper lungs with smaller drug particles (whether monotherapy ICS or combination therapy ICS-LABA) in patients with asthma is as good as with larger particles, but additionally allows a reduction in the overall daily ICS dose with smaller particles; indeed, some studies show that smaller particles achieve greater clinical efficacy[132].

It has been questioned whether improving the efficiency of inhaled aerosol deposition within the lungs, by targeting drug delivery to the appropriate airway regions and sites, actually improves the therapeutic response. That is, is there a risk that deeper lung delivery improves efficacy but may detrimentally worsen adverse effects? Derom and colleagues[141] compared the systemic exposure of small versus large particle ICS monotherapy and observed that smaller ICS particles (~1.1 µm) of ciclesonide did not significantly affect cortisol secretion, in contrast to larger ICS particles (~4 µm) of fluticasone propionate. Concerns have been raised about the clinical safety of aerosolized solution particles of tiotropium, a long acting muscarinic antagonist (LAMA)[142], that have recently been dispelled by the FDA at the recent American Thoracic

Society Meeting. Indeed, there is interesting pharmacokinetic data comparing slow velocity aerosolized solution particles of tiotropium to faster velocity aerosolized powder particles, where blood exposure is lower following the slow velocity aerosol[143]. Part of this may be due to the lower nominal drug dose with the more efficient slower velocity tiotropium aerosol.

7. CONCLUSIONS AND OUTLOOK

Development of a new drug for the treatment of lung disease is a complex and time-consuming process. The identification of potential drug targets, the screening for bioactive substances with sufficient target specificity, the formulation of the drug and the clinical validation requires contributions from numerous disciplines of basic, applied and clinical sciences including biology, chemistry, pharmacology, aerosol science, inhalation device, imaging technology as well as medical and clinical sciences. Only substances with clinically proven safety and efficacy profile will receive regulatory approval. Hence, only a very small fraction of the pre-clinically investigated substances will benefit the patient and successfully reach the marketplace.

The translation of scientific knowledge into clinical innovation is often delayed or even disregarded notably because of a significant gap between science and clinical relevance. For example, ventilation-controlled inhalation devices are only just starting to reach the clinics, even though the significance of inhalation maneuver for optimized pulmonary drug delivery has been well-known since the mid 1980s[60]. Reasons for this gap include language and methodology barriers. The transfer of knowledge between disciplines is hindered or even made impossible by the use of different terminologies and methodological approaches. While this is a common feature of highly interdisciplinary ventures, the high regulatory hurdles for novel drugs/therapies are specific to the clinical setting. Undergoing all phases of clinical trials involves a high financial risk. Pharmaceutical companies prefer modifications of established drugs, therapies and

technologies to the introduction of completely new ones, since this is associated with lower regulatory hurdles and consequently lower budgetary risks.

Overcoming the gap between different disciplines is essential for reducing the cost and time required for the development of novel therapeutic options. One key aspect is the establishment of a more comprehensive approach to drug development. This would include regular interaction and knowledge exchange between the various disciplines of basic, applied and medical/clinical science. This has now been recognized by many funding agencies as evidenced by the broadening of the scope of numerous calls for basic research projects. The successful consortium has to demonstrate clinical relevance and frequently even include clinical partners. Moreover, numerous interdisciplinary networks and platforms have been formed and some comprehensive pulmonary centers have been established bringing together experimental and clinical research units under one roof. As clinical trials have to be financed by industry, it is also important to include industry representatives early in the drug development process posing another “language” barrier as the scientific merit of new ideas/technologies has to be translated into budgetary terminology. For example, for ventilation-controlled inhalation devices, key “selling points” could be the reduced cost of clinical trials due to enhanced pulmonary drug delivery efficiency resulting in more efficient substance use. Moreover, the device-specific reduced variability in applied pulmonary drug dose strengthens the statistical power of the clinical study. This relaxes the requirements on the number of enrolled patients and hence reduces cost. Also conflicts over intellectual property rights might be avoidable, if industry partners are involved early on.

The workshop on Bridging the Gap between Basic Research and Clinical Efficacy of Inhaled Drugs, which took place during the 2015 ISAM Congress, also served to facilitate

interdisciplinary communication. The main conclusions from this interdisciplinary group of experts can be summarized as follows:

- Personalized, patient-specific (precision) inhalation therapy is within clinical reach. Basic research has identified most of not all relevant parameters for patient-specific regional drug targeting to the lung (ADD), which allows improved engineering of inhaler devices and drug formulations. Implementation of these advanced products in the clinical roam requires combination of modern 3D imaging and clinical pulmonology.
- Computational analysis of ADD is expected to play in important role in matching patient needs and device characteristics for disease-specific optimized drug delivery (precision medicine).
- The availability of patient-specific, easy-to-use devices has to be accompanied by thorough training of patients in device handling for optimized compliance, a prerequisite for the therapeutic success of inhalation therapy.
- The predictive power of preclinical substance testing for clinical efficacy should be improved by utilizing advanced preclinical *in vitro* and *ex vivo* lung models such as reconstructed/sliced tissue models from donors and patients combined with dose-controlled, aerosolized drug delivery under physiological conditions. This is expected to result in less false positive and less false negative drug candidates reducing the risk of conducting ill-fated clinical studies (reduced budgetary risk) or premature termination of the testing of clinically successful drug candidates.
- In the intermediate future, embracing the currently available advanced preclinical *in vitro* and *ex vivo* models of lung tissue may lead to the development of human-based lung models at the organ and even organism level paving the way for more reliable, more cost-effective, and animal-free preclinical drug testing.

- In the more distant future linking computational ADD models with pharmacokinetic, pharmacodynamics and systems biology models may allow *in silico* drug testing.

These findings demonstrate the crucial role of bridging the gap between basic research and clinical efficacy for optimized translation of fundamental research from the bench to the bedside (clinical outcome).

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FIGURE LEGENDS

FIGURE 1: Mean airway velocity (solid line) and residence time (dashed line) in the airways as a function of generations of an idealized dichotomous branching lung model (Weibel model A [5]). As a result the most relevant mechanism for aerosol deposition onto the lung epithelium changes from impaction in the upper to sedimentation and diffusion in the lower airway generations (see text for details). Data were calculated for an inspiratory flow rate of 500 ml/s.

FIGURE 2: Coronal slices of 3D Single Photon Emission Computed Tomography (SPECT) deposition data superimposed on x-ray CT images of asthmatic subjects illustrating the effect of helium-oxygen carrier gases (right column) on ADD in a responder (subject A06, top row) and non-responder (subject A05, bottom row) to helium-oxygen based aerosol therapy. Data from [144]

FIGURE 3: Available and proposed biological models of the lung stratified according to their level of complexity (or similarity to clinical settings). Different levels of complexity can be accomplished by *top-down* or *bottom-up* approaches. In the *top-down* approach, the model of lower complexity represents a section/part of a model with higher complexity. In the *bottom-up* approach, which is pursued by tissue engineering, lung models with higher complexity (e.g. tissue, lung organism) are reconstituted from primary human cells. Some of these *bottom-up* models are already available as prototypes in miniaturized form ('on-a-chip').

FIGURE 4: Image processing steps to generate quantitative anatomical ADD images. HRCT images of the chest were collected during apnea at maximal lung inflation and at the average lung volume during spontaneous breathing. These CT images where rendered and segmented into 5 lobar regions and the proximal fraction of the airway tree up to subsegmental bronchi. The bronchial tree was further segmented into 9 airway anatomical regions. The two 3D-rendered airway trees were also mapped to each other and the mapping function, combined with the spatial resolution characteristics of the PET scanner and co-registration uncertainties, were used to estimate the degree of blurring for each anatomical region. Such estimates were transformed into a “gray-scale” voxel of influence matrix, which specified the fraction of each anatomical region sampled by each voxel. Finally the VIM and the ADD image were used to solve for the activity originating from each anatomical region [(102)].

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Address reprint requests to:

Chantal Darquenne, Ph.D.

Department of Medicine

University of California, San Diego

9500 Gilman Drive, mail code 0623A

La Jolla, CA, 9309-0623

USA

Email : cdarquenne@ucsd.edu

Or to

Otmar Schmidt, Ph.D.

Institute of Lung Biology and Disease

Helmholtz Zentrum München – German Research Center for Environmental Health

Ingolstaedter Landstrasse 1

D-85764-Neuherberg

Germany

Email: otmar.schmid@helmholtz-muenchen.de

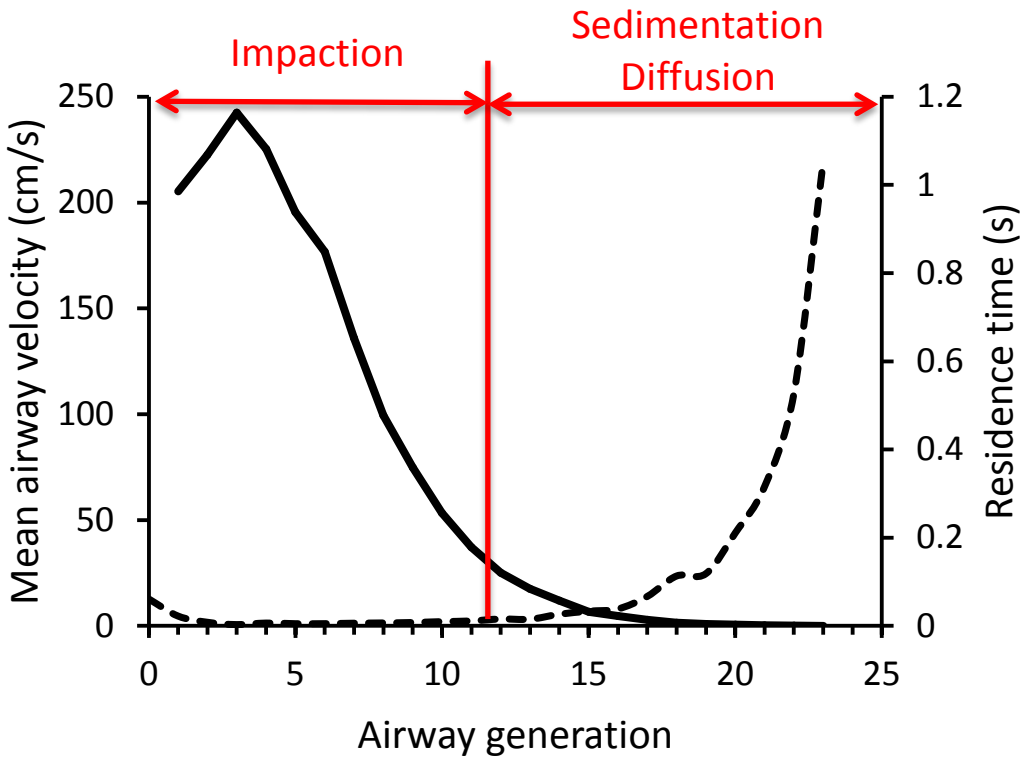

Figure 1: Mean airway velocity (solid line) and residence time (dashed line) in the airways as a function of generations of an idealized dichotomous branching lung model (Weibel model A [5]). As a result the most relevant mechanism for aerosol deposition onto the lung epithelium changes from impaction in the upper to sedimentation and diffusion in the lower airway generations (see text for details). Data were calculated for an inspiratory flow rate of 500 ml/s.

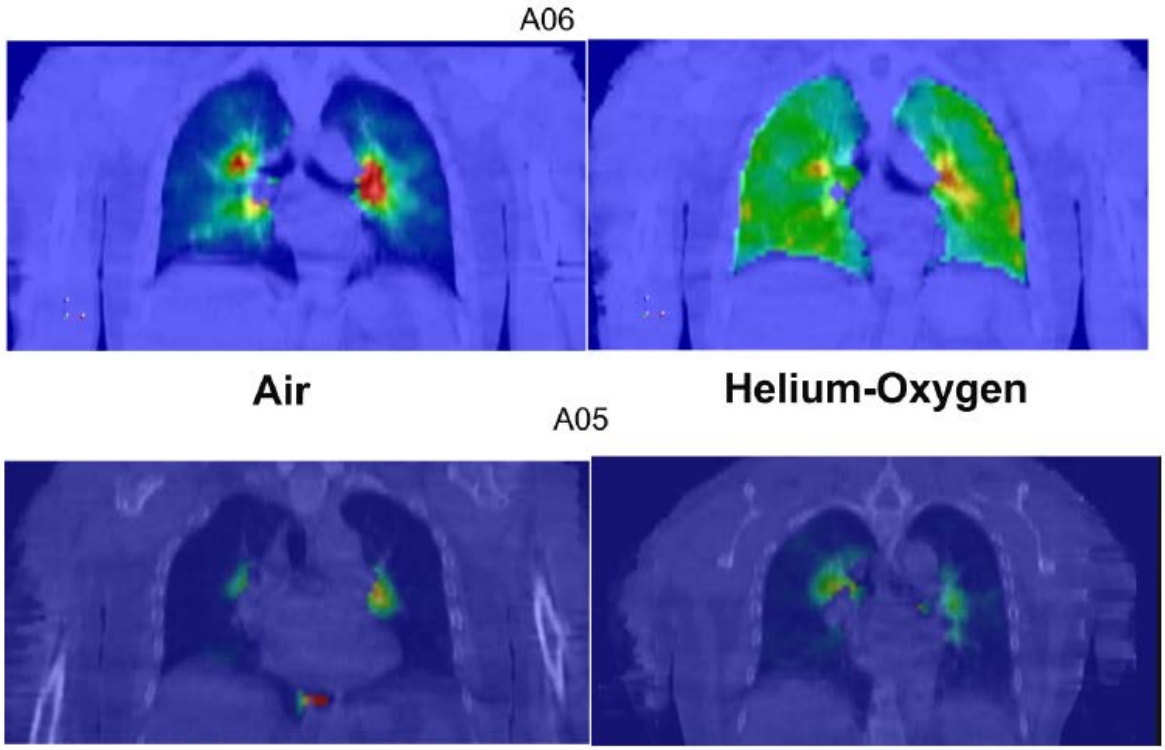

Figure 2. Coronal slices of 3D Single Photon Emission Computed Tomography (SPECT) deposition data superimposed on x-ray CT images of asthmatic subjects illustrating the effect of helium-oxygen carrier gases (right column) on ADD in a responder (subject A06, top row) and non-responder (subject A05, bottom row) to helium-oxygen based aerosol therapy. Data from [144]

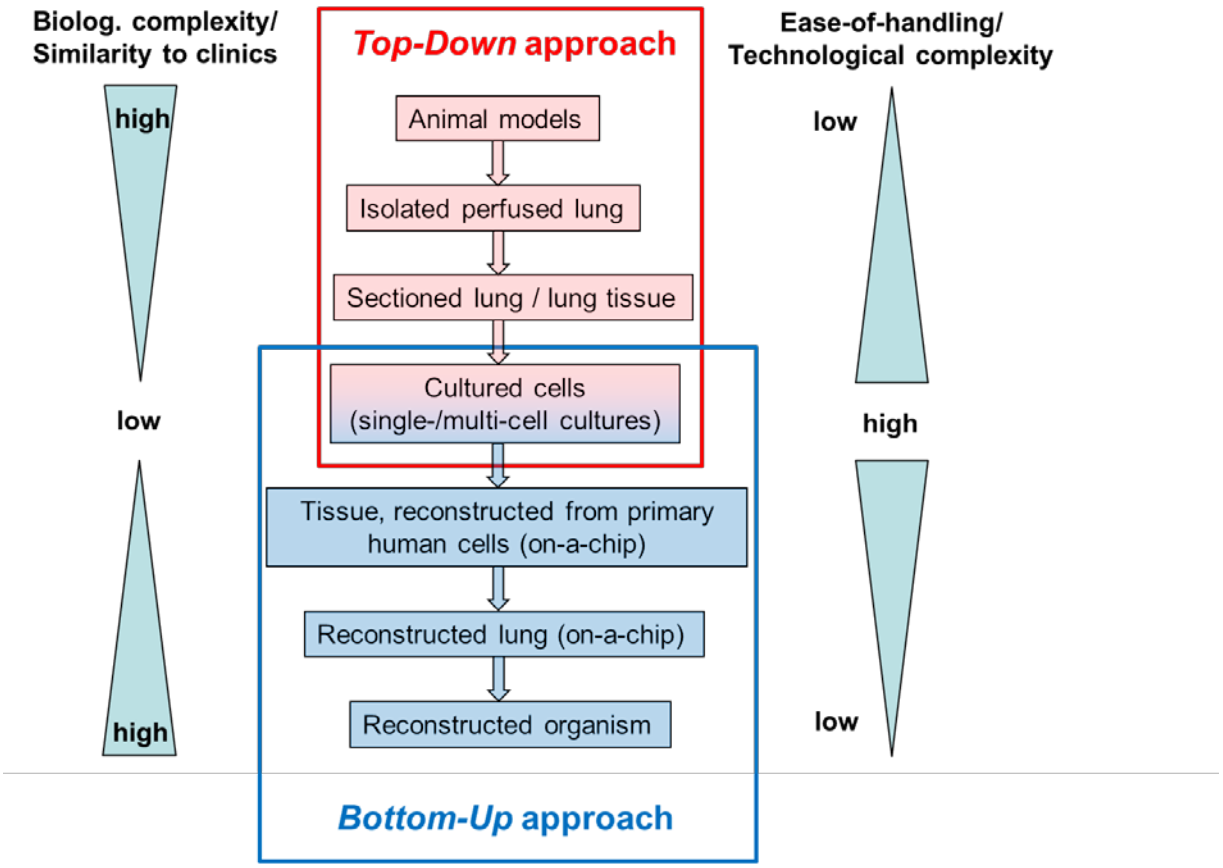

Figure 3. Available and proposed biological models of the lung stratified according to their level of complexity (or similarity to clinical settings). Different levels of complexity can be accomplished by *top-down* or *bottom-up* approaches. In the *top-down* approach, the model of lower complexity represents a section/part of a model with higher complexity. In the *bottom-up* approach, which is pursued by tissue engineering, lung models with higher complexity (e.g. tissue, lung organism) are reconstituted from primary human cells. Some of these *bottom-up* models are already available as prototypes in miniaturized form ('on-a-chip').

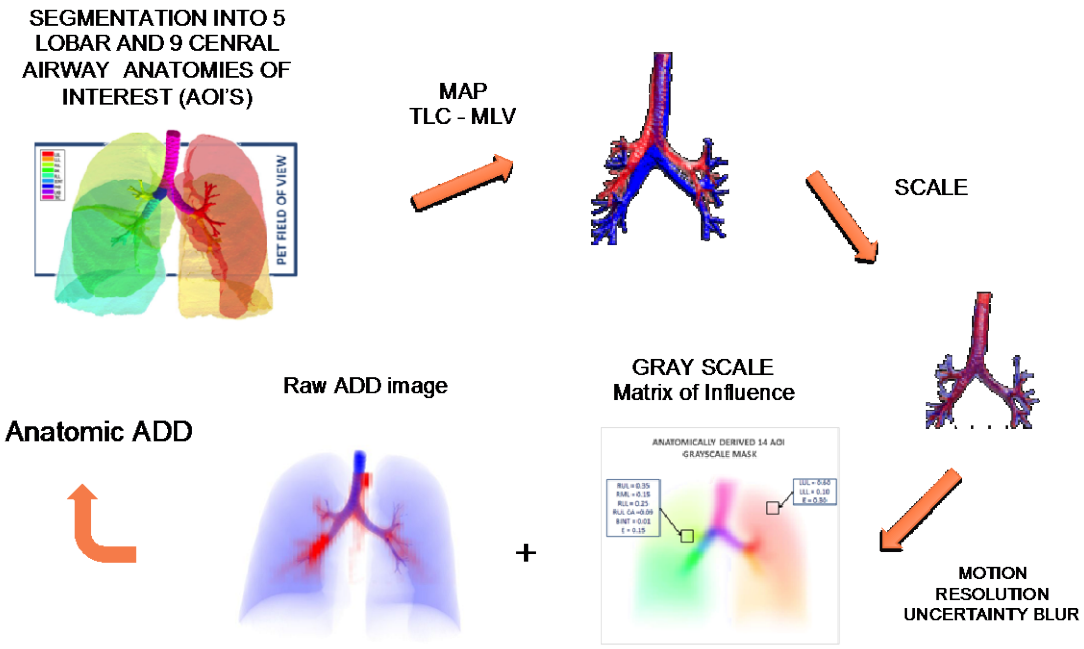

Figure 4. Image processing steps to generate quantitative anatomical ADD images. HRCT images of the chest were collected during apnea at maximal lung inflation and at the average lung volume during spontaneous breathing. These CT images where rendered and segmented into 5 lobar regions and the proximal fraction of the airway tree up to subsegmental bronchi. The bronchial tree was further segmented into 9 airway anatomical regions. The two 3D-rendered airway trees were also mapped to each other and the mapping function, combined with the spatial resolution characteristics of the PET scanner and co-registration uncertainties, were used to estimate the degree of blurring for each anatomical region. Such estimates were transformed into a "gray-scale" voxel of influence matrix, which specified the fraction of each anatomical region sampled by each voxel. Finally the VIM and the ADD image were used to solve for the activity originating from each anatomical region [102].