Advancing Drug Development by Reducing Reliance on Animal Testing

Case Example: Pre-Clinical Animal Models in Lung Toxicology

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Transcript

Innovations in Lung Toxicology Safety Studies: New Approaches in Pre-Clinical Models & Clinical Monitoring

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- **Alexandra Maertens, PhD, Assistant Professor, Bloomberg School of Public Health, Johns Hopkins University**
- **Megan LaFollette, PhD, Executive Director, 3Rs Collaborative**
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Susan Winckler: (00:00:09)
All right, everyone. Welcome back. Those who are coming on in, let's make sure that we move the slide so our virtual folks know that we are back from lunch. as you know this morning, we focused on the current realities and challenges associated with preclinical lung toxicology in animal studies. This afternoon, we are moving to talk about solutions and future possibilities, looking at innovative alternatives and human relevant approaches. We'll explore what can be learned from the development of alternative methods and human relevant approaches and how these innovations may shape how we evaluate and monitor lung safety moving forward. We are going to hear from these six people in rapid succession. I hope that you are energized post lunch and I am not worried at all about anyone falling asleep because there is going to be great content from each of these. I'm going to invite Dr. Mary McElroy, who is head of discovery pharmacology and toxicology at Charles River Laboratories to the podium. Dr. McElroy, kick us off.

Mary McElroy, PhD, MBA, Head, Discovery Pharmacology and Toxicology, Charles River Laboratories

Mary McElroy: (00:01:30)
Great. Thank you very much. Thank you very much to the Reagan-Udall Foundation for the invitation. I'm super excited to be here. I've really enjoyed the discussions this morning and looking forward to presenting what we're doing at Charles River. Today I'm going to cover just a general

introduction of NAMs and AMAP, which is a Charles River specific grouping, a quick overview of the lung models. And then I'm going to talk in more depth about work we've been doing under a grant from the American Chemical Council to validate a Lung NAM for assessment of upper respiratory tract toxicity, provide some results and then just conclude. As the first speaker this morning gave a broad definition of NAM... I know when we've talked about NAM definitions within Charles River, it's often been quite a long conversation because it can mean slightly different definitions to different groupings.

But I would say in general, the ICCVAM definition is useful first to any technology or methodology or approach and combination thereof that can be used to provide information on pharmaceutical chemical safety. It's very general, but I would think that we would all agree that however you define a NAM, it always comes down to impact on the 3RS, replacement, refinement and reduction. Within Charles River, we have a grouping at a very senior level called AMAP, which stands for the Alternative Methods Advancement Project. Part of it is prioritizing in vitro models for the scaled and routine adoption of NAMs. AMAP is Charles Rivers initiative dedicated to the scientific and technical knowledgical innovations that help us fulfill our commitment to 3Rs and development of alternatives in animal testing.

This group was originated in 2024. At that time, there was obviously the FDA Modernization Act. It's clear that the science of human cell biology is advancing hugely. So we know a lot more about tissue level mechanisms of homeostasis and repair and obviously AI. Since that time, there's even been more regulatory momentum for the introduction of NAMs or human-based models for risk assessment. But I suppose the conversations alluded to this morning, is you can see the scientific drive and societal drives, how does it work in practice and what does it mean in practice? Again, I think this reflects Charles Rivers perspective, reflects the discussion today that NAM adoption is likely to transition from augmentation to selective replacement of animal studies over time. We see it as an evolution, not a revolution. I think that reflects the general view from this morning and rightly so.

Just to go a little bit deeper into the overview of in vitro lung NAMs, they can start from the very simple 2D models, which have been used for many years and have found use, but in the intervening time, we have developed to these air-liquid organoid cultures or spheroids or organoids that are submerged to include lung-on-a-chip, which, I believe, was the first organ on a chip, which include physiological stretch, and then to the AlveoliX's system of alveoli on array, which is a great technological advance. As the systems, they go from simple to more complex, and also there's an increased complexity of the epithelium and of the fibroblasts and other cell types that together help to remodel in more detail the alveolar airways or the alveolar barrier. These models have been used for obviously a diverse range of objectives from discovery to understanding more cell or tissue level biology. So the fundamentals of how the lung cell works in injury and repair situations, and also for safety.

This has all been generally out with the strict regulatory space. At Charles River, my team have been working on the air-liquid interface organoid cultures, generally available from commercial suppliers like Mattek or Epithelix. Now these models had the advantage over the 2D models in that they've got more... the structure is more representative of the human trachea, so they're primary bronchial epithelial cells or small airway cells or even alveolar cells. The upper airway models are

pseudostratified and they have tight junctions, which is very key for the lung because unlike most organs, the lung is very, very sensitive to fluid. We do not want excess fluid in our airways. So that's a very important role for the function of the lung and obviously to prevent infection in general.

Again, we have the mucocele escalator and the surfactant. These models have been shown to have both metabolic and drug transporters that are representative of in vivo. There's a lot of pros for the organoid cultures and from a CRO perspective, the fact we can purchase the model, it comes with a certificate of analysis and it's a constant project. The effect of shipping is well known and it's not detrimental to the tissue. That means the barrier for our clients to use it is low, it's available model and the throughput is moderate because we can run 24 well plates quite easily. In our labs, we've been using these models for safety assessment and sometimes the safety assessment has been for IND applications as we run to GLP.

What we're finding is that they're used on a case by case basis, maybe in a weight of evidence approach to demonstrate a safe inhaled, for example, ingredient of a perfume fragrant, set the safe inhaled doses for that perfume ingredient. When the American Chemical Council announced their call for projects, it meant that if we submitted a regulatory type grant, it would mean that there was a chance then we could develop a standardized protocol that would take the guesswork out of the protocol. It would make it less case by case and more of an uptake maybe for... replace like the acute rat inhalation study. It was a long range initiative from the American Chemical Council. It was a collaboration between ourselves, Mattek, because they were US-based, Greek Creek Toxicokinetics, which is Richard Corley and Patel. We had a number of objectives and they were generally framed around the ICCVAM framework I put here on the right-hand side about how would you approach validation and qualification of NAMs and how would you accelerate their uptake?

Our context of use was that we would suggest or hypothesize with good reason that the human lung upper airway model would be able to predict the toxicity of directly acting inhaled chemicals. Those chemicals that you might expect to deposit mainly in the upper airways and just... I won't read all these objectives, but one of the first ones was to take the acute exposure scenario with a panel of 10 chemicals and assess the extent of toxicity in those models and determine if it would rank in the known or the identified potency in terms of hazard. That was the GHS system, and the studies we run in two labs, Charles River and Mattek. In addition, because Mattek also have a rat model, we could run the rat model in the same way, and we could compare the rat data... what's toxic in the rat and compare it to what we'd hoped was good in vivo acute toxicity data from the rat model.

We were also looking at some aspects of when you expose at the air-liquid interface, generally it's by liquid application, which is quick and cheap and easy to do, but non-physiological. If you were to expose by aerosol application, would that affect essentially your dose response? Again, moving into the other aspects of if you take a dose that's known to be toxic or not toxic in the in vitro model, how do you then translate it to an equivalent dose in the whole lung? That was this in vivo to in vitro extrapolations. One of our first, just for our first kind of, let's say, dose setting work was done in 2D primary cells, so 9 to 6 well plates, exposure for 24 hours. For this talk, I'll just talk about cytotoxicity as assessed by alamarBlue. The assays with 10 chemicals run in both labs and this next

graph is just a summary of those data. Basically, we've got dose response, viability on the X-axis... sorry, concentration on the X and viability on the Y.

Basically the two pink and blue are the two labs. You can see generally we've got very good concordance between the two labs. We modeled to show that statistically the data between the two labs were non-statistically significantly different, which is exactly what we wanted. We had one exception with the first isocyanate, isophorone diisocyanate, and that we identified as a formulation issue prior. The different labs formulated and it was different times on the bench before being applied. We did the same study, but this time we moved into the 3D model, which we thought will be more representative. So single application by liquid application, four, hour exposure, washed off. The data I'll present are on tier because of the tight junctions and the viability based on alamarBlue staining. Essentially, just as we saw with the 2D model, good concordance between the two labs with no statistical significance between the dose responses when modeled.

So then having sort of confirmed we can get a protocol that's reproducible between labs, we determined the EC effective concentration 50 that caused toxicity. Now, I'll just orientate you on this graph. So we have the 10 chemicals along the X-axis and we have the mean EC50 concentration on the Y. This is the mean EC50 from both labs and as you go from left to right, you're going from high toxicity to low toxicity. As you would imagine, then the EC50 concentration becomes higher for a non-toxic compound. Generally speaking, as the compounds go from toxic 1 to non-toxic 5, the EC50 concentration goes up. So you can take more in before you have damage, basically. There's just one exception, and that's the isophorone, diisocyanate, which is number one category, which is life-threatening, which we think it might be a product of the model, maybe lack of macrophages, or it might be something to do with the fact that it's applied in liquid format and not an aerosol. That's to be determined.

But generally speaking, for most of our chemicals, we're ranking them on the basis of their GHS categorization for toxicity. Another minor point when you're looking at assessing toxicity by either junctional integrity, if you like, or viability, junctional integrity is usually more sensitive than viability, and that's what this graph also shows. alamarBlue concentrations are generally higher than the tear, particularly for the more toxic compounds. So the next... We're running over. I'm going to have to move very quickly just to say that when we did the same type of study, with the rat model, we get very similar values between human and rat. Just to draw this in for the conversation that was this morning, we're taking the in vitro concentrations in the rat, the EC50 values, and we're using the MPVD models or computational fewer dynamics to compare the in vivo, say, deposited dose along the trachea, and particularly the upper airways, and then use this to compare with our EC50 values in micrograms per centimeter squared. We do it for some of the chemicals. We're getting equivalency between deposited dose in the upper airways when modeled versus the concentrations we get from in vitro.

In the interests of time, I will move across from the aerosol work and summarize that. I hope I've shown you that we can get equivalents between labs. We can rank the chemicals in terms of their hazard based on in vitro values. We're working towards extrapolating those in vitro values to compare with them in vivo values, and we've got concordance. Thank you for your attention. Sorry I had to rush.

Susan Winckler:
Excellent. Thank you, Dr. McElroy. You've kicked us off in our... This is our afternoon tour of what might be and exploring opportunities. So I want to turn next to Dr. Alexandra Maertens, who is assistant professor in the Bloomberg School of Public Health at Johns Hopkins University. You have presentation number two. Go ahead.

Alexandra Maertens, PhD, Assistant Professor, Bloomberg School of Public Health, Johns Hopkins University

Alexandra Maertens: (00:17:30)
Thank you. My name is Alexandra Maertens. I work at the Center for Alternatives to Animal Testing. I am their data scientist, their AI person. I'm going to talk to you today about how my variational auto encoder can completely replace animal testing. Just kidding.

Susan Winckler:
But you got everyone's attention.

Alexandra Maertens:
So we get a lot of pushback sometimes when we tell people that we can do toxicology without rats, but I want to point out that in a lot of ways we are bringing toxicology back to the future. Toxicology did not start out overly concerned about the health of rats. It started looking at people. Most of what we know about carcinogenicity dates back to early studies when we looked at exposures. The very first carcinogen dates to 1775, and that was when Percivall Pott realized that there was a very rare disease, scrotal cancer that was mostly seen in chimney sweeps. This continued with occupational exposures that established aniline, dyes as bladder cancer, et cetera, et cetera. So traditionally, what we know about carcinogenicity was first established in humans, often occupational exposures, not always, and we used animals just to verify the mechanisms. I think there's a couple of important points to this.

We can't have a toxicology that is human based, but also I've heard a little bit about the challenges of using an uncertainty factor or can we get a better point of departure? But I would actually ask a slightly different question. Is a point of departure, which is one number, is used to represent very complicated phenomena, really a good way to think about population level health effects. As a data scientist, I do not want one number. I want the distribution. And by going over that, we lose a lot of really important data. When it comes to... I work mostly in environmental health, and I think there's a lot of lessons that we can learn from environmental health that apply to clinical and preclinical safety. If you think about what we know about the Bradford Hill criteria, which is how we established epidemiological evidence to prove causation, that again, was also based on humans.

When they looked, they said, "Hey, we see this different rate of lung cancer. Could be cigarettes, could be air pollution. How do we know which one?" They said, "Well, if you really quantify exposures, we're pretty sure it's cigarette smoking." Of course he was right, cigarette smoking does cause cancer. It's one of the few things that we can say does cause cancer definitively in humans. But they were wrong to overlook atmospheric pollution. They just didn't have the necessary molecular granularity and details to actually establish that there was a link. We do know this now. If you are breathing in Washington D.C. air for most of your life, as I have been, you

probably have a very large number of driver mutations sitting in your lungs. What turns that into air pollution is actually promoted by non-mutagenic mechanisms, and that likely accounts for the increase that we've seen in lung cancer and people who have never smoked.

This would be invisible if you're looking at rats because you really need a database of many, many people to see this. You need in depth epigenetic detail on not just their tumor but their surrounding tissue. But we can establish links like this or causal mechanisms if we have the right data and if we have the right tools. I've also heard a lot of people talk about in silico models to talk about the importance of better dosing, and that is certainly true. I have spent a lot of my career actually working on structure activity relationships. In theory, all chemical toxicity is a function of the structure. If it is a function of the structure, we should be able to predict it based on the structure. Why can't we? There are a lot of good reasons for this. We have tackled this for a number of endpoints.

We've done skin sensitization, all sorts of things. I'm going to talk about respiratory sensitization because we have no really validated animal model for that. We do have very good in silico models of skin sensitization. I think this is a good kind of demonstration of how you can move away from animal testing. Both EPA and ECHA have validated alternatives to skin sensitization because the combination of in silico and in chemical techniques are as accurate as the animal models are. So have succeeded there. We asked to ourself, "Okay, the molecular initiating event is quite similar. Can we predict chemical respiratory sensitization?" And we encountered a couple of stumbling blocks that will be familiar to everybody who has done a deep dive in the literature. I've heard a lot about the importance of going back to literature, connecting it, but you have to be very careful because the terms that are used in the literature vary quite significantly over time.

We discovered that people would talk about respiratory sensitization in 2010, but if you go back a little earlier, they were talking about extrinsic allergic alveolitis. In a lot of instances, there was not a consistent demarcation about whether they were talking about irritation or respiratory sensitization. This is true. One of the complaints we have in computational toxicology is that everybody wants curated data, but nobody wants to take the time to go through and dig out through the literature back in 1970, which is a PDF that's really grainy or it's buried in the basement of the library, but if you don't do this, this data disappears. So it is very important to collect and curate your legacy data. When we did this, we built our little model based on our structural descriptors and we found out, yeah, okay, we could predict it with about 76% balanced accuracy.

You may think 76% balanced accuracy, it's not that great. The reality is that is as reproducible as the animal model is of itself, and it is more reproducible than the animal model is of humans. By definition, that is as good as the computational model will get. Before we really knew this, those of us who were in computational toxicology, we don't always get our models up to about 75%, 80% accuracy, wouldn't go anymore. It was only when we had very large scale data sets of the reproducibility of the animal assays that we found that they were also 80% reproducible. We were hitting that limit not because of our problems, but the dataset. We did go back in this dataset and find that some of the mispredictions were almost certainly errors in the data set when we really dug in and look at the literature.

We also found out that structural alerts overpredicted. This is very important because a lot of drug discovery starts with, "Hey, let's get rid of everything that has a structural alert." But the reality is, if you have an unbalanced data set, so a data set that is skewed towards positives or skewed towards negatives, the algorithm will fail. The standard structural alert that is used for skin sensitivity had been a benchmark on a dataset that was largely positive. Once you actually had a balanced dataset, the accuracy went down to about 65%. So it was a little better than flipping a coin, but it was not a useful tool and it was certainly inappropriate for an era of big data. It will not surprise anybody in this room who is a biologist that we had activity cliffs. In other words, we have places where the chemical structures are very similar and we have very, very different outcomes. That is just a feature of data sets.

I will emphasize that this was done on 2D modeling. 3D modeling is now tractable in a way that it wasn't just five years ago. So these are going to get better, but they will only be as good as the data set itself. One of the things we did with this study is we worked really hard to balance the data set so that we wouldn't have it skewed one way or the other. How do you do that? How do you find negatives? In the case of respiratory sensitization, almost 80% of the chemicals that we have a curated endpoint for are positives. So we went back, we found a bunch of high production volume chemicals. We said if there was any report of respiratory sensitization, if we didn't find one, we declared it negative. To my surprise, we actually got a lot of pushback from this.

People were not happy at this evidence of... There's no evidence of harm, that's not evidence of safety. I understand that, but I do think that there is a bigger issue because these exposures, if it's a high production volume chemical, are by definition taking place. We can't make gazillion tons of chemical and nobody's exposed to it. We just aren't capturing the data. This is not an AI generated image. This was from back when you could study the effects of smog on people. Something that IRB, we can't do that anymore. I don't know. We cannot do this on people, but the reality is we are all being exposed. We just aren't capturing that data. This is extremely important for clinical and preclinical toxicity because many real world adverse drug events are caused by co-exposures. All lung exposures in the real world are co-exposures because you're breathing the soup of volatile organic chemicals, benzoypyrine, that will affect the toxicokinetics, that will affect the tight junctions, that will affect the immune response.

Most of those, in fact, the vast majority of them are invisible in the electronic health records. There's no, do you live in a high pollution environment, et cetera. So think about all the data that you are missing that is extremely important. Going forward, I think that chemical safety, both environmental health and preclinical toxicity, has to be biomarker driven. We have to actually collect the exposures and the disease mechanisms. I will just say, because I work in environmental health, that we have to start thinking about all the data we're missing. Agricultural workers, cleaning stuff, nail salons, high exposures, no data. They just disappear. But this is going to be a very difficult balance between privacy and highly granular genomic and exposomic data. A lot of people, they donated tumors to say TCGA, that data's public. Guess what? We can use algorithms to get your genome right now.

It's not anonymous. It is extremely hard to de-identify data. We will require electronic health record mining to analyze this. This will necessarily be AI driven, but this is its own problems. Data doesn't grow on trees. It doesn't just come to you with no manipulation. Somebody has to decide who

counts, what we quantify, and how we encode that. Because of that, all data is biased. We can be more thoughtful about this fact, but we cannot get rid of it. AI often makes this less obvious. For example, for respiratory sensitization, do you think asthma is encoded with equal precision for men and for women, for people of different background? How useful is the encoding of asthma and non-asthmatic? Some people have very low level asthma. All of this matters if you're going to be building your prediction model.

There are some other challenges for AI. So I just used Claude, which is what I used. So I asked, "Hey, is BPA linked to respiratory sensitization?" Yes. PCBs? Yes. Lead? Yes. Mercury? Yes. It turns out every chemical is somehow linked to respiratory sensitization. You might think, "Okay, yeah, we all know AI is hallucinating." In this case, I would not say it is hallucinating. I would say that the stochastic parrot is reflecting back our own literature in a slightly distorted way. As we become more dependent on AI, we need to think about how we correct for that. This is the comprehensive toxicogenomics database. I just looked up and I asked how many chemicals are associated based on a curated pathway with respiratory sensitization associated based on a curated pathway with respiratory sensitization gave me a huge number that's curated. It's also, as somebody who's worked in this field, a largely impossible number. When we then asked it, okay, just based on any sort of genomic evidence, it inferred the connection between 23,457 chemicals. So there's two things going on here, and they're both important to think about.

One is that there isn't a lot of incentives to publish, "Hey, I did this study on a chemical and guess what? Nothing happens." So the data's biased towards positive. The other thing that's going on here is that genes fluctuate a lot. So if you are just going to put a chemical on a cell or lung tissue, or even in human blood, whatever, you will see genes go up and go down.

So we need to think a little bit more carefully as we make toxicology more molecularly-based, what do we mean when we talk about adversity? Because if we do not take care of this, we will eventually have a situation, like we do with cancer, where literally every chemical causes cancer.

And with that, I'm done. If you found this interesting, you can sign up for a seminar. 10 seconds, yeah.

Susan Winckler:
Well done. Thank you, Dr. Maertens. I do want to know if the parrot was AI-generated.

Alexandra Maertens:
The parrot was AI-generated.

Susan Winckler:
All right, just to confirm.

Alexandra Maertens:
Everything else is not.

Susan Winckler:
That's my guess. So that's our second of our six. And now we're going to turn to a virtual presenter.

Hello, Dr. LaFollette, who is executive director of the 3Rs Collaborative. We can see you and we can see your slides, so please proceed.

Megan LaFollette, PhD, Executive Director, 3Rs Collaborative

Megan LaFollette: (00:31:00)
Okay. Awesome. Thank you guys so much. I've been warned that I may have an unintentional auto advance on some of my slides. That could not be taken out last minute, so please bear with me. But today, I'm going to talk about some methods that we see at the 3Rs Collaborative on ways to reduce reliance on animal testing, specifically on inhalation talks.

Now, just in case you're not familiar with our organization, we are a US-based nonprofit whose mission is to advance better science for both people and animals. And we do this through facilitating Collaborative 3R's efforts: focused on refinement, reduction, and replacement.

And just a brief note, because there's quite a difference between the English definition of replacement, as well as the 3R's definition of replacement... Oops, let's see. Maybe I cannot actually go backwards. So sorry. I'll just hopefully at some point be... If you guys can make it go backwards to my 3R slide at all, that would be great. Sorry about that. I rehearsed this and I timed it, and apparently my timing saved.

Yes, but the 3R's definition. So technically, refinement and reduction only apply once an animal study is required. Refinement's usually more about animal welfare. Reduction technically is usually about experimental design and reducing the number of animals per experiment. So from the technical definition, a lot of what's being able to be achieved is replacement. Even if we have to use some animals, eventually we're having fewer of them overall.

Our efforts span across critical topics. I'll just touch upon the three in orange today. First, very briefly, translational digital biomarkers. This is above and beyond what we've been talking about today, but these are non-invasive 24/7 continuous monitoring technologies that we can use in animal studies. And I just mentioned this because they can expand the translational relevance of animal research and potentially reduce animal numbers. I don't have a long-talk, specific example of this, but wanted to mention them when we're talking about reducing reliance.

We do have some overview papers from our initiative on this, if you are interested in this, including a validation framework specifically for regulatory use.

Second, very brief topic is artificial intelligence. Obviously, this has huge broad scope, great potential. We also have an initiative on this that is specifically focused on risk assessment and safety, including of drugs, and we have all sorts of different stakeholders involved: end users, developers, regulators. And I'm just going to mention and repeat this concept that one of the earlier speakers talked about, as well as the speaker before me, that AI can be reused to integrate different types of data.

So that in vivo data, that in vitro data, human-based epidemiological data, and that is where it can be really, really powerful. This is a figure from a currently under review paper for us. But I'm mostly

going to talk about microphysiological systems, complex in vitro models, organo-on-a-chip, spheroid organoids. We have a very broad definition at the Collaborative.

And we really see these as this impactful emerging technology that are already being used to assess inhalation, toxicity, and efficacy, although lots of that is currently in discovery outside of regulatory purview versus actually in the regulatory applications.

Now, we have a huge initiative on this. It's mostly commercial developers, but it does have end users, regulatory agents, consultants, nonprofits, and more, lots of awesome companies that we have involved. And we also have an MPS tech hub to help you connect with tech providers, consultants, and enabling companies.

You can scan the QR code, filter by disease type and with a publication hub. Of these, 15 self-report that they have lung models, and we have two consultants. Now, if you're like, "Oh my gosh, that's a lot of MPS companies with lung models. How do I actually get started? What's the difference between them?" Well, we have some things that can help.

Back in 2023, we hosted a lung-specific workshop with presentations from the below. You can watch that on our website. It's super helpful. Going to go into way more depth than I'm able to do in just 12 minutes today. Of course, it's a little old, so not every company's represented, and they have new technology and new data, but this will still get you a start. Now, I'm going to go into some case studies and highlights. This is high level. This is simplified. It's not all models, but it will at least give you a little bit of the overview of what's out there.

So here's a list of the lung MPS providers when I contact and said, "Hey, give me some information on your platform." Note that CN Bio isn't on here because they have their whole own talk after this. But most of these have mechanical stretch. They have dynamic airflow. Lots of them have both epithelial and endothelial cells. Some are immunocompetent, some are vascularized, some have tumors, some have other organs involved, but a lot of really great things are happening with these models.

What they actually can be used for? Lots of potential regulatory applications. As other speakers indicated, these safety studies, specifically mechanisms, and explaining why might we have seen something in a rat, but not in the non-human primate. Also, to explain early clinical results. Why are we seeing this variability where some patients are responding negatively and some are positively? Lots of other things on here. Modeling healthy or diseased lungs, drug repurposing, personalized medicine, lots that you can potentially do with these systems.

This is what they actually look like, or at least some diagrams and some pictures. You can see here, I think it's pretty exciting to see the vascularization, alveolar cells, bronchial cells, how there might be liquid flow, how there's airflow, how there's cells undergoing stress. Again, these are just a few pictures from these awesome models.

Now, I'm going to go into just a few specific case studies. First one is going to be for Emulate. This is drug repurposing. This is not toxicity. This is more efficacy. They actually were able to look at a lung model that had a viral infection, identify a gene target, then identify a compound that was already shown to be safe, test that drug efficacy to see if it could rescue that infected lung MPS.

And what's very exciting is it only took 12 months from the initiation of that study to successful licensing, and that compound is in clinical trials right now. Again, this isn't talks, this isn't safety, this is efficacy, but still under regulatory purview. Still, a really great case study of how these models can be used. Oops, let's see if I can go back. I can just go back one slide. I clicked too soon. Awesome. Thank you.

This is second general case study. Don't have really specific details here, but AlveoliX did report that their model has had data included in a number of different IND submissions all on these different topics. Yeah. So lots of different things these models can do.

Other simple case study, Dynamic42. They had a two-organ model lung liver. They partnered with a company, looked at Paraquat as a reference compound, showed very low binding and showed this lung specific cytotoxicity. Oh, gosh, sorry.

Last, but certainly not least, is ImmuONE. This is a good example of getting really specific, and this is where those MPS can really shine, where they saw that a problem with ADCs, with these antibody-drug conjugates, is DIILD. And our conventional models are just not predicting these. They figured out the reason that they're not predicting these is they don't have these specific receptors, and they're not seeing that uptake.

They were able to make a model specifically for this project that has confirmed receptors of this type that are much more accurate in actually predicting this specific type of lung disease. Again, this is very mechanism-related, going really deep into why are the models failing, actually establishing a gap, and then coming up with a specific solution. So again, another great case study of how these models can be used.

Okay. So that was rapid fire case study of a number of different models and things going on. What do I see from my perspective as overall strengths of lung MPS? Well, they can really recreate these tissue-tissue interfaces. You can dose them in lots of different relevant ways. I actually didn't mention that on the earlier slide, but lots of different dosing strategies, and they can include immune cells. They're physiologically relevant. You can have that air-liquid interface, and often that mechanical stretch.

They're obviously human cells. Well, they can be human cells. They can also be rat cells to get comparison data, but they can specifically predict what's happening in the human. You can use primary tissue that's either healthy or diseased, with different features depending on your specific scientific question. Animal cell models can remove some of the major anatomical differences between, say, rodents and humans, and also just provides some of that validation data, that mechanistic insights, generally having this reduction replacement of animal use. And another advantage is they can potentially require less test substance for dosing compared to animal models.

That being said, they still have limitations. As many of the speakers said this morning, there's likely that we're still going to need animals for that overall safety package for specifically drug development under FDA's purview. This is different than potentially hazard identification. They do have limited cell types. They're not mimicking the entire respiratory tract. They may have limited capacity for long-term exposure. There's not huge consensus of, "This is exactly what you should

do. These are the performance standards on how to have a good lung MPS." That just hasn't been established yet. And although they certainly can model diverse populations, they may not at this state. Again, I've noted this is also a concern with animal models. These aren't unique.

So are they ready for the regulatory stage? This depends greatly on the context of use and validation data that is provided, whether it's even a regulatory context of use to start with, but they're already being used. They're already being in IND applications. They're already in clinical trials. But again, we may not replace the entire animal study, but just certain animal studies, portions of them to explain them. It's an additional, really important tool in the toolbox.

They also can be submitted to the FDA ISTAND program if they wanted to be broadly qualified. At the 3RC, we're working through this process for liver MPS. That includes eight different platforms. Happy to talk more for that as needed. And I also just want to note, we don't have to qualify our models to be used in regulatory submissions.

And with that, I am officially at my last slide. Call to action is we'd love for you to join us in advancing better science for both people and animals. This is a link to our newsletter. And although I don't have a link to the survey, we are about to release a survey that's actually on benchmarking, use of MPS in discovery versus development. We're looking for a lot more information on that, people to respond. That will come out via newsletter, LinkedIn, et cetera. And reach out to me, email me if you're interested in talking more.

Thank you so much for the organizers for having me, and thank you for this panel altogether. Thanks.

Susan Winckler:
Great. Thank you so much, Dr. LaFollette. And I'll note that you even finished a little early, so your timing was brilliant, even with technical challenges. So thank you.

We're going to go to our next virtual speaker, who is Dr. Emily Richardson, Biology group leader at CN Bio. Dr. Richardson, we can see you and we can see your slides. There. And now you're on the first one. Please proceed.

Emily Richardson, PhD, Biology Group Leader, CN-Bio

Emily Richardson: (00:44:00)
Wonderful. Thank you so much. And first of all, thank you for inviting me to this really wonderful event. It's been fantastic listening to everything so far, and some really great discussions going on. So I'm really fortunate to have followed Megan. She's given me a really good introduction already, and so fantastic to see the breadth of different NAMs. So I'm going to come at this with the perspective from a NAMs developer.

So the first thing I wanted to do was just give you a perspective about what we're thinking about when we're building new models. Obviously, first of all, we want them to be predictive of the human. We're not trying to replicate animals, we're trying to replicate humans. But as well as that, we also want them to be usable. We want them to be used by drug developers. So the first thing we want is that they are reliable and robust. We want to ensure that they are cost-effective within

the context of use. And also, we want them to have the right amount of throughput for that context of use as well. So first of all, it's really great to think about human biology complexity, but we really need it to be useful as well.

So then, obviously, being representative of human biology, being able to give out clinically relevant endpoints that are translatable to the human. Thinking about dosing of various different modalities, from standard small molecules to biologics, cell therapies. Thinking about whether we can mimic pharmacokinetics and also about different dosing regimens. Can we also consider chronic dosing, as well as just acute dosing? And then we can get even more complex and think about immunological effects and also population variability, which obviously, as we move into clinical trials, is so important.

And so firstly, what is organ-on-chip, or MPS, which are microphysiological systems? I'm going to refer throughout this presentation as MPS. As Megan previously, really nicely introduced, MPS come in many shapes and sizes. Here, I'm going to just tell you about some common features.

So first of all, they tend to be human, although actually not always. We have started to see development of animal MPS that is used in translation. They also tend to use primary cells derived from patients, but again, not always. We also see cell line-based MPS and MPS that are built on IPSC-derived cells, increasingly as that technology gets better and better. And these cells tend to be patent within the MPS, to provide a better microenvironment that is more relatable to the human organ that we are modeling.

You also then tend to have fluidic flow. The idea of this is to mimic the flow of blood, and this provides better oxygen and nutrient supply to the cells. And we can also provide environmental control, such as sheer stress, electrical stimulation, and control of other factors. And as the technology improves and increases and becomes more advanced, we're seeing more and more of these exciting technologies come out.

And also, I wanted to give some insight into where we think MPS is helpful within safety toxicology studies. So we know that within drug development, we start off with hundreds of thousands of candidates, and it's really not practical to think that MPS can be used at this point. We use our really great in silico tools, our really good high throughput in vitro tools, such as a simple cell line screening assay or a spheroid. These are great screening tools.

They tell you whether a cell dies or not, at different concentrations, whether candidates are a huge risk. But ultimately, where you want to use MPS is really when you get down to the point of progressing your lead candidates, and where you really want to understand the human relevant mechanistic data that will lead you into preclinical animal studies. And this allows you to make more informed stop-go decisions, de-risk your drug development before going into the animal. And also, we were talking about reduction of animals in this conference. It allows you to potentially narrow down molecule selection, or potentially concentrations of drugs, or dosing regimens as well.

And as I mentioned earlier, we're also starting to see development of animal MPS. And I think earlier, we were talking about contextualizing two species results versus the human. And that's, again, a place where MPS can be really beneficial is if you get conflicting results in your animal

studies, gaining further confidence to be able to progress into human trials. So altogether, this is really where we see MPS as being really beneficial moving forward.

And so today, I'm going to briefly touch on, as I said, a case study that's from CN Bio and the PhysioMimix platform. Here, on the left, you can see what the PhysioMimix platform looks like. So you have a controller, which sits outside of a standard incubator. This powers up to six plates, and each of these plates are multi-chip. So each of these wells you can see here in the barrier plate are individually controlled chips, so individual repeats.

We have various different plates which power different organ types. So here's just some examples. So here, you can see on the far right-hand side at the top, our Liver-48 plate, which has 48 individual Liver chips. So you can see that you can really increase the throughput based on your requirements, and that's a really important thing to consider when you're looking at MPS.

So the plates that we have are all open well. That means you can take them in and out of the incubator as you would a standard plate. You can sample, you can dose as required. The plates are quite large. So you have a 600 microliter volume to do various different biomarker testing and a huge amount of cells that you can do high-content analysis, such as omics analysis, high-content microscopy, low cytometry. And altogether, what this gives you is a really good basis for doing mechanistic studies.

And so in the barrier plate, the way that this works is that you can use standard inserts, such as those that Mary was describing in her presentation, such as Transwells. These can be placed into these wells. You can do liquid or aerosolized dosing. It allows you to grow the cells at air-liquid interface, and then you have this flow that moves around the basolateral side of the insert.

And so when we're thinking about modeling the lung, these are the sort of things we consider. We want to be able to model the various different types of the lungs, so from the airways down to the alveoli. We want to be able to see that air-to-cell-to-blood interface, so being able to model that is crucial. Ensuring that we maintain a functional viability over time. We also said in the beginning, we want to be able to do acute and chronic dosing. And when we think about that in terms of functional viability, we are thinking about the tissue itself, so formulation of that barrier, and also the individual cell types functioning, such as goblet cells producing mucus as an example.

And then importantly, I think when we're thinking about toxicology, we also want to consider whether we can include immune cells, both in terms of tissue resident cells and also circulating immune cells, as we know this is important when we think about the lung.

So I'm going to briefly introduce you to the PhysioMimix Lung MPS. We have a bronchial and an alveolar MPS. These are built around primary cells. So as I said, these are taken from donors, so we can use different donors to look at donor variation. We use epithelial cells on the apical side of the Transwell, and then the endothelial cells on the basolateral side to form that air-to-blood interface.

And this is what the tissues look like after 14 days differentiation at air-liquid interface and fluidic flow at 0.5 microliters per second. And what you can see is they form these beautiful structures that we expect to see in the lung.

Now, what's really interesting is when we do that same experiment in a static condition, with the same cells] in a liquid interface, with the same media of 14 days, you don't see this sort of differentiation. You don't see that pseudostratified epithelium. You don't see these structures that are formed in the alveolar MPS. And the reason we think behind this is that the fluidic flow is really allowing that increased nutrient oxygen availability to the tissues, and this is allowing faster differentiation.

And so when we characterize these models further, we can do various different types of analysis, as I said. We can look at microscopy, we can look at the presence of cilia, which we can see stained here in the IHC images. We can look at the expression of mucus. We can also collect samples from these MPS and look at the actual production of these different molecules as well over time. And we can also look at gene analysis as well to determine phenotypes.

In the alveolar model, you can see here that we've looked at both type 1 and type 2 cells, and we're able to keep the formation of both of these cells over time. This is particularly important for the type 2 cells, as these quite often in in vitro models transdifferentiate into type 1 cells, so you tend to lose that population. And type 2 cells are obviously very important when we think about alveolar biology with production of surfactant and having that immunological role as well, which obviously we saw in this morning's presentations as being a particular interest in toxicology.

And so just to briefly show you what we can do with these models, we can challenge them using different molecules. So here, we just used standard challenges of LPS and poly I: C. We can look at the structural changes. We can also look at the inflammatory response of these models. So very briefly here, you can see there are different responses in the bronchial and the alveolar tissues. Importantly, in the bronchial model, we don't see response to LPS, which aligns with what we know from in vivo, as the bronchial epithelium does not express the TLR4 receptor. So this really nicely recapitulated what we expect.

We can also, as I said, include immune cells. So here is an example where we included monocytes, both circulating in the media to represent a blood population, but also in the epithelial side, which you can see in the central image in purple, where we added monocytes into the model and they integrated into the tissue and acted like an alveolar macrophage-like cell. And you can see here again, we can look at the inflammatory response, see how it changes when we have those immune cells in. Here, you can see in green that we then get that response to LPS in the bronchial model.

So finally, just to conclude, hopefully what I've been able to show you in this very short presentation is how we can use lung MPS really effectively for safety toxicology studies. Lung MPS can really faithfully recapitulate human biology. The fluidic flow allows faster and cheaper differentiation and also incorporation of immune cells, which is very important.

Using these insert-based cultures, we can do liquid and aerosolized drug dosing, which obviously is important for when we're thinking about respiratory safety. And because we have these open-well formats, we can have this ease-of-drug administration and sampling over time, which really allows us to look very deeply into mechanisms. And these are very adaptable if you want to add different cell types, fibroblast, smooth muscle cells. They're looking at donor populations. You can

really adapt it to the context of use. So if you're also thinking about disease models as well, they're really helpful.

And so finally, just to conclude, these are human-relevant. They provide mechanistic data to inform stop-go decisions, to de-risk drug development earlier, look at reducing our in vivo testing, and ultimately gain translatable data to confidently progress to clinical trials. So I'll stop there.

Thank you very much for your attention, and if you want any more details, please contact me on the details below. Thank you.

Susan Winckler:
Great. Thank you so much, Dr. Richardson. That was a great snapshot of an example of what might be. So let's turn to our fifth discussion today, or presentation in this section. I'll call to the stage Dr. Rachel Eddy, who is imaging scientist in clinical development at Vida. Dr. Reddy, please proceed. Eddy. We're ready for Dr. Eddy. That's what I meant to say.

Rachel Eddy, PhD, Imaging Scientist, Clinical Development, VIDA

Rachel Eddy: (00:58:41)
I'll take it. I'll take it. Thanks very much, and thanks for having me here today. So what you'll notice on my slides here, I guess I'm going to take a zoom out at this point. We've heard a lot about microscale systems, and I'm going to take a zoom out to how you can use quantitative imaging, particularly CT imaging of the chest, once your therapies are in humans. Of course, based on the nature of this workshop, focused on toxicity, but a lot of the measurements that I'll speak about are also applicable if you're actually wanting to look at the effects of your therapy.

And now, the other thing I want to point out before I jump into it is just the two titles that I have here to hopefully show you that I bring a perspective of how these measurements have been developed in an academic setting, but now applied and translated in a standardized industry fashion. And I'll go from here.

And so in general, if you're looking to monitor lung toxicity, what's done typically is looking at symptoms. So there's a lot of validated patient-reported questionnaires, and you'll hear a lot more of this in the next talk by Dr. Fahey, and then of course, a more objective measurement using lung function tests. These are typically measurements that are made at the mouth using pressure and airflow, but this is one of my favorite graphics to show when we talk about the typical clinical measurements of lung function, because it highlights the true complexity of the lungs.

This is a resin cast of the lungs, and so you see the airways there. And there's over 1,500 miles of airways in the lungs. And then you see the vasculature as well on the other side. Not shown even are the over 500 million airspaces in alveoli. And so when you're making these measurements at the mouth, you're reducing that complexity, the sheer size and volume of the lungs, to these single measurements. And so this is where I always challenge where we can bring better measurements to this. And so my solution, of course, is using CT imaging of the lungs.

So what I'm showing you here is a single cross-sectional image of CT. We're at the apex of the lung, and we know this because you can see the trachea in the center there. And so CT, for anyone not

familiar, is actually the clinical gold standard for imaging the lungs in 3D. And so it's a very rapid test where a patient would be laying on a table, moving through the scanner and asked to breathe in and hold their breath. And the breath holds are on the order of eight seconds or less at this point. And so we're typically acquiring a 3D image from the apex of the lungs down to the base.

Now, all I'm showing you in this graphic is four different slices, if you will, through the lungs, but it is a tomographic or 3D modality. And so what this looks like from a... Hopefully, this video is going to play automatically for me. What this looks like in the clinic and what a radiologist would typically be doing is scrolling through. So you have a sort of lineup or a stack of axial slices and you'd scroll from the apex down to the base. And you'll see it get close to the diaphragm and just past the diaphragm here.

And so in the clinic, of course, that would be visually read for things like lung nodules. In this case, lung toxicity. And so before I move on about the measurements, I just want to make a note about terminology. And so you're hearing me call it CT imaging. You may have heard it called CAT scan before. You may hear it or see it published in the literature as MDCT or HRCT. These effectively really all mean the same thing at this point. And really the reasons we've moved through the same thing at this point. And really the reasons we've moved through this terminology is because the technology has advanced so far, that we're now in a high resolution state. And so I want to make a note about image resolution before I move on here. And so this is a graphic of just a 2D where you can see how the number of pixels in the image increases the crispness or the contrast that you can see of that letter R. And so what this means in the context of CT imaging is that now our pixels in the image or voxels in the case of 3D allow us to render the images in 3D and in the different imaging planes. And so what you see is the axial plane cutting cross-sectionally and the other planes there as well. So that's really the advance of the high resolution imaging that allows us then to measure and quantify different structure function in the lungs.

So a range of what we can pull out from a chest CT on any given person, see a range of vascular tissue, airway as well as functional measures. And in the context of lung toxicity, what I want to focus on is the tissue measures. But based on what we heard this morning, I did want to make a call-out for the functional measures. And so we heard this morning a lot about the particle size. And so I want to propose that you can also actually use quantitative CT imaging to model in a patient specific fashion because not only are mice just not small humans, each human is not the same. And so you can actually use CT imaging in a patient specific fashion to model how your inhaled drug will actually deposit, but I'll digress from that just to focus on the quantitative measures of lung toxicity here.

So the first case I'm going to show you is a scan from a healthy adult male. And I'm going to take a step back first just to orient you about what the intensity of this image means. So I'm showing you the scale of Hounsfield units on the bottom here. They're called Hounsfield units from the original inventor of CT. And so this is a linear scale of the intensity or the brightness of the voxels, which are directly related to the density of a given tissue within that voxel. And so it's anchored on this scale from -1,000 to zero where -1,000 would be air. So you can see the main airways are quite dark because they're filled with air, up to water. Of course, I've sort of truncated the scale at zero here for simplicity, it does go past zero where more dense things like blood, bone would have a brighter intensity, and so that's what this is showing you here.

So things that are darker have lower density and things that are brighter would have higher density. We talk about this because it is an x-ray based modality. We tend to talk about this in terms of the attenuation of the x-ray. And so something that is lower density would be lower attenuating like the lung tissue versus something that's higher density. And so it's actually a relatively simple computational problem at this point because of the contrast between the lungs that are low density and the higher density surrounding tissue to generate what we call an automatic segmentation of the lungs. And so all this really means is outlining the lungs from the rest of the surrounding tissue so that we're isolating our region of interest for quantitation to just the lung tissue. And so that's what you see outlined here in blue.

This can be done very rapidly and completely automatically at this point. And so then what we do for all of the voxels or pixels inside the lungs, we would plot the intensity of them on a histogram. So now what you see on the bottom are the Hounsfield units. Again, on that same -1,000 to zero scale, and simply just the frequency or the number of voxels that lie at each of those Hounfield units. And so we've defined within this -1,000 to zero range, different anchors of normal or low or high. And so for this healthy adult male, you see the thresholds of -950 and -700 Hounsfield units. And you see the bulk of that histogram falls within that normal range. Now, before jumping into toxicity, I want to show you what it looks like when it actually shifts more negative, and so it shifts to lower attenuating.

So here's an example of a patient with smoking related COPD and emphysema. And so you see the density curve then shifts more negative. It shifts to the left. And so then you can see in green there now, and then how we quantitate this on a density scale is so this is the low attenuating areas below or less than -950 Hounfield units. Now this threshold was determined and anchored against histology and shown to be very sensitive and specific for histologic emphysema. And I want to call out this recently published state-of-the-art position paper from the Fleischner Society that proposes ways to use this and other biomarkers in trials and so can share that if there's interest. But then jumping into toxicity then, so now showing an example where you see some inflammation, we heard about ground-glass opacities.

Now that's what you see where the density of the lungs actually shifts upwards now or shifts less negative. And so outlining this in yellow and showing the shift of the histogram now to higher attenuating. We would call this the high attenuating areas then. And so that falls within a range of -700 to -250. I'll just make a note that that 700 has actually shifted around to -700 from previously -600, and that was with the onset of COVID actually. And so attempting to be a little bit more sensitive to those earlier, maybe those earlier changes that might not be overly fibrotic, for example, but would be likely related to inflammation. And now the HAA measurement then as a quantitative biomarker has been shown that when greater than or equal to 10% of that total lung volume, and so you see it outlined with the yellow hash there, is sensitive and specific for clinically relevant abnormalities, and those were as deemed against a clinical radiologic view of CT scans.

Moreover, these lung density measurements, and that includes the range of the measurements that I'm showing you along the scale on the bottom, are highly repeatable. This has been shown in a recent publication where people actually underwent chest CT repeated over four to six weeks, and showing that those density measures were highly repeatable with coefficients of 0.97 and greater. And so beyond that then, I'm showing now that we can actually now take this from an academic

setting where the measurements have been developed and validated into an industry standard report. And so it can be a fit-for-purpose standard report. You notice the CE mark on this hopefully. And this is the same patient that I showed you where I showed you that yellow histogram. You can now see that their high attenuating areas indeed are greater than 10% of their lung volume, and so that could possibly be flagged for further follow up, further treatment, and so on.

And so just to make a note before I wrap up and sort of summarize what I told you about CT and what we can quantify, I do want to make a note about radiation dose. Of course, CT is an x-ray based modality, so it does carry some level of risk. What I'm showing you here are radiation doses for a range of different, what most of us would experience just in our background radiation, if you flew from the West Coast like I did. And so you don't have to appreciate necessarily the millisievert units here, but just appreciate the relative magnitude of just what you would experience on average living in the US, or even what you would experience having a clinical chest CT for your clinical care and follow up. We have developed standardized research chest CT protocols, and I use the less than five millisieverts symbol here because there's a few publications whereas CT technology have cited some papers there, one from 2016 and one more recently, just from 2025, that as CT technology is getting better and better, we're able to bring this radiation dose lower and lower and lower.

And so though not really shown here, there are some CT modalities and CT techniques that allow you to achieve the radiation dose on the order of a single 2D chest X-ray. So there's a lot of excitement to be able to maintain the 3D volumetric and quantitative information and reducing that risk to patients and research participants. So in summary here ... I'll bring up my summary here. So CT has evolved into a standardized, quantifiable and repeatable technique to measure lung structure and function. And so this is what I've hopefully told you today, that lung density measures are rapid and they are repeatable. We do have a clinically relevant threshold for toxicity using a high attenuating area of greater than 10%, and quantitative CT importantly can be done in a standardized way. This includes across different CT scanners, different CT manufacturers, and can be quantified in a standardized dose, or sorry, a standardized way, and that our radiation doses do continue to go down and reducing the risk to study participants. Thank you.

Susan Winckler:
Great. Thank you so much, Dr. Eddy. Really helpful to get a different way of thinking about the opportunities in this space. So the last of our six-person overview here is joining us on our virtual stage. We're going to turn now to hear from Dr. John Fahy, who is Professor of Medicine, Division of Pulmonary and Critical Care Medicine at the University of California, San Francisco. Dr. Fahy, now we have your slides up and ready to go. We can see you, so please proceed.

John Fahy, MD, MSc, Professor of Medicine, Division of Pulmonary and Critical Care Medicine, University of California-San Francisco

John Fahy: (01:13:04)
Thank you for inviting me to participate.

Susan Winckler:
There we go. Go ahead.

John Fahy:
Yeah. Great. Thank you. Thank you for inviting me to participate and to the Reagan-Udall Foundation for organizing this important meeting. So as mentioned, I'm a professor at the University of California, San Francisco in the Division of Pulmonary Critical Care. I think I might be the only practicing MD who's on the call today, so I'll try to provide some clinical perspective. I also provide the perspective of having founded Aer Therapeutics and having had some experience in the last few years at dealing with some of the issues that were raised by others earlier. So I'm going to briefly just review because it level sets my own talk, what the reasons are for the relatively high safety margins for inhaled drugs, or at least, what I perceive them to be from where I sit as a clinician and drug developer. And then review a little bit this monitoring issue, what we have currently for drug-induced lung toxicity monitoring and clinical trials of investigational drugs and what the sort of pros and cons of those are. And then really review lessons from the use of CT lung scans and clinical medicine in monitoring lung toxicity from approved drugs.

And just to follow up on what Rachel was saying there about CT scans. So I started practicing medicine in 1985, and I've qualified as a pulmonologist in 1993. And in the evolution of time since 1993, the use of CT lung scanning in clinical medicine has really evolved to where it's a very valuable tool now for the diagnosis and management of lung disease, so much so that it's taken over from tissue-based diagnoses in many cases. And so what I've noticed as I've entered this world of drug development is that the same advances in imaging have not been applied to drug development as they have been to clinical medicine. And so in particular, I'll probably end by making a point about CT lung scanning to emphasize Rachel's point, that we can use lung CT scanning to monitor lung toxicity for investigational drugs.

So to this point of where these safety margins came and where this conservatism comes from in terms of inhaled drugs versus other routes of delivery, so it seems like the relevant division at the FDA considers that safety margins of these levels, 10 in mouse and rat, six in the dogs, and five in monkeys that have been reviewed by others earlier, including Jeff and Matt and Adrian, the reasons for these high safety margins seem to relate to the fact that the FDA considers oral inhalation along with injections as having the highest risk among drug administration methods because the drug gets delivered directly to the surface of the organ. And the unique toxicity concern relates to this concept of non-monitorable. So it's considered that inhaled drugs can cause inflammatory responses and tissue changes in the lung that are not easily monitored. And because they're not easily monitored, we need to be very conservative in calculating doses for delivery to humans. And not just that, but you set maximum clinical doses as distinct from starting doses that are based on preclinical data.

All that reflects a conservatism that's part of this field that I think we're all beginning to wonder, is that serving patients and industry well? So if we think about monitoring for lung toxicity outside of the imaging just for a minute, we've typically relied on symptoms and quality of life, and there are ways to quantify symptoms and quality of life using validated questionnaire instruments. And I won't go into all of them here, but there's some well-known ones in our industry, including the St. George's Respiratory Questionnaire, which is a sort of a generic questionnaire for capturing symptoms and quality of life. You've got some specific airway disease questionnaires, including the asthma control tests and the COPD assessment test, and you've got ones for interstitial lung

disease as well, including the Kings, which is well known. And then of course, we have lung function tests, including spirometry, measures of lung volumes, diffusing capacity and oxygen saturation. So when we have these symptoms and lung function, why might this scenario have developed where the lung is considered non-monitorable?

I think the concern has been partly that these methods, symptoms, and lung function tests are both not sensitive enough for purpose here and maybe not specific enough. So you can have, perhaps if there's histological events going on in the lungs, pathological events, maybe they occur prior to symptoms. So you can't rely on symptoms as a sentinel kind of marker of that. And in terms of lung function tests, they also have some sensitivity issues, but they also have specificity issues. And of course, we're studying people with lung disease, and some of the specificity issues may relate to, well, are these changes in lung function a function of the disease or a function of the API? And they're non-specific as well, because you can have a reduction in lung function for lots of different reasons. So this is where lung imaging comes in. And you have chest x-rays, CT lung scans, and of course, we've magnetic resonance imaging. Chest x-rays are easily available. The radiation exposure is not very high. MRI is available and there's no radiation exposure. So those two have some advantages, but the main advantage of chest x-rays is not sensitive enough.

The big advantage of computer tomography lung scans were sort of outlined very nicely by Rachel, and I'll just go into some of them in more detail here for emphasis. So as Rachel mentioned, CT scans are superior to chest x-ray because they provide high resolution, three-dimensional cross-sectional images, and they allow for detection of small lesions and subtle abnormalities. You can pick up millimeter size lesions in the lung. You can pick up lung cancers, obviously, much more subtle abnormalities related to changes in attenuation that can be specific in the right clinical context. So you have this high sensitivity and pattern-based assessments in multiple planes, not just in the axial plane, but in the coronal and sagittal plane as well. So the CT scan samples the whole lung. And in that way, it's very valuable because you're not really just sampling the lung because we're taking slices ... Typically, 600 slices per person. It depends on the size of the person. You're getting, essentially, you're doing a whole sampling of the lung, and that's what gives rise to its sensitivity and power, really.

And in this regard, just to come back to interstitial lung disease, and we heard a lot earlier, we heard some passionate pleas earlier around idiopathic pulmonary fibrosis, which is one type of interstitial lung disease. When I first started practicing pulmonary medicine, you were not allowed to diagnose an interstitial lung disease without a biopsy. It was sort of like cancer in that regard. You can't start treatment without a histologic diagnosis. That's no longer the case. CT scans have largely replaced lung biopsies in the diagnosis and monitoring of interstitial lung diseases. And these interstitial lung diseases, it's a classification that describes a number of lung disorders characterized by inflammation and fibrosis that affect the interstitium and parenchyma. These are the kinds of drug toxicities that regulators fear. And the point I'm making here is that CT scanning can detect them. And you also heard Rachel talk about the Fleischner Society, which is a well-respected international body that does a lot of sort of standardization here. And the Fleischner Society has published the fact that they're comfortable now diagnosing interstitial lung disease based on imaging criteria.

There are exceptions, but in the large part, if you go to a doctor with shortness of breath, they'll do lung function measurements and they'll do CT scanning, and 95% of the time, you'll have a diagnosis, and you will not be subjected to a biopsy. So just an example of idiopathic pulmonary fibrosis, which is one of the most serious of the interstitial lung diseases. It's idiopathic, we don't know what causes it. Typically comes on in the fifth or sixth decade, has a lifespan that's not that different from cancer. Most people die within five or seven years. And so it's a feared diagnosis, and there are typical patterns of idiopathic pulmonary fibrosis that can be seen on CT lung scans. Some of them, to people in the audience who aren't physicians or are familiar looking at these, it can look a little subtle. Say if you take panel A here, this is an axial slice that's just in the region there up by ... I don't know if you all can see my cursor, but in the region up around A there, there's some subpleural increase in attenuation, this whiteness appearing where blackness should be.

Similarly, in B, this subpleural whiteness appearing where blackness should be. And that goes through the stack here. You can see here in panel F, that this so-called term of ground glass ... It's interesting how these terms come up. They obviously came up in olden times in radiology. I'm not sure any of us even know what ground glass is now, but it's a descriptor for an increase in attenuation. And I suppose it does look like ground glass. You can see the circle here showing whiteness where blackness should be. And as we'll talk more about ground-glass opacities, but the thing about this is the pattern of ground-glass opacification is important for the specific diagnosis of the interstitial lung disease. So in the case of IPF, we don't like to see subpleural opacification, and you can see some honeycombing from the traction bronchiectasis, and this appearance is very typical in the right clinical context.

So the other important thing is that many drug toxicities manifest as interstitial lung disease. These are approved drug toxicities. So there's this term drug-induced interstitial lung disease or DILD. Multiple molecularly targeted and immuno-oncology agents are associated with this interstitial lung disease, including more recently the immune checkpoint inhibitors, targeted cancer therapies, and there's a known pattern of interstitial lung disease that can occur across these drugs, including these different subtypes that will be recognized by clinicians and radiologists. I won't read them out here. I'm going to go into them in a little more detail. So when you have these sort of known patterns, many of them are characterized by this ground-glass opacity, GGO, or increased attenuation. And the pattern of GGO can vary across the different diseases. You can get a more consolidating appearance, which is more pure white than this ground glass, that's called an organizing pneumonia. You can get a hypersensitivity pneumonitis. You can get simple pulmonary emphysema or eosinophilia.

The importance is that this can be quantified and it can be quantified automatically and with a high degree of precision. And what's interesting about that, as I began to look at what's done in, say, even these preclinical animal models, when you look at the histopathologic measures of toxicity, they're typically on a relatively semi-quantitative scale that goes from one to five. So you get a severe effect as five and a mild effect as one. There's some subjectivity to that. It's not a particularly wide scale. These GGO percentages can go from zero to a hundred percent. So you've got a fairly large dynamic range, which is helpful, and it's automated and it's more objective. So you have these different kinds of patterns that you'd need to be a radiologist to determine, but you can

determine from the pattern of increased attenuation, what you're kind of dealing with, and you can determine whether it's widespread or focal. And so it's very sensitive to picking up small events that may be important.

You can get an organizing pneumonia, and then that can be shown to reverse after you treat the ... You can stop the drug and then treat with steroids in many cases, and you can show that the effect is reversible. So you can monitor, and what can appear like scary lesions, go away. And most drug-induced toxicities go away. And that's not private to the lung. That's true of any drug toxicity. There are some that don't, but in general, most toxicities, when they occur, when you stop the drug, they go away. Now, there's a particular example I wanted to bring up, which was this trastuzumab deruxtecan, this Enhertu product that came out and was approved in 2019. It's this antibody drug conjugate targeting ... Actually, it's not the EGFR2. It's a member of this family. It's HER2. And there's a drug cargo, a topoisomerase inhibitor conjugated to the antibody. And this drug has been very important for patients with metastatic breast cancer, and also it's been now approved in lung cancer and stomach cancer as well.

It causes interstitial lung disease. Sometimes fatal interstitial lung disease, and it's reversible with treatment most of the time. In the interest of time, I'll pass by this one. But one of the things that's come up with regard to should you use presymptomatic CT scanning to detect early onset drug toxicity? And there's now consensus in the field that this trastuzumab deruxtecan drug should have a proactive monitoring strategy aimed at early detection because these patients need to be treated for a year, and if you wait too long and they get very severe disease, they can die from it, from the drug toxicity. So now there's consensus that not only in monitoring symptoms, but they should have a CT scan every six to 12 weeks during that year of treatment to pick up toxicity, and a similar monitoring strategy could be employed for investigational drug development.

So if I could go back to the prior slide just once, the summary one. I don't know if I can go back. I just want to make a couple of summary points and then I'm finished, obviously. Can we go to the first slide, the summary one? Yeah. So the concern that inhaled drugs can cause lung inflammation or tissue damage that is not easily monitored in human clinical trials is actually not true. These inflammatory responses can be addressed using CT lung images, and this is a testing strategy that's widely available. Multiple CROs, not just VIDA have incorporated imaging into clinical trials, can be done repeatedly, radiation exposure is going down.

There's a world in which we could develop specific safety profiles that wouldn't necessarily have to sample the whole lung to the degree that we do for clinical testing, but would be fit for purpose for drug toxicity monitoring. So advances in CT lung imaging have reduced reliance on tissue histology in the diagnosis and treatment of lung disease, and I think we should incorporate them into drug development, and it provides a method to improve safety monitoring and reduce reliance on animal data. And it should allay concern that lung toxicity is not easily monitorable. And as a final point, I say, we should incorporate lung imaging into animal studies of lung toxicity. There's no reason we can't do that to allow cross-species comparisons of drug-related lung findings between rats and dogs and animals and humans. Thank you all.

Susan Winckler:
Great. Thank you so much to each of our speakers in that session. We wanted to provide a wide

array of options and think about the many, many alternatives that might be available. While I call our final panelists up to the stage, I'm going to ask each of our six presenters, it's going to be one question. You get less than a minute to answer. So let me tell you that question and then I'm going to hand you a microphone. What I'd love you to opine on is where do you see the greatest barrier to reducing reliance on animal models in lung toxicology and would it be scientific, regulatory, cultural, operational, or something else? I'm going to turn to Rachel first as I steal a microphone. So we'll do in the room and then we'll go virtual. But again, if you're on the final panel, head to the dais. Dr. Eddy.

Rachel Eddy:
I would argue that we have solved, at least from a quantitative CT perspective, we have solved operational. I think regulatory is still a lot of things that we face, and a different regulatory pathway in different regions, and some are more risk averse to radiation dose, for example.

Susan Winckler:
Great. Thank you. I'm just going to go down the row. Thank you. Alexandra.

Alexandra Maertens:
I would say that what I see as the biggest risk is, or sorry, the biggest problem is that I think people are pretty unrealistic about what the animal models can and can't tell us. They're also a little unrealistic about what in vitro models can and can't tell us. And we have to accept that uncertainty and realize that when you stick a drug in a human, there will be things you cannot predict, and you have to figure out a way to sensitively monitor that so that it does not become something that's irreversible rather than hope the magic model will save you.

Susan Winckler:
Great. Thank you. Mary.

Mary McElroy:
I think some of the models will have high technological barriers to overcome, I think defining a context of use that makes sense, and reproducibility. We've had the lack of reproducibility in the animal models. It'll be the same thing in the in vitro if we're not careful.

Susan Winckler:
Yeah. Great. Could we get our three prior speakers, the three who just finished their presentation? So if Dr. Fahy could answer that question. Dr. Fahy, let me know if you need me to repeat it.

John Fahy:
No, I think I got it. I mean I would comment on something Adrian said earlier about the massive amounts of drugs that we give to animals because of these very large safety factors and the conservatism around nominal dose and delivered dose. If we can get more comfortable that the lung toxicity can be monitored in humans, it could reset what kind of protocols we have to do in animals. Because I don't see us getting completely away from rats and dogs, and I don't know that we want to, but we might be able to get to where we could reduce the protocols, make them simpler, fewer animals, maybe even shorter periods of time if we get ... Because this large size of those protocols is driven by this conservatism, and I think we don't need to be doing such very large scale protocols.

Susan Winckler:
Emily and Megan, we can't see you, so one of you just needs to start speaking and answer the question.

Dr. Richardson:
I'll go first. Yeah. So I'm going to reply with just confidence as a barrier in what I see is what I kind of assume is regulators have not quite become confident, although maybe this workshop will make that change, in the need to change those regulations. Usually, it is about confidence and just feeling really like, okay, we can change the regulation, so that's why I'd say for regulatory use. And then just in general, confidence is really, it's a little ambiguous. It's related to validation and reproducibility and all of that, but that is hugely important for actually using these things in a regulatory context where the consequence of getting something wrong becomes quite high. And who's going to take that risk? Is it going to be the regulators, is it going to be the pharma, and taking that risk if you don't have confidence is very hard.

Susan Winckler:
Great. Thank you. So the emphasis on confidence. And Emily, if we still have you, you get the last word.

Dr. Richardson:
Yeah, I'm still here. Thank you. Yeah, I'll follow on from what Megan said about confidence. And I think the discussions we always have in the NAM space is around qualification, validation. It was mentioned earlier that that's more challenging in the lung space due to the availability of tool compounds. I think that's something we're all working on together as a field, and it's something that we need to work really closely with regulators, with drug development partners on, and ensuring we really have that confident understanding across the board, and we're speaking the same language.

What the Future Might Look Like: Regulatory Harmonization and Global Alignment

- **Lorna Ewart, PhD, DSc, Chief Scientific Officer, Emulate, Inc.**
- **Andrew Goodwin, PhD, Director, Division of Pharmacology-Toxicology for Immunology and Inflammation, FDA**
- **David Jones, FRSB, FBTS, Consultant, ApconiX**
- **Tim McGovern, PhD, Principal Consultant, White Oak Regulatory Tox, LLC**
- **Steven Rowe, MD, Chief Scientific Officer, Cystic Fibrosis Foundation**

Susan Winckler: (01:33:38)
Excellent. Thanks so much. So let's thank our last session speakers. So now we're going to go to our final panel where we are welcoming the insights of five leaders in this space to help us evaluate all that we've heard. If you can't tell by their facial expressions, they're all processing everything that was shared during the day. But we want to have a conversation and explore what's needed to move forward in our collective efforts to both improve product development and reduce our reliance on animal testing. So I am thrilled to sit among them and continue to learn. So you may have observed, we have three here in the room and two online. So let's begin with some grounding on why we are doing this work and where the greatest challenges might lie. So I'm going to start at the opposite end of the stage. Dr. Rowe, as Executive Vice President and Chief

Scientific Officer for the Cystic Fibrosis Foundation, you come to this topic with daily immersion in being both a patient advocate and a striving scientist. Why don't you kick off our discussion?

Steven Rowe, MD, Chief Scientific Officer, Cystic Fibrosis Foundation

Steven Rowe: (01:34:50)
Sure. Glad to do that. It's been a great day. I really enjoyed this presentation and thank you for the invitation to be here. I really love that we started with Dr. Kozlowski talking about the metaphor that we've heard several times today about the bamboo and this being planted. And we feel like now is the time. Now is the time to let it sprout and grow. And it feels like, based on all that we've heard today and our view of the field, that all the pieces are in place.

When we think about a patient perspective, the clock is ticking. There's been a lot of progress in CF therapies, but still about 20% of our patients do not have a drug that alters their natural history. And we've seen what success can look like, and it feels very much like we need to do more and accelerate that. We heard that from the patient providers with IPF, and that very much resonates. And the theme that we may have a period of overly conservative analysis of pulmonary drugs very much resonates for us as well. It feels like this is based on dated assumptions. It feels like it is based on conservative interpretations. And like we heard about IPF and the analogy with cancer, I would say that we need a more holistic evaluation given the progression of disease.

We've been successful. I'd like to just share a little bit of a vignette of how we collaborated with the FDA for the development of CF therapies based on sound science that preceded it in academia using the airway-liquid interface culture models or the 2D culture models that we've heard about a half dozen times today for the development of CFTR modulators, which have been transformative for those that have benefited. That model turned out to be highly predictive and in fact allowed us to develop CF therapies with a great deal of predictive capacity and essentially avoid efficacy models in the CF state, and we see no reason why the time is now to start to do that for toxicity.

As we start to pursue NAMs, I think we're at a point where we really need to build up the bridging data, just like we built up the bridging data for the efficacy data that allowed these multiple modulators to be successfully developed across different providers. And I view a couple of accelerants that I'd like to encourage us to think about.

One is requiring the use of these bridging. I know it's been suggested for submissions, but the number of submissions that have had those NAMs in the package that we're familiar with, that we get to see isn't adequate to develop the sort of bridging data that we would really like to see. The second thing that may be more controversial, a little more out there is to start to make that data public. I could imagine de-identifying those submissions in a way that protects the proprietary information, but then allows the NAMs to become public-coordinated, start to link that with AI information. And all of a sudden, I think we would see a massive acceleration in their use.

So those are the few things that feel like they resonate across the programs. Of course, there's plenty more to talk about.

Susan Winckler:
Yeah, great. And in my head, I'm like, "Accelerant? No, plant food." That's what we're speaking to.

Steve Rowe:
Yeah, we'll call it fertilizer.

Susan Winckler:
Yeah, there we go. Even better. Good fertilizer. And so that sends us just in the right direction as we think about pulling this together and what might move us forward. I want to turn now to one of our virtual panelists reflecting on the most recent session where we had a number of potential solutions or at least alternatives to consider in this space.

So Dr. Ewart, could we tap your expertise across drug discovery, development, and translational science and your perspective as chief scientific officer at Emulate to provide some pros and cons of our current and emerging alternatives?

Lorna Ewart, PhD, DSc, Chief Scientific Officer, Emulate, Inc.

Lorna Ewart: (01:38:52)
Yeah, absolutely. Happy to do so. So really, when I think about that question, I always want to answer it first with the animal models. I think this is something you've heard from a few speakers already and something I would really like to impart here as well, that we must recognize that animal models have been central to both hazard identification as well as dose response assessments, and many drugs that are on the market today have come from those testings.

But the interest really in alternatives is quite simply because we recognize now the limitations that they provide. And it's been eloquently described by several speakers already, Dr. Thielen, for example, Dr. Hornby at AstraZeneca as well. So it's incumbent upon us as we're now in the 21st century to be thinking about where the new science is going, what new technologies are available to us. But importantly, if they're human-relevant, what advantage can they bring? How can we think about being ethical and sustainable in our endeavor to discover and develop new drugs, and of course, responding to the many regulatory shifts or policy updates that we heard globally last year?

So with that in mind, I'm happy to talk about the pros and the cons. I'm going to talk specifically about organ-on-a-chip because that's really where my career has been in the last 12 years or so. But of course, it's not to dismiss these pros and cons when we think about other alternatives and 2D models, for example, that was nicely described by Dr. Mary McElroy earlier there as well. Also, thinking about in silico models, but thinking about precision-cut lung slices, which I don't think I've actually heard mentioned throughout the session today.

So thinking about some of the pros then for organ-on-a-chip technology, for me, it's really about the microenvironment. It's what I've learned over the last, as I say, just over a decade that I've been working in this space. It's about how we bring together four main concepts here. First of all, how we design and think about the extracellular matrix, how we think about the media, how we think about the flow, and that can be flow of air, and in the case of air-liquid interfaces for lung tissue models, it can be the flow through the vascular channels to keep the endothelial cells stimulated, and it can also really be about that mechanical stretch and that's been described as well. And we know that when we get these things right, when we get them balanced, we can see a very human-relevant physiologically appropriate model system. For example, it's been shown in multiple

publications that alveolar Type II epithelial cells can actually remain in culture when we have them in the right conditions rather than of course dedifferentiating into Type Is.

Secondly, it's about the expression of key proteins on the cell surface. Again, it's been shown in a recent paper, actually a nice paper by the FDA, in fact, by the CDER Group that the expression of drug transporters in these types of models is maintained, and these cells are correctly polarized. So these models open up the possibility to study in greater depth residence times of inhaled molecules. And again, that's really going to be important as we think about better risk assessments moving forward.

Also, I'd like to comment on the fact another pro of these models is that we can incorporate the immune component. And in fact, where lung-on-a-chip began in the Wyss Institute at Harvard was really one of the first and very early demonstrations that immune cells could be recruited from a vascular channel into the epithelial channel and mount a response to a bacterial infection. We've also seen publications from Roche, for example, where they've included immune cells to do a risk assessment of bispecific antibodies.

And then lastly, thinking about the fluidics, especially in unidirectional flow platforms, this allows drug developers to really control exposure to drugs and systemic exposure to drugs in particular when we're thinking about lung air-liquid interface models. And all of that allows us to get better dose demand predictions and pharmacokinetic predictions as well, all of which will really help on that risk benefit and assessment. So, those would be the pros.

In terms of cons then, these models are technically complex. As part of my role here at Emulate, one of my roles is really to simplify the workflow. How can we get users trained and up and running more quickly? Cost comes up a lot as well, but I'd still argue it's not as expensive as repeated, especially longer-term animal studies. Throughput's another consideration. And lastly, we have to think about standardization. And I think that this was actually exemplified beautifully in Megan's presentation. We often hear from pharma that there's a paradox of choice of platforms. So again, it's incumbent upon us as developers to really articulate why one platform over another. Maybe there's a role here for standardization as well.

And lastly, I'd like to say that we would like to see also more larger datasets that allow benchmarking of these models against one another, but also against more traditional methods. So hopefully that gives you a good indication of pros and cons.

Susan Winckler:
It does. Thanks so much, Lorna. So let's continue pulling this thread about different ways to think about this. I'm going to turn then to you, Dr. Jones. You're the first of our panelists, at least on this stage for this afternoon, with regulator experience, and that's an important part of our conversation.

So as you think about your prior role with the Medicines and Healthcare products Regulatory Agency in the UK, and now as an advisor, what did you hear today? What are your thoughts?

David Jones, FRSB, FBTS, Consultant, ApconiX

David Jones: (01:45:17)
Thank you. And thank you for inviting me today. I've enjoyed the meeting today, and certainly I think we all must agree that developing more predictive in vitro models is a clear priority for the pharmaceutical industry. Really and truly, the massive attrition rate that we see currently cannot be sustained forever, and it probably does delay good drugs getting into the clinic, and that benefits no one.

But sadly, as we have tended to hear throughout the day, there is a gap in the maturity of a lot of these in vitro non-clinical models. A lot of them are being developed in academic settings, and when you compare them to the sort of translational readiness for a model to be used for regulatory purpose, there are some gaps. There are fundamental shortcomings, I think, in robust validation, standardization. We heard a lot about scalability, but that is still an issue for many of the models. Most models probably do require further development before they can be routinely used in a regulatory context. We don't have gold standards, we don't have harmonized protocols, and many of these in vitro systems have sort of inconsistent endpoints and different assay conditions that actually hinder any sensible comparisons between different systems. How does the regulator know which is the best system that should be used?

There are limited comparative data against nonclinical and clinical data cells also, and that reduces the confidence amongst a lot of scientists. We heard earlier on about how some databases are actually skewed towards positive findings. When it comes to clinical toxicity and drug safety, the databases are actually skewed the other way. We don't take overly toxic drugs into the clinic. We don't tend to attempt to poison human beings. They don't like it that much. So we have a lot of negative clinical data. We don't have an awful lot of positive clinical data.

But there's little doubt that international regulators are increasingly showing more interest in NAMs, and they're beginning to fully understand what they actually can bring to the safety assessment tools. And I think meetings like this actually underscore their value and show that there's a real drive for the improvement and uptake of NAMs. Qualification and validation remains a problem, obviously.

And so to this end, I don't see these models replacing repeat those toxicity studies anytime soon. Although I would like to see some discussions around using instead of a battery of tests, having a hybrid approach where we have a battery of tests actually replacing one of the animal models. We still have the animals, that will give comfort factors to those who need the comfort factors with an animal, but we have some very good, hopefully, human data.

Now, I've long been skeptical about the usefulness and translation ability of animal models of diseases. We can cure every disease known to man in animals, but there's an awful lot of sick people out there that shows that these models are not the best models. And I really see NAMs, especially a lot of the NAMs we've heard today, playing a big role here, and especially if we have more biomarker developments. And I also see a great role for these models in investigating mechanistic data if we've seen toxicity in animals to actually show how human-relevant is that finding? We also heard that there's a great potential for inhalation toxicologists. Like a lot of people

in the room, I'm a member of the Association of Inhalation Toxicologists. I grew up as an inhalation toxicology, if you like. And so to use these models to actually better show disposition and localization of the drugs to help better define clinical doses, I think is another way where we can actually use these data.

It all comes down to a weight of evidence. So I don't think if we're going to use these models outside the proper safety assessment, if we're using these models to sort of provide human mechanistic data, I don't think they really need to be fully validated because after all, whoever validated the animal studies in the first place? I think as long as a model can be shown to be fit for purpose, as long as it can be shown to be reliable, as long as it can be shown to reproducible, I cannot see why we cannot switch to NAMs to replace an awful lot of our pharmacology, pharmacodynamic studies. And the more people use these in this way, then obviously that increases the confidence in what these models bring to the table, and that will open doors for greater use.

It's very easy to get pessimistic about these models. In 2015, I co-authored a UK government document on working to reduce the use of animals in science. And last year, 10 years after I wrote that report, the UK government published another report on replacing animals in science, and the aspirations were exactly the same as within my 2015 document. Having said that, I think that 10 years from now, we will have seen that there will have been a big, significant uptake in these technologies.

One thing I would say is that we've heard a lot there during the day about the Three Rs, and I'm a great advocate of the Three Rs. I've supported the Three Rs for most of my scientific life, but I don't think it's overly relevant here and I think it can be off-putting to some people. I think we really need to put the emphasis here that these models generate human-relevant mechanistic data, and that's where I see these models going forward.

Susan Winckler:
Thank you so much, and helping us just think about the bridging and where these might be used versus not.

So let's continue the regulator conversation, and obviously the voice of the US Food and Drug Administration is an important one. So thank you, Dr. Goodwin, for joining us. Outside of today, well, actually today, but you probably didn't get much work done, but in your day job, you're the director of the Division of Pharmacology and Toxicology for Immunology and Inflammation within the Office of Immunology and Inflammation, within the Office of New Drugs, within the Center for Drug Evaluation and Research at FDA, which is not the longest title I've ever had to read for someone at FDA, but it's really close.

So we know from Dr. Kozlowski this morning that the overall topic of reducing the use of animal models is a priority, but it's a transition that happens in specific stages and in discrete circumstances. What did you hear? How would you reflect on the information shared today on our shared learning? What comes up for you as the regulator?

Andrew Goodwin, PhD, Director, Division of Pharmacology-Toxicology for Immunology and Inflammation, FDA

Dr. Goodwin: (01:52:35)
Sure. Well, thank you for the opportunity to be here, and that's why we have so many acronyms at FDA.

Susan Winckler:
Indeed.

Dr. Goodwin:
So FDA, just stepping back, we do take our dual mandate very seriously, right?

Susan Winckler:
Yes.

Dr. Goodwin:
We're helping to partner with industry to bring new medicines forward while also protecting patient safety. And under Commissioner Makary, we now also have this renewed focus or newly emphasized focused on reducing animal testing and we're trying to weave that in where scientifically justified without compromising either of those other two goals. And I think sessions like today are very productive in that effort.

So I thought I could just reflect briefly on each of the sessions from today. And then as we go forward, I think you also want to be more forward-looking, but-

Susan Winckler:
Great.

Dr. Goodwin:
but I just wanted to provide 60 seconds on each of the sessions.

So Dr. Reed and Dr. Tepper certainly gave us a great technical foundation and the approaches that are used to evaluate the non-clinical support for proposed clinical trials for these products. There are benefits to a consistent structured approach, but we acknowledge the values they described are estimates and are intentionally conservative. I think everyone understands that. And I think there's already been some ideas about how to engage going forward, continue that, modernizing that discussion and seeing what data is out there to support that.

And certainly, FDA reviews, we are somewhat unique in that we do conduct a review of the raw data from each of the studies. We get all the study reports and we pour over them and the reviewers do exclude findings that are known to be species-specific and things like that. Obviously, as others have mentioned, there are judgment calls and differences of opinion, and that does happen. We do consider other weight of evidence factors as appropriate. And I think over time, the emphasis on that has increased, especially in serious diseases. And with that, we are always keeping that therapeutic context in mind. Is this a phase one study in healthy volunteers? Is this a serious disease like IPF or others we've heard about today? Are we treating children? Right?

Susan Winckler:
Right.

Dr. Goodwin:
Our division covers some neonatal indications even, and there's very different considerations.

So perhaps that segues a bit to the second session with the case studies, and obviously I can't respond or discuss any of the individual case studies. I won't attempt to or try to provide a different perspective on any of those. I did take the opportunity to look at a higher level across the types of questions we ask sponsors during IND reviews and the clinical holds that do occur from time to time because you might get the sense from listening today that those questions and those holds are overwhelmingly driven by rack toxicology findings. And really, that's not true for inhalation products. I looked over and the things that actually become questions or problems are varied, and I think they reflect the fact that these are very complicated drug development programs for many different reasons. There are device issues, there's product quality issues, there's inactive ingredient issues, there are pediatric ethics issues, there are sometimes a lack of proper data for these ... We heard about some reformulations where you're changing as an existing product to now be inhaled. And overall, study design issues with the studies that were submitted.

There could be other toxicity problems like genetic toxicology concerns that have nothing to do with the fact that it's inhaled and on and on. Drug development is hard and inhalation drug development, I think, has additional complexities. I think Dr. Curran and others gave us some, everyone in the room, some challenges of how to work together in the future to get past some of these issues and we're happy to participate in those discussions.

So moving to the afternoon session, I enjoyed, as everyone did, hearing about the progress that were being made, and there is a lot of reason for excitement. I think many of us have expressed that there's not any one of these assays that's a one-to-one substitute for the GLP toxicology studies, but that's okay. And there's still a lot of utility in these studies at this time, especially we heard a lot about how certainly on the efficacy side, they're very impactful. And even on the safety side from our industry colleagues, how they're using them early in their discovery processes to put better quality candidates into those animal studies, and that's all before they even think about bringing any data to the FDA. But if we do that, if we improve the candidates on both the safety and efficacy side, we are reducing animal use.

And I think a request to our industry colleagues is, one, find ways to share that data with us and with the public in whatever way is possible because those discovery screening assays, we don't see them. They're not a part of those ultimate IND submissions. And also, I think maybe we could do a better job of emphasizing that aspect of animal use reduction. And I don't know how you can quantify it or characterize it, but we've heard it in different forms. We have different discussions with industry groups on various topics and the same theme comes up. They're employing these novel methods in their discovery phase and we're getting benefits from that, but how can we let the public and other stakeholders know that that is an area of success?

Certainly, others have mentioned, but there are some FDA publications on NAMs and how to think about defining context of use and validation, and there's been a lot of great discussion on that.

Then the last section on clinical monitoring, I think there's been several times today where we've discussed terminology and things being lost in translation. And I think this is one of those moments for us because when our reviewers and our clinical colleagues are communicating with sponsors about findings being unmonitorable or difficult to monitor or whatever word you want to use, they're not saying that they're undetectable. And this maybe gets back to the goal of the AstraZeneca studies that were presented earlier. If our clinical colleagues feel a certain toxicity identified in the animals is not an acceptable risk for the patient population, and that judgment's obviously going to be different depending on what the clinical protocol is, then being able to detect it by any of these technologies that were discussed wouldn't necessarily be a satisfactory outcome because the injury is already there.

What we're really looking for is the ability to monitor for something, some sort of biomarker or whatever the case may be, that here we have a change and can we stop the drug or stop dose escalation at that point before there's a progression into whatever finding is of concern to the clinical team? And that sort of assessment, as was mentioned, could be in the non-clinical study, in the clinical study, or both, but it is more complicated than just looking for damage on a CT scan that's already there. So I'll stop there.

Susan Winckler:
Okay, yeah. No, that's very helpful in thinking through all it is that, as a regulator, you are aspiring to assess. And so thank you for giving us the perspective from the shoes you stand in of the information shared today.

So Dr. McGovern, you've been very patient and I promise this becomes more of a dialogue. It's just what we do to introduce everyone. So you're bringing former regulator and now working with regulated industry as a principal consultant at White Oak Regulatory Tox. Help us weave this together. We've had a couple of observations about the need to share more information, to have some more bridging of the opportunities to what's possible here. That's all I need you to do. Help us weave it together.

Tim McGovern, PhD, Principal Consultant, White Oak Regulatory Tox, LLC

Tim McGovern: (02:02:20)
Yeah. I'll pull it all together, solve all the issues, and we'll be out of here in about 10 minutes. Yeah, thanks for the opportunity to participate. A great session today with all of the speakers and a wide variety of topics to cover.

I thought I'd take one moment and just talk about some of the historical perspectives of the approach that Jeff characterized, the FDA kind of approach in terms of evaluating inhalation products. It preceded Andrew, so don't blame him. He inherited some of this stuff. We decided-

Susan Winckler:
There's no blame today.

Dr. McGovern:
We've decided to blame Luci Pace since he's retired and not here. And frankly, I joined the agency in '97. The approach that you have currently in use now, frankly, 30 years or so was put in place a

year or two prior to that, derived from the Wolfe-Dorado paper that was published, '93 or so. And it was non-clinical doesn't work in a vacuum, but it's in conjunction with the clinical review team as well. And so particularly things like deposition factors, Jeff went through the data showing where that 10, 25% came from. It's a blunt tool, no doubt about it, depending where you are and that particle size distribution, that can vary. The clinical assumption of 100% deposition, as Jeff said, everyone understands that's not the reality of the situation, but based on the variability in the data, it was the clinical position to say they were comfortable using the 100% deposition as that extra measure of safety conservatism.

And so that's one thing. So that's one area that, particularly today, we're focused mainly on the nonclinical issues, but a lot of this does overlap with clinical. Deposition factor, number one, monitorability, the risk-benefit assessment. So I think as we move forward in these discussions in whatever forums are approached**,** it's really going to be important to pull in the clinical parties on both the industry and the regulatory side as well to provide input on that.

And frankly, I've given presentations when I was at FDA on these issues. Many of these concerns, questions have been coming up for 30 years or so. So I think it's really good to have this type of workshop now. As Aiden mentioned, a great amount of data has been generated, a lot of it ex-US in clinical trials based on the same non-clinical data. Can we learn from some of that information and can FDA do some data mining internally? Can industry pull data together? Other regulators that may have data that FDA hasn't seen, pull that data together and say, "Have we learned anything over 30 years where you can at least make a reasonable judgment?" And I think Per said before, there's not a right or wrong approach, but is a more conservative verse less a conservative? Have we lost anything or have we learned anything from applying one approach in the US particularly, a more liberal approach outside of the US that can say, "Okay, we had these concerns at one time, but maybe they haven't played out over 30 years"? And maybe that can help to inform a revision of rethinking of the approaches.

Things like adversity, for example, that has been somewhat of a moving target over time. There was a period of time when I was in the pulmonary division where a simple increase in macrophage accumulation in the lung was deemed adverse in nature. And a lot of back and forth with industry, review of datasets in-house that we'd had with previously approved products. And we at least got to a position that ... And some of that, again, based on clinical concern, moving into an already compromised patient population, but got to a position to say, "At least that, we can live with that." If you go up to more mixed inflammatory cell response at a higher severity level, that would be considered adverse in nature.

So it is somewhat subjective. It can be a moving target as you learn more, depending on who the decision-makers are at a given time as well in terms of their position in terms of conservatism. And frankly, I think Jeff brought up the point, calling a NOEL based on the findings in that animal model as opposed to projecting it to a compromised patient population. I think there has, at least in some cases, the identification of a NOEL from non-clinical inhalation studies has been colored by the fact that it is going into a compromised patient population. And is there room to pull back on that? Is a relatively low-severity inflammatory response in an animal model, can that at least be identified? Can you identify a NOEL in that study? Because sometimes these studies are repurposed for other indications as well. So at least you have a study with a identified NOEL and the next step might be

applying additional safety margins based on the patient population, and so going from there. So that's certainly one area for reconsideration.

The role of alternative models can certainly ... We talked that it maybe not be ready for prime time at the moment in terms of replacing animal studies, but can they further inform that weight of evidence to where you're going from the animal studies to moving into the clinic can at least inform on clinical relevance of those findings and perhaps even what degree of clinical monitorability might be acceptable based on that information. So yeah, I'll stop there.

Susan Winckler:
Okay. So what I'm struck from all of you and then the day is that to achieve these dual goals, it obviously requires change and change from a lot of parts in the ecosystem. So I want to turn to each of you, and I need you to each be more rapid fire this time, to provide some thoughts on that change, about how that's driven, and is there some staging required? Just how should we be thinking about that change? We've had different phrases used about bridging and other components. But Dr. Ewart, I'm going to turn to you first. So pick that up. How would you help drive this change?

Dr. Ewart:
Yeah. So we think about it in a framework and David actually spoke a little bit about it. So I'm going to try and say it in the way that we think about it here at Emulate, but recognizing that today animal models, as we said, play an important part in the first time in human decision, for example. However, as we go through a new wave of regulatory change, as we go through qualification programs or provide validation data and build confidence in different alternatives here, we talk about a period of coexistence. So maybe there's a new word that we can talk about today.

And that coexistence really means that... So for example, if we have, as Emulate does, a Liver-Chip going through the qualification ISTAND program, it's in the final stage. It's about separating out structural analog toxicities. So if we have that very good model and we're fast following, and let's call it, let's say a pick a target, a BTK inhibitor, let's say we're a fast follower, BTK inhibitor, we know those have had liver toxicity. If we're structurally similar to ones that have liver toxicity, then the regulator surely needs to know that, "Okay, what is the risk that this might cause liver injury in humans?"

And if we have a tool that's been validated to say that is good at picking it up, then we'd far rather run it before we go first in human to get us all on the same page from a risk assessment perspective. So that's how we think about the coexistence. But how we move forward from there really relies on having many more organ models developed, lots of large data sets I mentioned before, comparisons showing that it's equal to or superior than some of the other assessments are already out there. How does it really add value to that weight of evidence argument, et cetera? When we get to that point, we might be able to remove one of the species.

Yeah? And then in the future, I'm not sure I'm quite there myself yet, but maybe the rise of artificial intelligence and silicon models, digital twins, however we want to term it, may actually get to a point finally where we've got a good suite of in vitro models or combinatorial NAMs is another term

you'll hear that we may get to a position eventually where we don't need to use animals. But I don't think anyone's there yet, but I think we're very much at that coexistence phase.

Susan Winckler:
Yeah. All right. So our word cloud has coexistence. Let's do McGovern, Jones, Roe, Goodwin. Tim.

Dr. McGovern:
Yeah. So yeah, as part of this, I was reflecting back on the last six years or so when I was at FDA, I was involved in nitrosamine impurity assessments, which kind of hits drug products pretty much across the board. A lot of research efforts underway. FDA working with Health and Environmental Science Institute developed a roadmap and kind of laying out kind of what are short-term goals, long-term goals, some that are higher impact, lesser impact, and trying to prioritize work based on that.

So I think certainly some short-term goals might be things like reevaluating deposition factors based on updated models. We had some conversations earlier. One thing that could readily be done is looking at, do we incorporate more realistic clinical deposition factors as opposed to assuming 100%? Do we look at some of these newer models that have been derived? They may confirm some of the data that's already been generated, but they're worth looking at anyway to see if those deposition factors are truly relevant and maybe more regional specific in terms of, and line that up with particular findings from toxicity studies.

Certainly maintaining open lines of communication across the various disciplines and stakeholders, coordinating those efforts as we were talking about. Groups like HESI, other private public partnerships, meetings like ACT, SOT, the [inaudible 02:16:39] Tox groups can all be kind of further these discussions as well. And then kind of advancing in that stepwise manner, incorporating things, where can NAMs be incorporated now and what's kind of the path forward for more fully integrating them into the evaluation process.

Susan Winckler:
So coexisting and then in gridding out potentially this pathway. We know there's the overall FDA roadmap, but thinking about in this space some options. Dr. Jones, to you.

Dr. Jones:
Thank you. I mean, traditionally the MHRA has never fixated on NOAELs safety margins when it comes to generating, calculating the first in human doses. We've always fixated, if you like, on the science. I think it's quite sad that most safety evaluation studies are actually designed to generate an NOAEL rather than to actually figure out what is the problem with a drug. So I think NAMs giving us sort of more mechanistic data, I think is definitely the way forward.

The other thing the MHRA has always sort of recognized is that regulators have two major roles. On the one hand, yes, we have to keep off unsafe and inefficacious drugs off the market. But on the other hand, we have to remember there's a massive unmet clinical need out for a lot of diseases and we must never put sort of roadblocks in front of that. So sometimes we have to remember that the non-clinical studies are there to show that a drug is acceptably safe to test in humans. That's where we will find out if it works or not. That's where we find out. So as long as it's acceptably safe to go into humans, then using more and more NAMs I think will help us get there.

Susan Winckler:
Great. Dr. Rowe.

Steve Rowe:
Well, I think we use the word bridging and it's now been stated coexisting and I totally endorse that approach. I think I would break it up systematically in terms of airway diseases, alveolar diseases, et cetera, and generate some general guidelines for program, NAM programs that the agency would like to see.

And then let's move from suggest to insist or and then make that data public. To me, if that were to happen, then we would accomplish the goal that brings everybody to this meeting today, which is to start to not just move away from animals for that, for safety's sake, but because they're only mediocre and I think the science can allow us to do better.

Susan Winckler:
You get the last word on this question, Dr. Goodwin.

Dr. Goodwin:
Sure. Well, I mean, I think it's clear from the day, there's plenty of work ahead for all of us here, the NAM developers to really hone in on the context of use for the assays they're developing, where they fit into this picture and addressing those validation questions and really assessing their tools and the data they generate from, among other things, from the eyes of a regulator in terms of the ways in which we'd look at the relevance and the technical aspects of the assay and the data integrity and all these things and transparency and data sharing too.

And the industry will need to do that as well. They need to get themselves together and put together the data we need to move forward. And they can blame their lawyers and get over that and move forward and work with us to... If there's a better way to do things, let's look at the data and find that. Encouraging them to, as I said before, submit the data they're already generating and using in their packages. And the industry also needs to be aware of the context of use and make sure they're not mislabeling things for a context of use that they aren't, and give us high quality reports and data like we expect for the GLP tox studies.

And I think through all of this for the animal, I think we're all saying that there's still going to be animal studies for the foreseeable future. For all of those studies, and this goes for anything, it's ethically and scientifically imperative that we get them right the first time. Right? You take the time, get your species selection, get your study designed, deal with the excipient qualification issues you need to for your inhalation program so that the worst study of all is a poor quality one that doesn't achieve its objective.

And for ourselves, look, for FDA, we need to work with both the product developers and the industry colleagues to move forward. And that's been happening and we have good success in other areas. And I think we can do that here too. We need to, as others have said, make sure the professional development training is ongoing for ourselves and we're open-minded to incorporating these where appropriate and we'll collaborate with industry to take these next steps.

Susan Winckler:
So it sounds too like this is a space where we hear often from the regulator that an early conversation is far better than a late conversation in the, just the process of applying anything new.

Dr. Goodwin:
Yes, absolutely. And also, I think what we're hearing today is the importance of this sort of bigger picture, broader conversation, not one application at a time, but let's look at the data. As everyone have said, there's so much data out there. Let's look at it in a holistic way.

Susan Winckler:
Yeah. Which I'm glad you raised that because that was a piece I thought about this morning that we know the product by product is inherently then siloed, but this seems like a place where the broader conversation can be particularly helpful and help all of those products move forward, or at least have better awareness. Dr. Roe, do you have any thoughts on that?

Steve Rowe:
Yeah. I was just going to say one more thing, and that is, if it felt like mandated transparency, that highly aspirational goal, given the current environment, right? Was a bridge too far. I just found the data shared by the AstraZeneca team on the power of the retrospective look at some of the assays that they shared and how predictive they turned out to be in helping them make forward-looking decisions thereafter could very much be employed now. And what that might look like would be funding to implement NABs in retrospective data sets and share them in a transparent way that then builds up the predictive data set.

Susan Winckler:
And maybe even on alignment on what are the key questions that you might want to explore or some component there. All right. One of the pieces we did want to talk about, Dr. Jones, I want to come back to you because we know. Right? Product development is a global enterprise and there are regulators around the world.

You mentioned a bit about how MHRA looks at Knowles and other things. Is there anything else you want to call out that we should think about that we can learn here in the US? We can learn from what MHRA has done here. I promise we do listen and we do learn. He's laughing.

Dr. Jones:
I mean, I'm laughing because no, the FDA has nothing to learn from me. The MHRA works very, very closely with the UK's National Center for 3Rs. We've worked with it since the day it was instigated, and we make sure that we're in all their projects and we encourage data sharing. And as we've heard, it does tend to be the lawyers that are the biggest sort of bug bears in that case.

But we're seeing more and more, I think, acceptance that data sharing helps everybody. One of the arguments against again was, yes, we can anonymize the data, but that takes an awful lot of work and we haven't got time to do it. I think that is changing. And I did welcome the AstraZeneca talk this afternoon. I thought it was an excellent talk. I think we will see more data sharing as the industry realizes that actually it helps everybody.

And so like I said, the MHRA encourages data sharing. I did talk to the UK government about, "Could we make it mandatory?" It didn't go very far. No. Unfortunate, no. Everybody was just worried that we'd be sued out of money by the industry who really don't want to be mandated to do anything. It has to be voluntary. So I cannot see mandatory data sharing, but I can see a great encouragement that data sharing does happen and is happening even more and it needs to happen more.

Susan Winckler:
Yeah. That makes a lot of sense. I will say is perhaps the only person who held a microphone today who's trained as a lawyer that yes, we are responsible for many of the problems and aspiring to protect the structures for which attorneys are responsible. What I want to do now, I want to ask, because even though we had a fabulous steering committee and a lot of input in structuring this discussion, I want to turn to each of you.

I want to give you an opportunity. Is there anything that should have been raised throughout the day that was not? Was there something that should have been said or put on the table? We obviously can't have a 30-minute talk on it, but was there something that we should just be thinking about that we should have raised and that we didn't?

Dr. Ewart:
Susan, I don't mind jumping in first with that one, but just because I wanted to say something in connection with the data sharing as well. And I think it actually is a segue into what we've not discussed, but I talked earlier about 21st century science and the combination of biology and engineering really giving us some unique insights into biology. Something else that's 21st century is our ability to have what's called federated data sharing. So that's where data isn't ticked up. And that might make lawyers more happier, perhaps. I'm not sure, but-

Susan Winckler:
It does.

Dr. Ewart:
Yeah. The Eli Lilly TuneLab I think is a really good case study here. So data can be put in and it's never sort of shared, if you will, but it is computed upon and then the results are given back. And I think that that, if we can sort of move more in that direction as a community here, particularly to bring out some of the historical in vivo data, as well as companies like ours and others that are developing and generating data on in vitro models, I think that would be really a powerful way forward for data sharing. So that's maybe something that we didn't talk about, how we go about federated data sharing, et cetera, but also that hopefully I build on what was said before.

Susan Winckler:
Yeah. Excellent. Thanks so much, Lorna. Other thoughts? Yeah.

Dr. Jones:
I mean, one thing I... Oh, no, after you, Tim.

Susan Winckler:
Nope, go ahead, Simon. Then Tim will follow.

Dr. Jones:
Okay. It's David, not Simon, but never mind.

Susan Winckler:
I'm sorry. Yeah. So it was a different NPS meeting where Simon Jones did a lot of talking. So now David Jones, my bad, corrected forever. Dr. Jones.

Dr. Jones:
Not to worry. I mean, like Tim, I spent a lot of years in ICH writing non-clinical guidelines, revising non-clinical guidelines. And one obstacle that we often hit was clinicians, clinicians insisting that they needed the comfort factor that a drug had been in an animal.

And I think we have to sort of... We've heard a lot today about convincing nonclinical people and convincing regulators that NAMs are a good way forward. I think we probably need to do a lot more work convincing medics and doctors that actually NAMs will give them better data than an animal.

Susan Winckler:
Excellent. Thank you, Dr. David Jones. Tim.

Dr. McGovern:
And I'll go back to the data sharing and lawyers issue before. I know back in FDA's July workshop on NAMs, Holly Saber brought up the issue with biologics, for example, where there's no 505(b)(2) pathway where you can't reference information from previously approved products. One issue, and this is brought to my attention with the 505(b)(2), so small molecules.

And it might not impact a large number of products, but at least in certain scenarios where if there's the ability for a follow-on sponsor to reference more of the detailed information from a summary basis of approval, for example, in scenarios where they, for example, may be pushing the dose, the clinical dose higher than what's previously approved, but there is actual non-clinical data in that package that could support that.

As currently, a sponsor would need right of reference to that data, but is there... And we'd frankly probably require changes to the regulations, but that's at least one area where in the context of reducing animal use that changes to regulation could allow for that. Whether the primary sponsor that generated that data would be comfortable with that or not is another issue, but it's at least one thing to consider in terms of the thoughts along reducing animal use.

Susan Winckler:
Very helpful. Anything else that we forgot? Yep, go ahead, Doctor.

Steve Rowe:
As a disease-facing foundation, we often find ourselves having to think in the pragmatic or practical terms. And if it looked like basically that the complexity of the task at hand was making it problematic to make success, then one thing we haven't really focused on today is, okay, which aspect of pulmonary medicine would we imagine going after first where you could eliminate or

reduce animal model use sooner or generate the tighter linkage to the NAM and use that as a starting point rather than trying to boil the ocean or accomplish all of respiratory delivery.

Obviously, as a respiratory scientist interested in the airways, I may be biased, but we certainly heard a lot of talks about the air-liquid interface culture model potentially providing the branch point, whether it was added to a chip or added to a more complex physiologic environment like shear stress to then deliver. So I would encourage us to think about maybe there's a subdivision that would really be well suited to start and push the NAM science with that and the regulatory linkage and the data sharing that would accelerate that area.

Susan Winckler:
That's great. You going to pass on that one? It's okay.

Dr. Goodwin:
Sure. Sure.

Susan Winckler:
Well, so then actually I want to pick up on that, Dr. Rowe, because I want to challenge you each with the final question. So here's, you're each going to get two minutes. And I may set a timer. So two minutes, but what I would like you to address is what action or effort do you think is most important to pursue our dual goals? And so I think this might help with what might we pursue. So are we good on the question? Yeah? Okay. We're going to go in the order McGovern, Roe, Jones, Ewart, Goodwin.

Dr. McGovern:
Okay. They're all important.

Susan Winckler:
Yeah, that's the premise. Yes.

Dr. McGovern:
Which ones are most important? And I'm thinking more in terms of, again, short-term, long-term type of goals and low hanging fruit versus more complicated things. Certainly short-term, I think kind of in terms of enhancing product development, maybe greater flexibility, kind of that reevaluation of deposition factors. Right there, you could make the room for greater allowance of dose estimation or dose allowances in the clinic, evaluation of adversity calls.

Are we really focusing on the animal model versus projecting to the clinical patient population? And then in terms of the moving towards incorporation of NAMs, in particular, where can we really fit a particular model, in terms of context of use, a particular model to address a particular issue, and then at a later stage starts focusing more broadly in terms of validation for more widespread use.

Susan Winckler:
Excellent. Dr. Rowe?

Steve Rowe:
Those are great examples. So I won't repeat those, but certainly the ideas that you just presented

about NAMs make a lot of sense to me. Another example where I think we could make progress is in the setting where the holistic analysis rather than the traditional analysis might allow a drug to go forward, perhaps because of a NOAEL limit or a deposition dose, and we're going to make an exception and start to pursue that.

Then to really couple it with disease monitoring, such as with CT scan, would make sense to me as a place where we can step our toe in to moving the needle on what a regulatory approach would be for a drug that looks important, perhaps in a highly severe disease with maximal urgency to go ahead and make a step in that regard and perhaps publicly with notice obviously to the human volunteers that we're going to have a much more intense safety evaluation, but we're going to go forward anyway.

Susan Winckler:
And I wanted to pull on that too then, then also sharing the results.

Steve Rowe:
Absolutely.

Susan Winckler:
And so that we have that full learning. Great. Dr. Jones?

Dr. Jones:
Thank you. I think most inhalation toxicologists will agree that actually calculating dose is one of the dark arts of toxicity to see. See NAMs being used in this way, I think is a massive step forward. And for us to get the correct doses to take into the clinic, I think will be beneficial all around. So yeah, and that's what I would say.

Susan Winckler:
All right. And I don't know that I expected to hear about the dark arts of dosing toxicity, so brilliant. Dr. Ewart?

Dr. Ewart:
Gosh, lots of things have been said already, which are really important. For me, I think it would be trying... And actually just thinking throughout the day, there's been a ton of really useful examples where we've got to get better at it. I think the one that sticks with me most was the warrior lady who spoke about pulmonary fibrosis. It's a tough old nut to crack.

I'd love to start there, but I think what I would actually urge us to do is coalesce around something that's a quick win. And I don't mean by cutting coroners or doing anything, sloppy science, however you want to call it. What I mean is if we can get a quick win in one area, then I think it might actually open up the way that we're thinking about these things and allow us to get to those more complex diseases like interstitial pulmonary fibrosis, which was truly devastating. And I think the lady spoke very, very eloquently earlier.

Susan Winckler:
Which I helps us think about if there are steps that can be taken that we share and know about

them, and then that helps with the enthusiasm and the moving forward and continued energy in the space. Yeah.

Dr. Ewart:
Yeah. It's just a way of getting some momentum going and it's like a snowball rolling down a hill. If you can get it going, it's going to be a lot bigger by the time it gets to the bottom. So thinking somewhere that's, as I say, a quick win, something that's maybe easier, tangible, measurable, that type of thing, and then it might just inspire us to know how we can tackle those more complex thorny issues.

Susan Winckler:
Exactly. All right. Dr. Goodwin?

Dr. Goodwin:
Sure. No. I mean, just on the disease focus, I think the CF Foundation is certainly one example where I think they've played a big role in, correct me if I'm wrong, but ensuring some technology availability and consistency for different programs and also collective data resources that really benefit the entire landscape for that indication.

And I think that's example for other disease areas. And I think for all of us, I guess the last word is the conversation needs to continue. Right? I think we know what we want to achieve, what the objectives are, but it's going to take some more work and we need to figure out the right venue to continue those conversations, how to bring that data to bear and move forward a step at a time.

Susan Winckler:
Which I'm struck with that idea that it perhaps pulls together... Do we put this on a roadmap to have folks collaborate to say what might that path forward be. And then think about where. Right? Not every conversation can be at a public meeting or should be on a street corner. There can be constructive conversations that then yield results that are great, that are important to be shared, as well as the data to be shared and the experience so that everyone can learn from all of that. So I have to observe from all of this, coming into these, I don't ever know if we're going to leave encouraged, discouraged, confused, or just tired.

And I'm hoping that folks can reflect on the day and feel encouraged that there is certainly a lot of work going on to expand into achieve the dual goal of improving product development and decreasing the use of animal models, that there is a lot of activity. And then there are things that we need to do to better share perhaps among industry, among the groups that are doing this work, the research and among regulators to assure that we're all kind of keeping pace with what's happening, and then to also look at what we know from prior use of animal models and how we might better learn from that with some key questions. So let's take a moment to thank our great panelists who have helped us think through this.

And in something that I'll just say, I wouldn't have expected when we set up this meeting that I might give you five minutes back to your day. So I do want to express our deep appreciation to the speakers, the steering committee, and the sponsors for coming together and having this discussion. As a reminder, we will be posting the recording, the slides, and the transcript on the foundation's website next week, so that if there were folks who should have heard some things or

you want to go back and hear some things, that will all be available. And with that, we are going to say thank you so much. Thank you for joining us and have a great day.