American Thoracic Society Documents

An Official ATS Conference Proceedings: Advances in Small-Animal Imaging Application to Lung Pathophysiology

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EXECUTIVE SUMMARY

The American Thoracic Society convened a workshop, "Advances in Small Animal Imaging: Application to Lung Pathophysiology," to identify cutting-edge research in imaging technology and the potential applicability to the study of lung pathophysiology in small-animal models. The goals of the conference were as follows: (*1*) to summarize the current state of small-animal models of lung pathophysiology and their applicability to human disease; (*2*) to identify all potential modes of noninvasive imaging; (*3*) to explore the potential for current and future applications; (*4*) to discuss and debate current controversies; and (*5*) to identify future research directions and opportunities for, and applications of, imaging technology to facilitate the use of small-animal models for the study of lung diseases.

The first part of the workshop focused on the current state of knowledge of mouse models with an emphasis on "What are the big questions?" and "How good are the models?" Presentations described four major animal model systems of lung disease: (*1*) reactive airway disease, (*2*) chronic obstructive pulmonary disease (COPD) and emphysema, (*3*) interstitial lung disease, and (*4*) acute lung injury (ALI). The second part of the workshop reviewed those "state of the art" imaging modalities that would be most likely applicable to lung disease with an emphasis on the questions "What is the cutting edge of the imaging modality?" and "What can we measure with this imaging modality?"

The related presentations focused on six imaging modalities that have received the most recent attention: (*1*) videomicroscopy, (*2*) magnetic resonance imaging (MRI), (*3*) micro-computed tomography (micro-CT), (*4*) micro-positron emission tomography (micro-PET), (*5*) optical imaging, and (*6*) molecular markers.

The final part of the workshop was devoted to discussion and interaction between those investigators focused on development of imaging modalities and those using small-animal models of lung disease. The discussion included (*1*) the quality and applicability of current small-animal models of lung disease and (*2*) how to better adapt currently available imaging modalities to study lung disease in small-animal models.

Workshop participants concluded that noninvasive imaging of health and disease in living organisms can span several domains, including anatomic, physiologic, metabolic, and molecular imaging. In parallel, technologies have evolved that allow us to query biological processes at multiple levels, including X-ray/CT, MRI, nuclear imaging (single photon emission CT [SPECT]/PET), ultrasound, and optical imaging (bioluminescence/fluorescence). "Molecular imaging" refers to the measurement and characterization of specific molecules, molecular processes, and molecular events, over time and space, in living organisms. Furthermore, whereas imaging modalities may be applicable to small animals, the currently used small-animal models of common human lung diseases remain limited in terms of their ability to truly recapitulate human pathophysiologic conditions. Further development is required for small-animal models of human lung disease as well as the integrated use of imaging modalities.

The following recommendations were made for future work on animal models:

- Continually reassess current animal models of lung disease.
- Delineate appropriate criteria for animal models of lung disease.
- Develop animal models that better duplicate human respiratory disease.
- Focus on an integrated approach, from the submolecular level up to the organ level, in the animal models of lung disease.

For imaging modalities, the workshop ended with the following recommendations:

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- Increase interaction between the integrative biological science community and the imaging science community.
- Utilize imaging modalities to investigate the topography of the lung pathophysiology.
- Utilize imaging modalities to investigate intracellular lung pathophysiology *in vivo* and in real time.
- Utilize imaging modalities to investigate and study temporal pathophysiological events.
- Utilize combined imaging modalities to better relate spatial and temporal events (e.g., CT with PET or SPECT).
- Utilize imaging modalities to determine how well various animal models reproduce the human disease condition.
- Explore technical advances for several imaging techniques, such as validation, increased resolution, and increased speed of data acquisition, to name a few.
- Develop better quantitative analysis tools for image analysis—for example, the application of stereological techniques to imaging datasets.
- Investigate the use of bioinformatics analysis techniques to the large datasets produced by image acquisition.
- Expand future workshops to include other imaging modalities and their potential application to respiratory disease.

BACKGROUND

Before 1990, the use of small animals (e.g., mice) was limited mostly to postmortem histology. Over the past decade, there has been a dramatic expansion in the use of small animals, and specifically, murine models, to study lung disease. Current research advances have made it possible to address research questions that require both premortem and longitudinal data.

The newest techniques for imaging and quantifying small-animal morphology are being developed in other fields that are not specifically related to lung research. However, many of these advanced imaging techniques are either currently directly applicable to lung imaging or, with alterations, could be modified to image the lungs. However, there is currently no means by which scientists who now perform lung research in small animals can easily interact with scientists who use various imaging techniques in other fields of biomedical research to facilitate the future development of lung-related small-animal imaging techniques.

We describe a workshop that served to bring together leading research scientists from around the world who have developed cutting-edge techniques in small-animal imaging modalities, but who may not be conducting lung research, together with leading research scientists within the American Thoracic Society (ATS) community who have developed and used small-animal models of lung disease.

METHODS

Presenters at this workshop were invited based on internationally acknowledged expertise in their specific area as well as their research publications in leading journals and presentations at international conferences. They were asked to review the current state of the science by searching the current literature and conference presentations in their respective areas of expertise. The literature was assessed by electronic and manual searches. Consensus on the recommendations was reached by active discussion at the workshop.

In the following section, we describe the four animal models that workshop participants reviewed. In the subsequent section, we will describe the six imaging modalities that workshop participants reviewed.

Small-Animal Models of Lung Disease

Asthma models. The use of mouse models has led to an explosion of information on the mechanisms of reactive airway disease due to inflammatory events (1). As a result, a great deal is now known about the signals, cells, and mediators that lead to a state of hyperresponsiveness of the airways. Most investigators use some form of antigen sensitization and challenge, commonly ovalbumin with an acute challenge protocol, although many other models have been described. More recently, investigation has moved to more "natural" allergens—for example, *Aspergillus* or dust mite and the use of chronic exposures (weeks to months) (2). As such, these models are most germane to allergic but not other forms of asthma. These models have many weaknesses, including the following: mice do not have naturally occurring asthma; airway hyperresponsiveness is typically not as severe; mouse airways do not have much or any innervation, little if any bronchial circulation, thin airway epithelium and minimal smooth muscle, few if any mucous glands, and other differences in cell and molecular biology (3, 4). On the other hand, the mouse is a very attractive model system; indeed, when compared with previous animal systems, the mouse has many advantages as seen by the explosion of articles using mice as a model system (5–7).

Several interrelated issues involve the motivation behind the current investigations to better understand the underlying mechanisms of airway hyperresponsiveness using various imaging modalities. Structural alterations due to the ravages of inflammation ("remodeling") are correlated to lung function in asthma and are the focus of current investigations that seek to better understand the mechanisms that cause a loss in lung function that appears to be progressive or permanent. In mice, a complete picture of the structural alterations is readily available, but the measurement of lung function is more precise but clearly different than the FEV_1 measured in humans (8). The influence of airway wall structural changes, interdependence, and lung volume, as well as the mechanical properties of the airway wall, are believed to be pivotal events (9). Accordingly, imaging, together with computational modeling, is an important tool to address these questions of structure and function.

By altering lung volume and imaging the airways or by assessing ventilatory gas distribution, insights into the role of airway wall or parenchymal mechanics should be forthcoming (10). Considerable attention has been devoted to the axial location of the effects of inflammation (11). In this regard, imaging techniques are singularly important to pinpoint the location of the effects of inflammation and its pathogenesis. Imaging studies suggest that the entire airway tree is involved, but the role of severity and treatments effects are not well studied. Recent studies using antigen sensitization and exposure models suggest that airway closure, not airway narrowing, is a significant aspect of increased impedance and, especially when in combination, is directly related to airway hyperresponsiveness. Various imaging approaches can be used to assess and explore the mechanisms that cause airway hyperresponsiveness.

COPD. Unlike many other animal models of lung disease, attempts at producing animal models of COPD has been going on for decades. Also unlike in other lung diseases in which the etiology is in question, the cause of COPD is not. However, aside from exposure to cigarette smoke, investigators have also used proteinases, such as elastase or collagenase, chemicals, particulates, and, more recently, proapoptotic agents to cause emphysema (12–15). It is important to keep in mind that some of these models are modeling upstream (e.g., cigarette smoke), whereas others are modeling downstream (e.g., elastase or cadmium chloride) events of the pathogenesis (15, 16). Accordingly,

some agents cause inflammation, whereas others do not (e.g., vascular endothelial growth factor [VEGF]-2 blockade). As such, each model system has its advantages and disadvantages, but clearly, cigarette smoke is the most defensible as it simulates the best-known etiologic agent of COPD.

Exposure to cigarette smoke leads to neutrophilic inflammation, mucous metaplasia, airway fibrosis, and definitive airspace enlargement in several species. Genetic strain is particularly important because the response to cigarette smoke can be very strain dependent (9, 10). In addition, there are several naturally occurring mutant strains that exhibit airspace enlargement which have been used, including tight skin, blotchy, and beige mice (4). Mice are particularly useful animals to investigate COPD because genetic manipulation and overexpression of various cytokines, such as IL-13 (17), IFN-γ (18), and tumor necrosis factor (TNF)-α (19), have all been shown to cause airspace enlargement (emphysema).

There are several disadvantages of the current models: cigarette smoke takes many ($\sim$6) months of exposure to develop emphysema, which, even after the period of exposure, can be quite subtle in some strains; the notion that a single cytokine can cause all the manifestations of COPD seems simplistic; there are limited studies of the lung function of these various COPD models in the mouse; and the connection between airspace enlargement and loss of static elastic recoil or airflow limitation is often not apparent, drawing into question the relevance of this particular structure–function relationship (20). Nevertheless, these models provide unparalleled opportunity to study pathogenesis with developing imaging modalities. In particular, CT, given its prominence in COPD diagnosis and ability to adequately resolve the structure of the mouse lung (10), seems particularly promising.

Interstitial lung disease. Animal models of idiopathic pulmonary fibrosis (IPF) have provided substantial insight into biological mechanisms. The pathogenic paradigm in IPF has shifted from being that of a chronic inflammatory disease to a disease that involves a failure of epithelial–mesenchymal cross-talk and tissue repair, explaining, in part, the lack of success of antiinflammatory or immunosuppressive therapy to suppress the progression of fibrosis (21, 22).

The most common and best-described animal model is the intratracheal bleomycin model, which involves ALI that leads to a relatively early (14–21 d) fibrotic response (23). However, it is dubious whether this model is a good reflection of human IPF, and it is well known that it does not involve progressive fibroproliferation. In further contrast to the human condition, there is evidence for a partial reversibility of the fibrotic lesions in this model (24). Although it is still the standard model for investigation of potential antifibrotic drugs, better models are clearly needed.

Using genetically modified and replication-deficient adenovirus vectors, investigators have been able to transfer profibrotic genes into rodent lungs via intratracheal injection (25). This methodology causes transfection of bronchial and alveolar epithelial cells and allows transient transgene expression for a period of 7 to 10 days. The most marked changes occurred with transient overexpression of transforming growth factor (TGF)-β1, which caused the appearance of abundant fibroblastic foci in the lungs, and induced progressive fibrosis over 35 to 60 days post-challenge. This is in striking contrast to the bleomycin model of pulmonary fibrosis (24).

Despite our knowledge about the molecular and cellular pathogenesis of lung fibrosis in animal models, there is a lack of understanding of the functional consequences of these events. The only consistently used "functional outcome" of the models is survival, which is probably most often related to inflammatory and not fibrotic changes. Physiologic parameters have not been established in animal models, and thus are not routinely used. IPF models, in which density changes in the lungs occur with disease progression, would seem to represent a largely untapped area of application for imaging modalities.

Acute lung injury. ALI is characterized by hypoxemic respiratory failure due to noncardiogenic pulmonary edema. Acute respiratory distress syndrome (ARDS) is simply defined as a more extreme hypoxic event of ALI in humans (26). ALI has remained a major challenge, with multiple known risk factors and a mortality of 30 to 40% (27, 28). However, the mechanisms of injury and repair remain poorly understood. The histopathologic hallmark of ARDS is diffuse alveolar damage, with evidence of diffuse injury and necrosis of type I and type II alveolar epithelial cells and activation of the endothelium (29). Over the past several decades, numerous models of ALI have been developed, each with its own distinct advantages and shortcomings.

The three most commonly used agents to induce ALI in rodents are bleomycin, hyperoxia, and endotoxin. These three agents also represent the general classes of agents known to be directly injurious to the lung in humans, as follows: (*1*) drugs; (*2*) toxic/environmental exposures; and (*3*) inflammatory triggers of innate immunity, such as sepsis or pneumonia, respectively. Although endotoxin is perhaps the most widely used model of ALI for its reliable deployment of neutrophil-mediated inflammation, other models, such as saline lavage, intravenous oleic acid, hyperoxia, acid aspiration, and bleomycin, remain widely utilized (28, 29). Although late bleomycin injury is perhaps more widely recognized as a model of pulmonary fibrosis, the early pathology of intratracheal bleomycin resembles that of ALI. Imaging approaches to assessing the extent and character of injury have classically involved histopathologic examination of formalin-fixed and stained tissue specimens. Confocal microscopy can make use of a *z*-plane to generate stacked images that progress deeper into the lung tissue. More recently, the use of CT imaging has helped to better characterize the pathophysiologic differences between many of the commonly used models of ALI. For example, CT imaging has demonstrated that, despite the reliable presence of acute inflammation, different animal models of ALI manifest in unequal degrees of alveolar edema and collapse, as well as differing responses to recruitment (30).

Mechanical ventilation is critical to the survival of most patients with ALI (27), and improper use of mechanical ventilation in the context of ALI may contribute to added injury through a process referred to as ventilator-induced lung injury (VILI). One field of research in ALI has been devoted to understanding the mechanisms of VILI (27). With respect to these mechanisms, CT imaging has contributed greatly to our appreciation of real-time lung recruitment during mechanical ventilation and how recruitment with sustained inflation and positive end-expiratory pressure might contribute to or minimize VILI (30, 31).

Imaging Modalities

This section describes the following imaging techniques: videomicroscopy, MRI, micro-CT, micro-PET, optical imaging, and molecular marker imaging.

Videomicroscopy. The study of dynamic alveolar mechanics examines the behavior of alveoli during ventilation in the normal and abnormal lung (32). Physiologic properties of the lung that play a key role in both normal and abnormal dynamic alveolar inflation include pulmonary surfactant function and

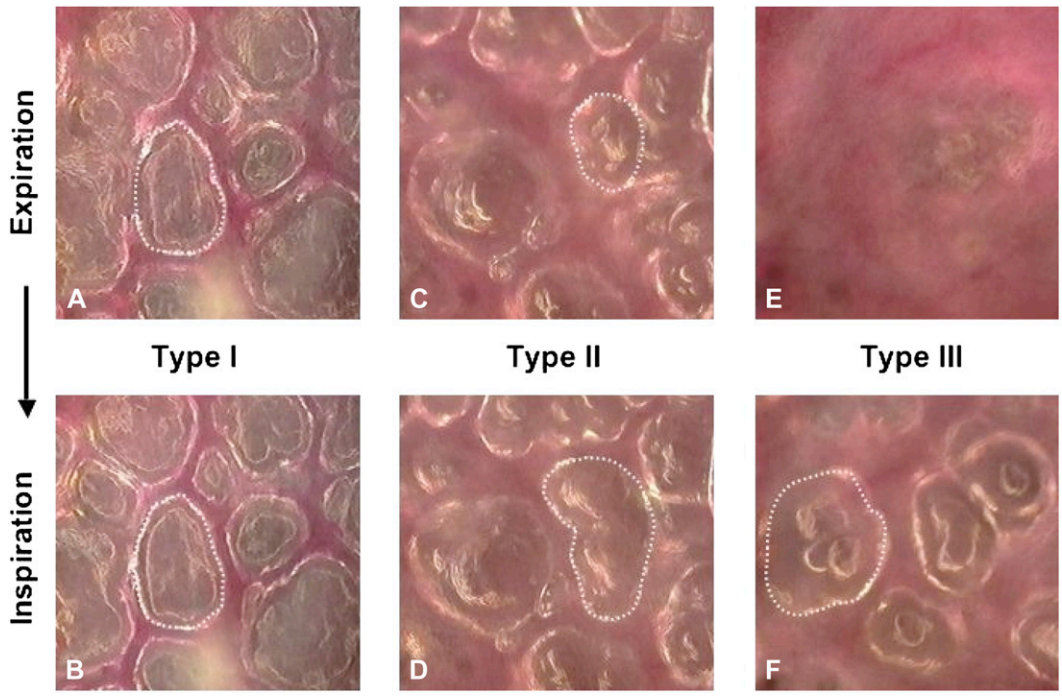

Figure 1. Photomicrographs depicting individual alveoli as they are inflated from end expiration (Expiration) to peak inspiration (Inspiration) during tidal ventilation in the normal (*A, B*) and acutely injured (Tween lavage) (*C–F*) lung. Alveoli of interest have been outlined with *white dots* and represent the same alveolus at expiration and on inspiration. Alveolar inflation patterns were separated into three types depending on the appearance of alveolar area changes with tidal ventilation. Type I alveoli change volume imperceptibly from end expiration (*A*) to peak inspiration (*B*). Type II alveoli change volume from end expiration (*C*) to peak inspiration (*D*) but stay inflated at end expiration (*C*). Type III alveoli collapse totally at end expiration (*E*) and reinflate with inspiration (*F*). In the normal lung, all alveoli exhibit type I inflation patterns. Reprinted by permission from Reference 64.

dispersal, the elastin/collagen supportive framework, and the three-dimensional architecture of the alveolus and alveolar duct.

The ideal investigative tool for the study of *dynamic* alveolar mechanics is one that can measure the three-dimensional changes that occur in the alveolus and alveolar duct continuously throughout tidal ventilation. Because this idealized technique does not presently exist, studies have traditionally used the assessment of signal-averaged populations of alveoli via serial CT imaging or pressure–volume curve measurement. Alternatively, static morphometric evaluation of individual alveoli in histopatholgic sections has been used, but this technique can be greatly limited by the potential for artifactual distortion during tissue fixation (33). A third option is to evaluate changes in the orthogonal projection of subpleural alveoli during lung inflation and deflation via real-time *in vivo* videomicroscopy, a technique that can be used during mechanical ventilation (Figure 1). This technique provides a unique insight into the dynamic alveolar mechanics of the living animal under normal and injured conditions (32). Although it has been debated that edema-filled alveoli negligibly change configuration during normal tidal inflation, *in vivo* microscopic evidence from animal models has helped confirm that repeated opening and closing of injured alveoli is a real phenomenon and likely contributes to the pathogenesis of VILI (32). Perhaps in its greatest potential, *in vivo* microcopy will help determine which modes of protective mechanical ventilation (e.g., high-frequency oscillation, airway pressure release, high positive end-expiratory pressure) or other adjunctive therapies (i.e., exogenous surfactant) optimally recruit alveoli and maximize alveolar stability during tidal inflation (34).

MRI. Over the past decade, MRI has been transformed from a modality with little utility in lung imaging to one that appears poised to measure pulmonary structure and function at exquisite resolution. This change has been made possible through the introduction of "hyperpolarized" gases whose signals have been enhanced by a factor of 100,000 or more through a variety of atomic physics methods (35). These agents can be inhaled (or, in the case of liquid state agents [36], injected), after which rapid imaging proceeds in a manner similar to normal MRI. The most commonly used hyperpolarized species are the gases ^{3}He and ^{129}Xe, since the hyperpolarized state of each is persistent enough to be useful. Unusual properties of these gases can be exploited to create images that reflect a variety of functional parameters. In combination with conventional structural images acquired from the tissues, MRI could become a very powerful platform for sensitive and specific evaluation of small-animal models of lung disease.

Simple hyperpolarized gas density imaging gives very illustrative pictures of the ventilated airspaces and highlights areas of decreased airway lumen and air trapping. In the context of small-animal imaging, these techniques have significant inherent resolution advantages over SPECT/PET. Exquisite, background-free ventilation images with near-alveolar resolution have been demonstrated (37). Quantitative measurements of lung ventilation can be acquired through comparison of differing numbers of contiguous hyperpolarized breaths, yielding a map of the fraction of gas replaced during each ventilation cycle (38). Various scanning approaches have also been developed to create dynamic images that reflect the flow of inspired gas and air trapping. Another set of techniques is used to map regional alveolar oxygen concentration by exploiting that molecule's ability to destroy the hyperpolarized gas signal. Carefully controlled measurement conditions (39) or mass balance considerations can be used to determine the rate of oxygen uptake into the blood as well, and accurate measurements have recently been demonstrated in small animals. A third protocol measures restricted gas diffusion in the lung airspaces (40) to reflect alveolar size and connectivity. The acquisition can be prepared such that microscopic-scale structure is encoded onto the macroscopic images.

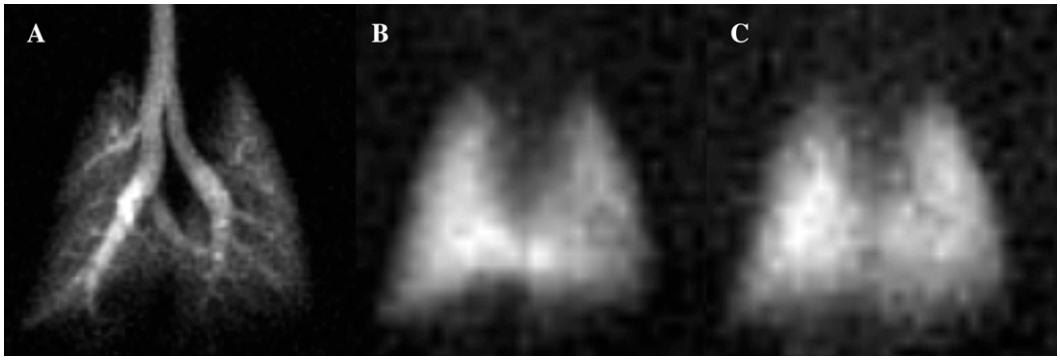

Figure 2. Images of hyperpolarized ^{129}Xe in three compartments of the rat lung. (*A*) Airspace, (*B*) lung tissue, (*C*) capillary red blood cells. ^{129}Xe exhibits three distinct frequencies in these lung compartments making such discrimination possible.

Although it has received less attention and has a somewhat lower inherent signal-to-noise ratio, the solubility and measurable frequency shift of ^{129}Xe in the blood and tissue make several novel and unique measurements possible (Figure 2). For instance, direct detection of the dissolved state ^{129}Xe fraction in tissue and red blood cells enables detection of blood–gas barrier thickening of less than 5 μm. Indirect measurements of ^{129}Xe exchange dynamics allow relatively high-resolution mapping of increased tissue thickness and gas exchange surface-to-volume ratio (41, 42). Conventional proton MRI is already useful for detecting edema (43) and, using ultra-short-echo sequences, even the low-density parenchyma of the lung can be imaged (44). Contrast-enhanced (45) and spin-tagging MRI of the pulmonary circulation allows pulmonary perfusion and blood volume measurements to be made, although, until now, this has not been applied to small animals. MRI is well suited to supply both structural and functional image content in a noninvasive and reproducible manner with the clear advantage of rapid acquisition times.

Micro-CT. Of all the imaging modalities, CT is perhaps the most familiar and yields the highest resolution, which is particularly important for thoracic imaging. Several commercially available, *in vivo* micro-CT scanners are capable of using a signal from an animal ventilator to trigger the scanner X-ray acquisition, known as respiratory gating (46, 47). The primary advantage of respiratory gating is not the reduction of breathing motion, as many presume, but rather, imaging the animal at near full inspiration instead of at near full expiration (48). Imaging a rodent at near full inspiration dramatically improves the visible lung tissue contrast and allows much more reliable detection of pathologies, such as lung lesions, compared with images obtained during free breathing.

The use of commercially available blood pool contrast agents, such as Fenestra (Advanced Research Technologies, Inc., Montreal, PQ, Canada), or more recently developed iodinated liposomal contrast agents (49) can improve the quality of thoracic images obtained from *in vivo* micro-CT scanners. Very often, the ability to distinguish vasculature from other pathology in lung images is important in the animal model under investigation.

Lung tumor volumes can be assessed using commercially available software applied to *in vivo* micro-CT lung images (Figure 3). Tumor volume measurements can be used to characterize tumors in terms of parameters such as growth rate and doubling time. Longitudinal images of animal models can yield helpful insights regarding the process of tumor development in new animal models. In addition, the newer contrast agents can increase the attenuation of even submillimeter vessels in the lungs (49).

Although CT is often the modality of choice for lung imaging because of the high resolution and natural inherent contrast between lung tissue and air, the radiation dose delivered by CT scanners can be quite high, especially if high resolution is required to visualize the anatomy. The tissue response to this radiation burden could have an impact on the animal model and the outcome being examined. CT imaging is also limited to observing changes in electron density, which is only an indirect measure of metabolic changes *in vivo*. The ability to distinguish different tissues is limited primarily to soft tissue, bone, lung, and fat. CT does offer fast and inexpensive imaging, with data relatively simple to evaluate because the anatomy is fairly obvious.

Micro-PET. Imaging of small animals, particularly mice, has become commonplace with current PET technology. At

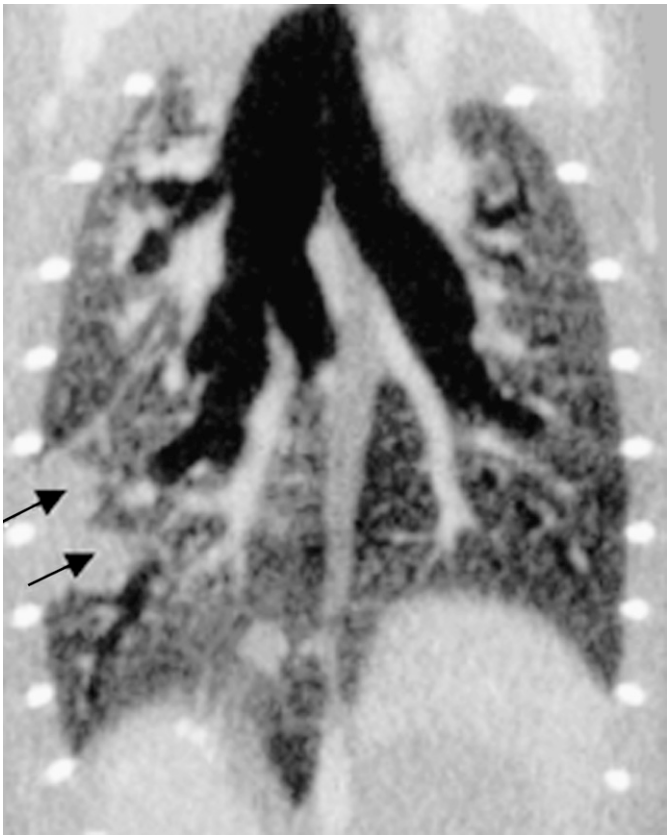

Figure 3. This micro-computed tomography scan was performed using Fenestra, which is a vascular blood pool contrast agent, and is responsible for the pulmonary vessel enhancement in this coronal thoracic view. Several lung tumors can easily be detected (*arrows*) and are readily distinguished from the surrounding vasculature structures.

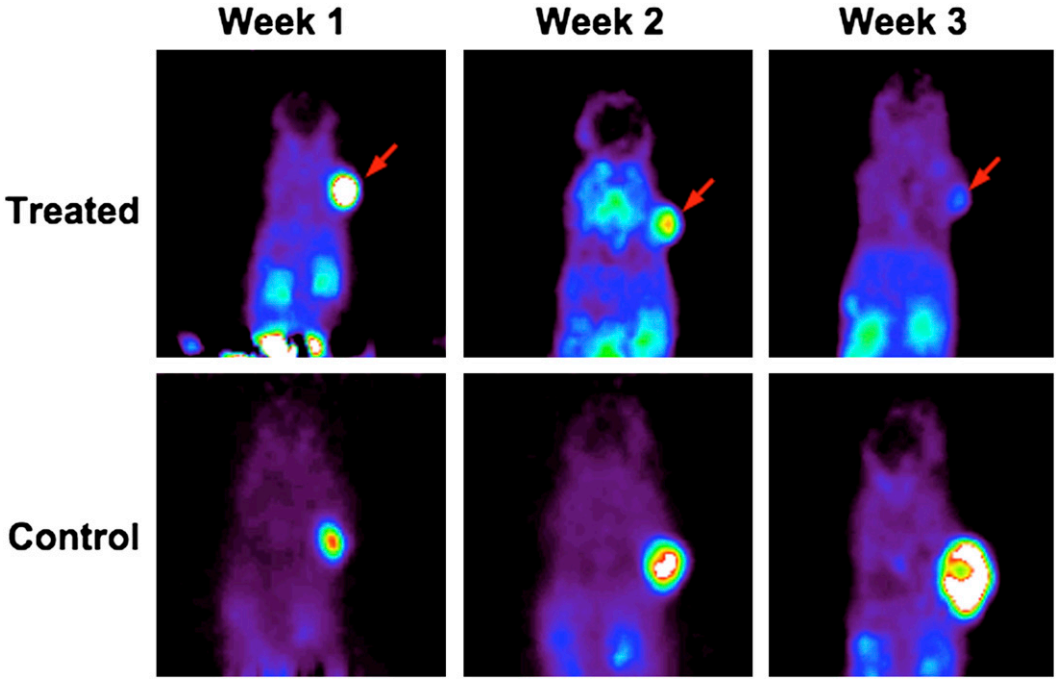

Figure 4. Micro-PET imaging of [^{18}F]fluorothymidine of a mouse treated daily with vehicle or ErbB-selective kinase inhibitor PKI-166 for 3 wk. *Red arrows* indicate location of subcutaneous A431 vulvar carcinoma tumor. Reprinted by permission from Reference 65.

present, small-animal PET imaging systems are undergoing a change, already seen in clinical settings, that pairs PET and CT imaging systems (50, 51). Molecular imaging research is increasingly using more than one probe or imaging method to interrogate the metabolic processes in living animals (Figure 4).

The strength of PET imaging is in providing noninvasive metabolic information about *in vivo* processes (52). It is most useful for comparing baseline versus treatment conditions or for measuring changes over time. The most common application is for oncology research; however, there is a large range of PET-related research, including neurotransmitter receptor assays, brain injury and repair, bone degeneration, antibody interactions, cell trafficking, and much more (53). PET can be used to estimate metabolic rate constants and is excellent for biodistribution and dosimetry experiments. With the ability to track rapid changes over short times, micro-PET imaging can also measure fast metabolic processes, such as transit time in a beating mouse heart, perfusion of probes in tissue, and uptake and clearance of probes to measure blood flow.

Limitations with PET imaging include low sensitivity to small or weak signals or small changes in signal strength, the potential for substantial radiation dose, the difficulty in providing absolute quantification measurements, and the fact that imaging information comes from the radioisotope, which may not be the imaging probe used due to metabolism. PET also requires a nearby cyclotron and radiochemistry center to create the isotopes and conduct the radiochemistry to make and purify the imaging probes.

Another imaging modality similar to PET is SPECT imaging, which uses single photons rather than coincidence detection. SPECT has the ability to image multiple probes at the same time using energy discrimination, and can have very high resolution at the expense of sensitivity. Recent advances in SPECT technology have pushed this method toward quantitative measurements and shorter acquisition times with greater sensitivity by using multiple probes. SPECT can often make use of longer half-life isotopes and kit chemistry for simple probe labeling, and thus may be a lower cost alternative or adjunct to PET imaging.

Neither PET nor SPECT have seen widespread use with pulmonary imaging, in part because these methods have primarily focused on oncology research using implanted xenograft tumors. The resolution also hinders accurate measurement of object sizes in mice; thus, tidal volume and tumor sizes can be hard to determine. Fortunately, the contrast with CT in the lung is good for these sorts of measurements. Both SPECT and PET are excellent for detecting tumors and monitoring their growth progress. Spontaneous tumors can be difficult to detect, stage, and monitor with nuclear medicine–based methods due to sensitivity, cost, and throughput considerations. Fortunately, implanted, injected, or inhaled delivery of cells, chemicals, or other agents can be delivered in a controlled manner, enabling imaging experiments at suitable times to follow *in vivo* responses. The most widely used PET imaging agent, [^{18}F]-fluorodeoxyglucose ([^{18}F]FDG), often has considerable myocardial wall uptake, masking out much of the lung region in mice. With proper experimental design, heart FDG uptake can be switched over to fatty acid energy usage by fasting, substantially reducing myocardium uptake and enabling better visualization of small signals in the lung.

Optical imaging. Optical imaging has rapidly grown over the past 6 years from a little-used method to become, today, one of the most frequently used *in vivo* imaging modalities. The imaging system is simple and easy to use and understand. It consists of a light-tight enclosure with a high-end digital camera to capture the light coming out of the sample, with excitation light provided for fluorescent imaging (54–57).

Optical imaging can take several forms, most commonly bioluminescence, where light is emitted from within the body, and fluorescence imaging, where a fluorophore is excited by an outside light source and a wave-shifted photon is emitted and imaged. Fluorophores can be produced *in vivo* by gene expression or they can be injected (Figure 5). Bioluminescence imaging is based on the expression of an enzyme in tissue that catalyzes the light emission reaction after a systemic injection of substrate.

The strength of optical imaging lies in the sensitivity, cost, and ease of use. Bioluminescence imaging is very sensitive to small signals, because the background signal is so low. Unlike

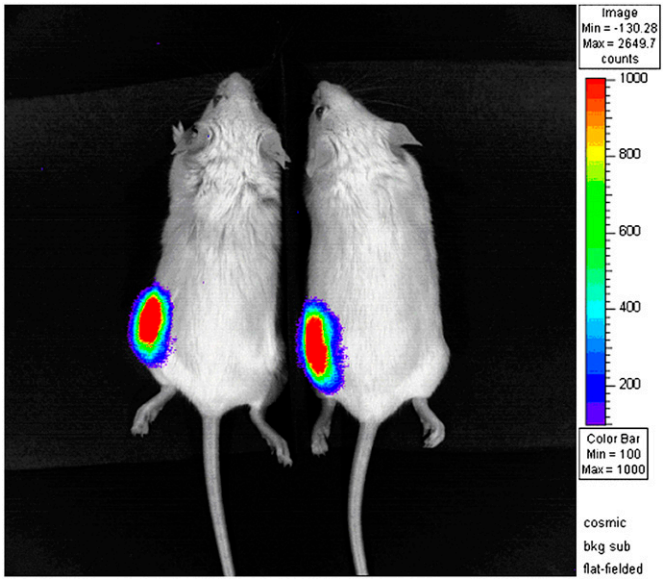

Figure 5. Bioluminescent optical imaging in tumor-bearing mice using luciferin. Images are composed of both photographic and luminescent overlay.

PET, where the radioactivity is always present somewhere in the animal, with bioluminescent imaging light is only emitted where the enzyme, oxygen, and substrate are present. Optical methods are good for qualitative or semiquantitative research, in which changes in a signal are followed over time. Light expression can also be verified by *ex vivo* imaging.

The weakness of optical imaging lies in the absorption and scattering of light, which is substantial. The detected light is therefore blurred into a relatively large region, and may not correspond well with the emission location, because light is variably absorbed and transmitted in different tissues. Approximately 1 in 1,000 photons makes it through a centimeter of tissue, so optical techniques are currently useful for small animals or surface imaging only, with most work restricted to mice. Fluorescence imaging suffers from substantial, nonuniform autofluorescence background noise, arising from hemoglobin, collagen, and the animal's fur. Autofluorescence can be removed by the use of multiple wavelengths, which can also be used to image several imaging probes at the same time.

Pulmonary imaging using optical techniques works reasonably well, because there is little intervening tissue between the lung and surface of the body. The scatter and absorption of emitted light require that mice be imaged from more than one side to completely cover the lungs; however, this is easily accomplished. Because the lungs are highly vascularized, there is good delivery of optical substrates to the tissue; thus, it is well suited to optical imaging in small animals. Clinical applications are very limited, because the light propagation means that animals much larger than mice will absorb the entire signal before it can be detected.

Optical systems vary greatly in size and shape. Some are optimized for imaging only a single animal at once, and others are suitable for many animals at once and are useful in high-throughput environments. Optical systems are increasingly being used to test whether molecular systems are functioning as planned, to see where and if signals are present, and to decide what probes to move into more quantitative assay methods.

Molecular markers. Molecular imaging in the lung can benefit from the low background signals typical for normal lung tissue. Tumors or inflammatory processes can readily be visualized using a variety of molecular markers. Molecular imaging approaches can be divided into two general categories: direct imaging (of endogenous targets) and indirect imaging (using "reporter genes" that generate a detectable signal to monitor endogenous processes). Direct imaging can be based on the presence of elevated levels of receptors, enzymes, and kinases, and so forth, at the target tissue. For example, elevated expression of glucose transporters and hexokinases leads to increased phosphorylation and retention of FDG in metabolically active tumor cells or activated immune cells during infection and inflammation. Increased expression of thymidine kinase leads to trapping of [^{18}F]fluorothymidine in highly proliferative cells (in tumors, bone marrow, and other tissues.) Overexpression of specific proteases (e.g., cathepsins) in tissues can be assessed using self-quenched peptide probes that contain appropriate cleavage sequences, and detected using fluorescence imaging (58). One of the most important reasons for developing molecular probes for *endogenous* targets is that these approaches are amenable to clinical translation (Figure 6). A significant limitation of using molecular imaging in lung disease is the sparse number of receptors, enzymes, and kinases due to the limited amount of lung tissue in general. Therefore, with the current state of molecular imaging technology, the signal-to-noise ratio for molecular imaging in lung tissue may limit the ability to produce significant data.

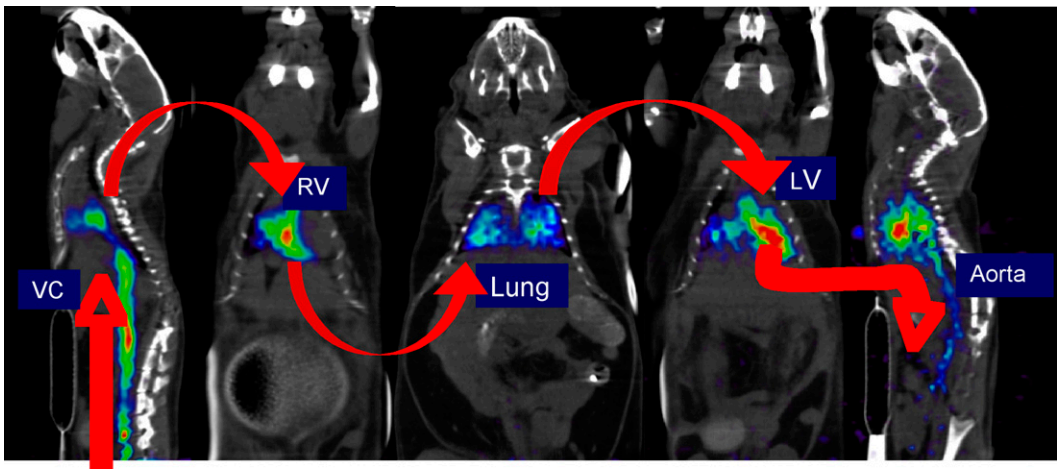

Figure 6. High temporal sampling, using coregistered micropositron emission tomography and micro-computed tomography. Mouse was injected intravenously via the tail vein with [^{18}F]fluorodeoxyglucose, although any radiolabeled probe would provide similar results. Passage of the bolus through the vena cava (VC), right ventricle (RV), lung, left ventricle (LV), and aorta is evident.

Reporter gene signaling can be linked to events of interest, such as gene expression or protein–protein interaction, and can provide a readout of these processes in living animals (59–61). For example, a common approach is to place a luciferase gene downstream from the promotor that drives a key gene, such as hypoxia-inducible factor (HIF)-1α. Reporter genes can be used to mark immune cells to follow their fate after administration to animals in models of disease or to visualize protein–protein interactions (62).

Many imaging agents have been developed from drugs. Some of the properties required of an imaging agent and a therapeutic agent overlap (specificity, affinity, bioavailability), but others differ and must be optimized for imaging (clearance from nontarget tissues). On the target identification side, genomics and proteomics research is yielding candidate markers for development of molecular probes. On the probe development side, powerful approaches, such as combinatorial chemistry and phage display, are allowing rapid identification of specific binding partners that can be turned into imaging probes for novel targets in disease, including conditions involving the lung.

Clinical molecular imaging is currently almost synonymous with PET with the glucose analog FDG (FDG-PET). FDG-PET has become an established test for staging the majority of malignant diseases and shows great promise for monitoring tumor response early in the course of therapy. Furthermore, FDG-PET has been used to assess the activity of various inflammatory processes. Because endogenous activity in the lung is very low, elevated FDG-PET signals can provide evidence of disease, particularly in focal processes. However, activity in myocardium may obscure structures in close proximity, such as those in the lung. Other processes that can be imaged clinically by PET include hypoxia, amino acid transport, lipid metabolism, and cellular proliferation, as well as the expression of somatostatin receptors and αvβ3 integrins (63). These types of processes occur in several types of lung disease, and molecular imaging may yield important information about disease mechanisms in the future.

CONCLUSIONS

The participants agreed that animal models of lung disease developed to date have been exceptionally useful and have led to a new understanding of disease pathogenesis. The replication of human respiratory disease in small laboratory animals varies significantly. Although the models appear to be accurate representations for some respiratory disease, such as asthma and COPD, other models remain less satisfactory, such as those for IPF. Therefore, further investigation and development of more accurate models of human disease were suggested. The participants concluded that there are unlimited opportunities to apply imaging techniques to better develop and explore small-animal models of respiratory disease, especially in real time in which the normal and unique architecture of the lung is preserved.

There was also a consensus that there were considerable current efforts to apply various imaging modalities to small animals, but that this effort struggles with issues of signal-to-noise ratio due to the small size of the rodents used. The unique structure of the lung creates both opportunities and difficulties in applying imaging techniques to studies in small laboratory animals. There are several technical issues that must be addressed and resolved. Although it was not possible to discuss all imaging modalities (e.g., ultrasound, quantum dots, whole body optical plethysmography), it was agreed by the participants that these should be included in future workshops. It was also agreed that experimental conclusions using imaging modalities will need validation and independent verification. Although some modalities have adequate resolution (CT), others are more limited (PET). Moreover, all the participants agreed that the time has come to move past qualitative pictures to quantification of the image data. By moving to better quantification of imaging data, standardization and/or technical guidelines will prove useful to future investigators. Future studies will provide unique opportunities for multidisciplinary research teams to explore the pathogenesis of respiratory disease.

RECOMMENDATIONS FOR FUTURE DIRECTIONS

Workshop participants agreed on the following recommendations for future work in the areas described in this document.

Animal Models

- Continually reassess current animal models of lung disease.
- Delineate appropriate criteria for animal models of lung disease.
- Develop animal models that better duplicate human respiratory disease.
- Focus on an integrated approach, from the submolecular level up to the organ level, in the animal models of lung disease.

Imaging Modalities

- Increase interaction between the integrative biological science community and the imaging science community.
- Utilize imaging modalities to investigate the topography of the lung pathophysiology.
- Utilize imaging modalities to investigate intracellular lung pathophysiology *in vivo* and in real time.
- Utilize imaging modalities to investigate and study temporal pathophysiological events.
- Utilize combined imaging modalities to better relate spatial and temporal events (e.g., CT with PET or SPECT).
- Utilize imaging modalities to determine how well various animal models reproduce the human disease condition.
- Explore technical advances for several imaging techniques, such as validation, increased resolution, and increased speed of data acquisition, to name a few.
- Develop better quantitative analysis tools for image analysis—for example, the application of stereological techniques to imaging datasets.
- Investigate the use of bioinformatics analysis techniques to the large datasets produced by image acquisition.
- Expand future workshops to include other imaging modalities and their potential application to respiratory disease.

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