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DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

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CENTER FOR TOBACCO PRODUCTS

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BIOMARKERS OF POTENTIAL HARM: A PUBLIC WORKSHOP

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8:30 a.m.

FDA White Oak Conference Center
Building 31, Room 1503
10903 New Hampshire Avenue
Silver Spring, MD 20993

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SESSION 1: BIOMARKERS OF POTENTIAL HARM OVERVIEW

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SESSION 2: CARDIOVASCULAR DISEASE (CVD)

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SESSION 3: CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

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M E E T I N G

(8:30 a.m.)

DR. DRESLER: It's 8:30, so we'll look at getting started, please. So welcome, everyone, both in the room and online to our biomarker workshop. This is our second workshop on biomarkers, and this one is a workshop on biomarkers of potential harm.

So what I would -- my name is Carolyn Dresler, and I will be moderating the workshop both today and tomorrow, and the way I look at it is my main job is to make sure that we are efficiently on time and then helping to ask questions to help move that forward. So that's why you'll be seeing me up here.

So the first person that I would like to introduce is our Director for the FDA Center for Tobacco Products, Mitch Zeller. Mitch Zeller has been with our Center for approximately 3 years, and we are always fortunate when he opens up our workshop to tell us the importance of why we're doing this work for the work of the Center.

So Mitch.

MR. ZELLER: Thank you, Carolyn. And good morning, everyone. On behalf of FDA's Center for Tobacco Products, I want to welcome all of you in the auditorium and viewing the

webcast to our second public workshop on biomarkers. FDA held the first workshop on biomarkers last August. That session focused on biomarkers of exposure, markers that can be used to assess actual human exposure to tobacco constituents that FDA has identified as being harmful or potentially harmful.

Today's second workshop focuses on biomarkers of potential harm. These are short-term markers that reflect long-term outcomes such as cancer, cardiovascular, and pulmonary diseases. They could serve as a basis for estimating the health effects of tobacco products before more definitive data from long-term epidemiological studies become available. The information gathered at today's workshop will inform how FDA might use biomarkers of potential harm in the scientific evaluations and regulatory decisions we will need to be making going forward.

We have a number of questions and thoughts for discussion related to the topic of biomarkers of potential harm that are part of why we feel today's session is so relevant and so very important. One area of discussion may be on which biomarkers have been used to predict the risk of tobacco-related disease in other areas of research. Also, to what degree can these biomarkers identify the impact of tobacco use separate from

other causes? And finally, how else do these biomarkers need to be evaluated for our purposes, for tobacco regulatory policy purposes.

For example, a biomarker of lung injury that leads to lung disease could serve as an interim marker and help guide our regulatory decision making, especially when reviewing marketing authorization applications for newer tobacco products, such as e-cigarettes. These newer products have not been on the U.S. market for very long and thus would not have those long-term epidemiological studies on health risks.

Another important regulatory authority we have is an area of so-called modified risk tobacco products. There our job is to ensure that tobacco products marketed with claims of reduced harm or risk of tobacco-related disease actually do reduce harm or disease, risk of disease. This is an important responsibility. When reviewing modified risk tobacco product applications, we will need a scientific basis to conclude the claims in marketing about the risks of tobacco products are properly substantiated so that the public are not again mislead about the relative risks of tobacco products as was the case with low tar and light cigarettes. Suitable biomarkers of potential harm may be part of the overall assessment of

modified risk applications.

Finally, biomarkers can play an important role in identifying and evaluation of potential product standards, an authority given to FDA to require the reduction or elimination of an additive, constituent, or other component in tobacco products. The appropriate biomarkers of exposure and potential harm could potentially link a change in the product itself to the biological response in humans and ultimately disease risk. At CTP, our mission is to reduce the disease and the death that results from tobacco use, and FDA decisions are based on rigorous scientific review.

As FDA acquires additional scientific information about tobacco products, including findings based on biomarkers of potential harm, this will assist us in carrying out our responsibilities under the law. So again, on behalf of everyone at the Center for Tobacco Products, thank you for participating in today's workshop, and I wish you a very productive session. And now I'm going to turn it over to Cindy Chang for an overview of the workshop itself. Thanks.

(Applause.)

DR. CHANG: Good morning. I'm Dr. Cindy Chang. I'm an epidemiologist in the Office of Science at the Center. I'm

also the lead of the OS biomarkers working group that planned this workshop. I'd like to welcome everyone and take a few minutes to set the stage for the workshop.

This is my disclaimer.

Many of you have seen this figure before, but this illustrates the different types of measures that can be used to assess tobacco products from external exposure on the left to disease outcome on the right. Biomarkers fall somewhere in between these two ends. At our first biomarker workshop last August, as Mitch mentioned, we focused on biomarkers of exposure.

For the next 2 days, we're focused on what we're calling biomarkers of potential harm. We're using this term for the purposes of this workshop, but we do acknowledge that there may be other terms that are in use. So to paraphrase the definition from 2001 IOM report, biomarkers of potential harm are a measurement of an effect due to an exposure. They include early biological effects, alterations, and clinical symptoms consistent with harm. Examples range from C-reactive protein, as measured in the blood, to pulmonary function tests. When we're talking about the health risk due to tobacco use, clinical outcomes such as cancer, cardiovascular disease, and

COPD are definitive endpoints.

The challenge is that they take decades to develop, and thus they're not always practical in the regulatory setting. As Mitch has already mentioned, biomarkers could serve as intermediate endpoints for assessing health risk of new and novel tobacco products while we're waiting on more definitive data.

This workshop is meant to be an initial discussion assessing the state of the science so that we could make any determinations on biomarkers. The objectives are to gather information on approaches to assessing and selecting biomarkers of potential harm; to start the process of identifying biomarkers of potential harm that may be useful in tobacco product regulation; and to identify areas of research which may help move the field forward. Note that FDA is not seeking advice or consensus at this time but just an exchange of scientific information. Over the next 2 days, we'll hear presentations from government, industry, academia, and other organizations.

Here's a preview of the workshop, which is divided into five sessions. Session 1 provides some context on biomarkers of potential harm and includes perspectives from clinical and

industry research, as well as lessons learned from FDA's experience with biomarkers at the Center for Drug Evaluation and Research, also known as CDER.

Sessions 2 to 4 will focus on both established biomarkers and biomarkers in development for cardiovascular disease, COPD, and cancer. These diseases or disease areas account for the majority of the smoking-related burden in the U.S. We do acknowledge that there are other important health effects, such as adverse pregnancy outcomes and addiction, but we just don't have enough time to cover everything.

Each session will start with an overview. Session 2 focuses on CVD, and this includes discussion on individual tobacco constituents and biomarkers, and findings from industry, clinical research, and CDER regulatory perspectives. Session 3 focuses on COPD biomarkers, including imaging biomarkers, biomarkers measured in bodily fluids, exploratory biomarkers, and CDER's regulatory experience with FEV_1.

On Day 2 are Sessions 4 and 5. Session 4 focuses on cancer, including findings on inflammatory, epigenetic, chemo prevention, and gene expression biomarkers. Finally, Session 5 focuses on new areas of research, especially advances in high throughput methods including Omics technology. We'll hear

about their potential uses at NIOSH and EPA, how they can be relevant to tobacco, and the CDER biomarker qualification perspective.

Finally, I want to note that while the sessions are loosely organized by disease, there's definitely an overlap across the sessions. For example, all three disease areas share some of the same biomarkers or pathways such as inflammation and oxidative stress. Another example is that some of the lung biomarkers may be, might be relevant to both malignant and non-malignant lung disease. So we encourage discussion across the sessions.

I'm going to stop here and have Carolyn go over logistics and questions for the speakers and panelists. Thank you so much, and we're looking forward to the talks and discussions.

(Applause.)

DR. DRESLER: Okay, so some of the logistics. We'll start at 8:30 in the morning, and I try and be prompt for that. We'll have coffee breaks in the morning and in the afternoon. There's a kiosk right outside, so in the morning, at your morning break, coffee or tea.

I would recommend that you also purchase your lunch there because you can pick out what sandwich you want and pay for it

and then it will be ready for you, because if you don't do that, you'll be standing in line during your lunch hour waiting to order your lunch and sandwich, so I'd recommend that you do that during your lunch break, and it will be ready for you then at lunch.

The restrooms are down the hall, so if you go out here and around the kiosk, the restrooms and water fountain are down that way.

Tobacco products, including electronic cigarettes or waterpipes, are prohibited during the meeting or on the FDA ground.

Please do turn off your electronic devices, and I need to remember to do that on mine also.

The questions, what we will do is we will have some index cards, you can raise your hand, and we can get you an index card if you want to write it and pass it in, or you can come to the microphone during the panel sessions. After each session, we will then have a panel discussion in order to ask the questions, okay?

The other thing is this timer that's up here, probably most of you are familiar with it, it's green. You all know how long you have to speak, as do the people controlling the timer,

so they will set that up, and green means you're good to go, keep talking; yellow means wind it up; and red means I stand up, and if you keep talking, then we turn the microphone off, so -- no. That is just to be fair to the people that are coming on; we all know how that works at conferences. So that's the reason for the timer.

If you have -- I've shown the morning speakers for the podium. You can see the pointer that I'm using here. We use this mouse pointer so that the people online can see what you are pointing to, so let's use that. If you have difficulties with it, which I sometimes do, just, you know, look at me or say I'm having problems with the pointer, and we'll get that fixed right away for you.

So I think that's the logistics, and with that, we'll move right on into our first session.

And I should have gone through that. Sorry, Cindy. So I think that we went through this already. If you have questions, I will say down at the bottom, if you're online, please look at that last line. If you have any questions, we welcome online questions, and we'll get those asked during the panel also, so send that to that website, workshop.CTPOS@fda.hhs.gov. Okay?

So now on to our first session, which is Biomarkers of Potential Harm and an overview of that. And I will say, I want to give kudos to Cindy and the team who put this together. We have spectacular speakers, and we'll start out with one of the most well known in the field, and that is Dr. Dorothy Hatsukami from the University of Minnesota, and she'll be speaking on Characterization of Biomarkers of Potential Harm: Perspective from a Clinical Scientist.

Dorothy.

DR. HATSUKAMI: Thank you, Carolyn. So I just want to preface this presentation by saying I'm not a biomarker expert, but what I will do is give you my perspective as a clinical scientist on biomarkers. I have no disclosures.

So the charge for my presentation was what criteria, such as biomarker characteristics and pathophysiology, should be considered when identifying, evaluating, and selecting biomarkers of potential harm?

First of all, what I want to do is just give an overall definition of biomarkers, and this is one that was provided by the IOM report *Clearing the Smoke* in 2001. And the report stated that biomarkers are a characteristic that is objectively measured and evaluated as an indicator of a normal biological

process, pathogenic process, or pharmacological responses to an intervention.

Now, as Dr. Chang had pointed out, there had been a prior workshop on biomarkers of exposure, and we are concentrating mostly on -- or primarily on biomarkers of potential harm. And she gave a nice definition of biomarkers of potential harm, so I won't go into that.

But as she also indicated, there are other definitions that have been provided for types of biomarkers, and these definitions include biomarkers of risk, which is defined as a biomarker that indicates a risk factor for a disease, as well as surrogate endpoints. A surrogate endpoint is a biomarker that is intended to substitute for a clinical endpoint. And clinical endpoint is defined as a characteristic or variable that reflects how a patient or consumer feels, functions, or survives. So a surrogate endpoint is expected to predict clinical benefit (or harm or lack of benefit or harm) based on epidemiologic, therapeutic, pathophysiologic, or other scientific evidence. So we'll assume the biomarkers of risk and biomarkers -- the surrogate endpoints under biomarkers of harm.

So why are biomarkers necessary? Well, as Mitch Zeller

had pointed out, it's critical for the evaluation of potential public health impact of modified risk tobacco products; in some cases, new products; product standards; and also in some cases, substantial equivalence.

Now, these are the main questions that are addressed by biomarkers. They include: Does use of a product lead to decrease in exposure to harmful substances in tobacco? Another question is, is this decrease in exposure associated with decrease in harm? And, of course, the million dollar question addressed by the 2001 IOM report is are these surrogate indicators of this harm to health, and can that be measured in a time frame sufficient for product evaluation?

So in the 2001 IOM report, they stated that although candidate disease-specific surrogate markers are currently available, further validation of these markers is necessary. In addition, they indicated other biomarkers that accurately reflect mechanisms of disease must be developed to serve as intermediate indicators of disease and disease risk.

Now, this was a report that was written about 15 years ago, and I'm not really sure we made tremendous progress in the area of potential biomarkers of harm, but you know, hopefully during the course of this workshop we'll learn what are some

biomarkers that show great promise.

So the question is what are the criteria for biomarker qualification? And these are criteria that I had modified from the Hill 1965 report that he wrote and the IOM 2001 and 2012 report. And so these are criteria that can be used to support causation of a biomarker to disease. And the questions that were addressed, that need to be addressed include the following:

Does the biomarker reflect pathophysiologic process as a consequence of exposure? And this would include addressing plausibility; that is, the question is, is the data elucidating the biological pathways from exposure to effect plausible? And there's the issue of coherence, that is, is the cause and effect interpretation of the data not seriously in conflict with the generally known facts of the natural history and biology of disease?

There also is the issue of sensitivity and the question is how sensitive is the biomarker in assessing alterations in biology and in its ability to detect disease?

And the issue of predictiveness, that is, to what extent are the biomarkers predictive of disease?

Then there are questions related to exposure, from tobacco

exposure, and there's the issue of temporality; that is, does exposure precede the effect?

The issue of experimentation: Is there evidence showing the removal of exposure lessens or removes the effect?

And also the issue of biological gradient: Is the magnitude of exposure proportional to the magnitude of effect? In other words, the dose-response relationship.

There's also the question of specificity: Is the effect specific, or are there other known causes?

And analogy: Can inferences be made based on data from other agents?

And finally consistency: Is the relationship reproducible and observed by multiple investigators in different populations using different methodologies?

There is maybe some other considerations, too. These considerations include the following: The utility of biomarkers depends on the assumption that they not only correlate with the clinical endpoint of interest but will also fully capture the complete effect of an intervention on a clinical endpoint.

We also need to think that no single biomarker is likely to fully capture a clinical endpoint.

And a single biomarker could be a predictor of many diverse conditions, so there could be biomarkers of systemic inflammation, other immune dysfunctions, or oxidative stress that could be related to cardiovascular disease, cancer, as well as lung disease.

And then also, other biomarkers could be specific to certain conditions such as HDL and LDL for cardiovascular disease.

So now I want to turn your attention to looking at these criteria related to tobacco exposure as well as product developed, product evaluation.

So on the issue of plausibility and coherence, there have been pathways of causation for cancer by carcinogens in tobacco smoke that have been determined, and this is an illustration of the carcinogenesis related to tobacco, and it will be further described by Dr. Hecht, Stephen Hecht.

But I just wanted to point out that biomarkers can occur along different pathways; that is, you can have biomarkers that target different pathways, and for example, they can include looking at DNA adducts or looking at mutations in p53 tumor suppressor genes.

I also want to point out that there are also multiple

pathways to cancer, as well, and so it would be really important to be able to capture these multiple pathways, and they can include such things as inflammation and oxidative stress.

Another example of plausibility and coherence is the pathways that have been developed to understand mechanisms of smoking cause acute cardiovascular event, and this is one that Dr. Benowitz will describe further. But again, what I want to point out here is that there are multiple pathways which include inflammation, platelet activation, endothelial dysfunction, coronary vasoconstriction, increased blood pressure, and so on that result from exposure to different harmful and potential harmful constituents of cigarettes. So again, potential -- biomarkers of potential harm can occur along any of these pathways but must capture all the critical pathways associated with disease.

Now, here's the ideal biomarker. This is a biomarker that lies along the only causal pathway of a clinical endpoint's process, and the biomarker mediates the intervention's entire effect on the clinical outcome. But this is really an ideal biomarker because not very many of these biomarkers exist.

As I pointed out before, we need to think of multiple

pathways, and therefore we need to capture these multiple pathways or else we wouldn't really have a good idea in terms of what the product's potential harm might be. There are other errors that occur besides not capturing multiple pathways, and they include using biomarkers that are not associated with the disease process or biomarkers that are insensitive to or do not reflect the effects of biomarkers of exposure.

Now, to determine the sensitivity of a biomarker of potential harm, sensitivity, and predictiveness, you need to consider the distribution and levels of biomarkers in a population without the disease, and it's important to think of what the distribution of this biomarker may be.

You also need to consider the distribution between those who have the disease versus those who do not have the disease and determine what the discrimination is between the -- the discrimination, the biomarker may be between those with and without the disease and the overlap. And then what you need to do, too, is to take a look at the level of biomarkers that might be predictive of disease or the occurrence of biomarkers that might be predictive of disease.

In terms of looking at temporality, experimental -- experimentation, biological gradient associated with exposure,

there are several ways to do this. One is to look at users versus nonusers of tobacco products, and again, it's important to take a look at the distribution between users and nonusers for the biomarker. You need to take a look at what the change is with cessation of the product. A dose-response relationship; that is, looking at levels of harmful and potential harmful constituent levels or levels of exposure biomarkers and how they relate to biomarkers of potential harm. And then to look at change with reduction in use or in constituent yields.

This is a very good example in terms of illustrating how biomarkers of harm for cardiovascular disease has been categorized in each of the ways that I have described. So on the left you'll see the biomarker, what it measures, and then looking at what is the scientific data that support smokers versus nonsmokers, change with smoking cessation, dose response, and change with reduced smoking. And that's in the 2014 Surgeon General's Report.

So in terms of specificity, the majority of biomarkers of exposure -- biomarkers of potential harm are not specific to tobacco and can be influenced by diet, cooking methods, ambient air, occupational settings, and physical activity and so on.

In terms of analogy, there are several non-tobacco agents which have demonstrated that lowering biomarkers of potential harm will reduce disease risk, such as hypertension. And then in terms of consistency, there have been some biomarkers that have shown similar results across different study methods, tobacco products, and investigators, such as white blood cell count.

Now I'm going to turn your attention to study designs for biomarker development and testing, and this could or could not include smokers. And so typically when you're trying to develop a biomarker, you have a number of phases that it goes through.

So Phase 1 could be preclinical and exploratory, and this is the phase where you try to identify a potential biomarker of harm, and you also develop and validate assays to assess this biomarker.

Phase 2 involves clinical characteristics of this biomarker, and this is typically a cross-sectional study in which you compare people that are with or without disease.

Phase 3 will involve typically an existing cohort study where there are bio-samples that are available for analysis, and they're usually case-control studies. And these are samples that you can look at that are pre-disease, so you look

at pre-disease biomarkers and then determine how these biomarkers perform in terms of differentiating people who eventually actually develop the disease and then versus those who did not develop the disease.

And then the last phase would be the longitudinal study, and that would be the prospective study where you take a look at the biomarker and then determine to what extent they are predictive of disease.

Now, in terms of study designs to assess biomarkers of potential harm in tobacco users, and these are users that are -- that experience different levels of exposure, and it could be exposure within tobacco products as well as across different tobacco products.

And these are the types of studies that could be done: they include laboratory studies that look at acute effects; residential studies, so you put these smokers in a residential setting, and that could last for a few days. Sometimes these are target or tagged with non-residential studies, or they can stay in for several weeks. And the benefit of these two studies is that you can control the compliance to a particular product, and you can control confounding factors.

There are also clinical trials that can be conducted, and

these tend to be either short-term or long-term studies. And then there's cross-sectional observational studies that can be conducted, so for example, comparing smokers versus nonsmokers on these biomarkers.

And there there's cohort studies that can be conducted. These -- for example, the Population Assessment of Tobacco and Health, where you can follow these people to see a change in tobacco status, as well as change in the types of tobacco products that they use. And then finally the case control studies, both cross-sectional and longitudinal, which are the types of studies that I have previously described.

There are other considerations for studies testing biomarkers for potential harm. One is you need to consider the population recruited. It should be representative of a broad cross-section of the population, or they could be a population at risk for disease outcome or current diagnosis of disease, especially if you're taking a look at clinical endpoints.

The trials should be informed by the half-life of the biomarker and incorporate a range of biomarkers that are reflective of disease condition or different disease conditions. You need to consider stabilization and compliance with tobacco product use, and then assess confounding

environmental, behavioral, and individual factors such as diet, environmental exposures, obesity, level of physical activity, sex, age, race, BMI, and so on and so forth.

So in summary -- I'm going to beat this red light here -- biomarkers of potential harm should be based on our understanding of pathophysiologic processes associated to tobacco exposures; sensitive to and predictive of disease risk and conversely demonstrate reduction in disease risk if the biomarkers are positively affected; they should be capable of distinguishing different degrees of tobacco and constituent exposures within and across products; and finally, sensitive to the impact of lowering tobacco/constituent exposures.

Thank you.

(Applause.)

DR. DRESLER: Thank you, Dorothy.

Our next speaker is Dr. Kimberly Frost-Pineda from Altria Client Services speaking on Utility of Biomarkers of Potential Harm in Tobacco Product Regulation.

DR. FROST-PINEDA: Good morning. My name is Kimberly Frost-Pineda. I'm an Associate Principal Scientist at Altria Client Services. Altria Client Services provides regulatory affairs support and other services to Philip Morris USA, USSTC,

Nu Mark, and John Middleton.

I'd like to start by thanking the FDA CTP for sponsoring this workshop and for the opportunity to participate in the presentations and panel discussions on biomarkers of potential harm that may be useful in tobacco product regulation. My presentation today will focus on the biomarkers of potential harm that we included in the Total Exposure Study.

The presentation is organized as presented in this outline. Questions to be addressed include, based on our learnings from the Total Exposure Study: What are the well-established biomarkers of potential harm? What factors should be considered in the data analysis and interpretation? And what are some of the challenges and strengths?

In the background section, the relationship between exposure and disease and the mechanisms believed to underlie smoking-related diseases are described. Next, a description of the Total Exposure Study, which I'll refer to as TES, is provided, as well as the biomarkers that we measured and a summary of the results.

There are three things I'd like people to take away from this presentation: What are biomarkers of potential harm and how they relate to exposure and disease risk? What factors

influence BOPHs? And finally, which three BOPH may be the most useful based on our analysis of the Total Exposure Study?

So let's start with some background information: There is overwhelming medical and scientific consensus that cigarette smoking causes lung cancer, heart disease, emphysema, and other serious diseases in smokers.

Chronic diseases related to smoking generally develop over decades, which makes long-term prospective studies of tobacco products difficult.

Researchers have been attempting for decades to reduce the harm associated with tobacco products, and the scientific basis of tobacco harm reduction was reviewed by the Institute of Medicine and published in 2001.

Both the IOM, now known as the Health and Medicine Division of the National Academies, and the World Health Organization have recommended the use of biomarkers of potential harm in the evaluation of tobacco products, and researchers have begun to use them.

As you've heard, the IOM defined biomarkers of potential harm as "a measurement of an effect due to exposure; these include early biological effects, alterations in morphology, structure, or function, and clinical symptoms consistent with

harm." As you can see, this is a broad and inclusive definition of a BOPH.

This figure shows the relationship between exposure and disease. Even when there's a strong relationship between exposure and disease, there are genetic and other factors that play a role in every stage of the development. BOPHs are important early indicators of disease risk. Differences in BOPH are expected in those with and without exposure, and favorable changes in BOPH may result from reductions in exposure.

The mechanisms believed to underlie smoking-related adverse health effects include oxidative stress, inflammation, platelet activation, and abnormal lipid metabolism. Considering the broad and inclusive definition of BOPHs, there are numerous other markers that are also associated with disease and risk. These include things like heart rate, blood pressure, and lung function.

The Total Exposure Study or TES is the largest published cross-sectional study to date that included such a large number of measures of BOE and BOPH in the same population.

The primary objectives of the TES were to compare exposure among people with different -- who smoked different FTC tar

category cigarettes and also to estimate exposure among U.S. adult smokers. A secondary objective was to compare selected biomarkers of potential harm and look at cigarette smoke exposure. In the design of the Total Exposure Study, BOPH were identified through a review of the existing science.

Selection of BOPH was based in part on having validated measures and the feasibility of measuring the BOPH in such a large study population. The focus in this presentation is on the BOPH that we measured and reported, and this is how we identify some of the specific BOPH that we believe have the most utility for assessing potentially reduced harm products.

I will quickly summarize what we did in the TES and then get into some of the specifics. We measured 29 biomarkers of potential harm; 21 of them were significantly different between adult smokers and nonsmokers. Nicotine equivalents, which is a biomarker of exposure, was statistically significant in the multiple stepwise regression model for 19 of the BOPH. And 10 of the BOPH had R^2 values greater than 0.10. Nicotine equivalents was the most important factor for two of the BOPH and was the second most important factor for one other BOPH.

The TES included BOPH measurements in approximately 3600 adult smokers and almost 1100 nonsmokers. The mean age of

purchase -- in the TES was in the early forties with a range of 21 to 88 years old. Nearly 60% of participants were female; 75 to 80% self-reported race as white; and the mean body mass index or BMI was 28.

Among smokers, the mean number of years smoked was 22 years. Mean cigarettes per day based on the number of butts returned was 16. I've also provided average machine yield tar, nicotine, and CO of usual brand cigarettes, and we've provided the complete TES dataset to the FDA CTP, and additional details can be found in the published literature.

The tables on this and the next slide include the 29 BOPH measured in the TES grouped by system and function. The 21 that are marked with an asterisk were significantly different in adult smokers versus nonsmokers. As you can see, we included markers of oxidative stress, platelet activation, inflammation, and markers related to cardiovascular risk.

Abbreviations I will use in reference to the BOPH in this presentation include 8-epi for 8-epi-prostaglandin F2alpha; 11-dehydro for 11-dehydrothromboxane B_2; WBC for white blood cells; and HDL for high-density lipoprotein cholesterol.

We also included measures of lung function, kidney and liver function, hematology, and metabolism. In future slides,

I will use FEV_1 to refer to forced expiratory volume in 1 second.

So now let's take a look at examples of some of those differences between smokers and nonsmokers. Presented here are the mean values and percent differences for white blood cells, fibrinogen, 8-epi, 11-dehydro, von Willebrand factor, HDL, triglycerides, heart rate, and FEV_1. The largest percent differences between smokers and nonsmokers was 42% for 8-epi, 29% for 11-dehydro, and 19% for white blood cells. Among BOPH that are different between adult smokers and nonsmokers, most are higher in smokers as compared to nonsmokers, but there are some exceptions. For example, HDL and FEV_1 are lower in smokers as compared to nonsmokers. Therefore, favorable changes in BOPH generally means a reduction but could mean an increase in something like HDL or slowing the rate of decline in lung function in smokers.

There are a number of factors to consider in the analysis and interpretation of BOPH. Smoking-related factors include cigarettes per day and smoking duration. We found statistically significant differences in BOPH by cigarettes per day, categories for white blood cells, 8-epi, and 11-dehydro, as some examples.

Smoking duration was significant in the multiple stepwise regression for 17 of the 29 BOPH that we measured.

There are other individual factors such as age, gender, and BMI, and behaviors such as diet and environmental factors that influence BOPH. Some of these factors are easier to assess and include in statistical models.

Our multiple stepwise regression models in the TES included age, gender, race, BMI, smoking duration, and number of cigarettes per day as independent variable in Model A, and the same factors with nicotine equivalents in milligrams per 24 hours in Model B instead of cigarettes per day. Cigarettes per day and NE were used as surrogates for daily smoke exposure. We used estimates of standardized regression coefficients to determine the most important factors in the models.

R-squared values for the models with nicotine equivalents were slightly higher than the models with cigarettes per day. And 12 of the BOPH had R^2 values greater than 0.10. Of the smoking-related variables, only smoking duration was significant for von Willebrand factor. And nicotine equivalents was significant in the models for 19 of the BOPH.

BMI was the most important factor in the model for 12 of the BOPH including 8-epi, fibrinogen, HDL, triglycerides, and

heart rate. Nicotine equivalents was the most important factor in the model for WBC at 11-dehydro and the second most important factor in the model for 8-epi.

BOPH are not without challenges. One challenge in interpreting BOPH is the significant intra and inter-individual variability. Some BOPHs are not consistently different between smokers and nonsmokers. As I've mentioned, there are many factors that influence BOPH, and there are also many factors that influence exposure and disease risk; therefore, interpretation of the findings should consider these factors that may impact study outcomes.

Another challenge is that few studies have looked at the impact of smoking reduction on BOPH. And as the IOM has stated and has been previously mentioned, no BOPH or surrogate endpoint is the perfect substitute for a clinical endpoint. Finally, it takes significant time and numerous studies to substantiate a new BOPH.

There are also many strengths to consider. Decades of research supports the link between oxidative stress, chronic inflammation, and chronic diseases such as cancer, cardiovascular disease, and pulmonary and neurologic diseases.

Many studies do report differences in BOPH between tobacco

users and nonusers. And Dr. Hatsukami's published review summarizes changes in BOPH that have been reported with smoking cessation, and as you can see, there's a number of them. There is also evidence that certain BOPH may change rapidly after smoking cessation or switching to a reduced exposure product. And finally, as science evolves, potentially useful BOPH continue to emerge.

In summary, in the TES, we found statistically significant differences between adult smokers and nonsmokers in 21 of the BOPH that were evaluated. BMI was the most important factor for 12 BOPH.

The largest percent differences between adult smokers and nonsmokers were observed for 8-epi, 11-dehydro, and WBC. Based on the F values, nicotine equivalents was the most important factor in the models for WBC and 11-dehydro, and the second most important factor for 8-epi.

Of the 29 BOPH presented here, these three BOPH appear to have the most evidence to support their use in future clinical studies evaluating modified risk tobacco products.

In conclusion, biomarkers of potential harm, particularly those related to inflammation and oxidative stress, may be useful in the assessment of the health impact of new and

modified risk tobacco products.

And I'd like to conclude by acknowledging some of my ALCS colleagues who have contributed to the analysis and reporting of the BOPHs that were presented here. And I've also included the references in these slides.

That's it.

(Applause.)

DR. DRESLER: Thank you, Dr. Pineda.

Okay, our next speaker will be Dr. Robert Temple, who has been with the FDA for quite a while, and he'll be speaking -- from the Center for Drug Evaluation and Research, and he will be speaking on Surrogate Markers at FDA – Lessons Learned.

DR. TEMPLE: Well, good morning. I'm not going to try at all to talk about what biomarkers and surrogates might be related to alternatives to smoking and things like that. I'm going to speak more generally about our experience with surrogate markers and what we've learned over the years.

We've actually -- we and NIH together have tried to get better definitions of these terms because they're used interchangeably and variably and things like that. And we produced a document together called "BEST: Biomarkers, Endpoints, and other Tools," which is an effort to assure

consistent and accurate use of terminology. So in all these matters, there's a critical distinction between a biomarker, which is a measure of normal or abnormal biologic processes, or sometimes a response to an exposure to something, but it's not an assessment of how a person feels, functions, or survives. It isn't a clinical endpoint.

So we try to distinguish between these biomarkers and what are actually surrogate endpoints, which is where we use a biomarker as a substitute for direct measure of how a patient feels or functions or survives. These are endpoints used in clinical trials to approve drugs.

So surrogates, surrogate endpoints, these are measurements that somebody uses to try to get a drug approved, are considered for use as evidence of effectiveness in two ways. They can sometimes be used to support full approval of a drug because we are absolutely certain that the effect on the biomarker predicts clinical benefit. So that's one.

And surrogates can also be used to support what's called an Accelerated Approval of a drug where we think the biomarker/surrogate is thought to be reasonably likely to predict a clinical benefit. And that's based on epidemiologic, therapeutic, pathophysiologic, or other evidence. Accelerated

Approval is a more rapid pathway to approval to reserve for new drugs that are used to treat serious and life-threatening diseases where the drug has what appears to be an advantage over available therapy. When we use Accelerated Approval, there's a requirement that there be clinical studies afterward to show that the surrogate really did predict what we hoped it would. So those are -- that's what surrogates are used for.

I want to make a distinction, though -- this seems relevant to the tobacco area -- between a surrogate marker, which is a measure of an effect of a drug that we think corresponds to some outcome, and the use of biomarkers as epidemiologic predictors and things like that. You don't have the same level of proof when you're not trying to approve a drug, and that's obviously going to be relevant to a lot of tobacco considerations.

So the critical differences between a surrogate and a biomarker is that we believe the biomarker predicts a benefit when a drug affects that biomarker. Now, considerable experience has shown that potential surrogates, very plausible changes in some characteristic induced by a drug, turned out not to be predictors of clinical benefit. And as a general matter, again, when you're talking about approving a drug to do

something, there's usually considerable skepticism until clinical studies confirm that the change in the biomarker leads to a benefit.

And this issue has been around for really decades and decades. And the reason is, the reason there are doubts about the biomarker is because we're not always sure about whether the biomarker is on the causal chain; that is, you believe in a surrogate when you believe that the marker tells you that the drug affects something that leads to the disease, and there's always debate about it, including things we now take for granted.

Maybe some people in the room will remember this, but in the '60s there was something called the New York School, which raised very major doubts about the effectiveness of lowering blood pressure because they said elevated blood pressure was a compensation reaction to arterial disease and that if we were to lower it, it would lead to more heart attacks and strokes.

This was very broadly accepted, and actually it was about the time I was in medical school, so I remember it. That went away promptly when the VA studies in the late 1960s showed that lowering blood pressure, in fact, reduced stroke, heart attack, and death, and that fixed that. But even there, the NIH NHLBI

ALLHAT study showed that not all blood pressure drugs are exactly the same. Some do more of one thing than another, and of course we know that some blood pressure drugs delay renal dysfunction in diabetes and others don't. The trials that showed that angiotensin receptor blockers delay loss of renal function in diabetic nephropathy included other antihypertension drugs, modipine, and it had no effect at all. So they're not all the same; you never quite know what's blood pressure and what's something else.

And then we've been -- yeah, okay.

Probably not too many people are going to remember this, but in the '90s, just before there were definitive cholesterol lowering drugs, that is, drugs that lower LDL cholesterol, statins notably, before the definitive studies that showed that this led to benefit, the 4S study and the WOSCOPS study, there was a book written about how lowering LDLs with statins would be worthless, wouldn't do any good at all. The book, I imagine, became remaindered quickly after the 4S study showed that lowering cholesterol was very good for you. But we read skeptical articles in books all the time about whether statins are really as good for you as all that, and there's great doubt about it. So even well-established surrogates can have their

doubters.

And whether -- for example, lowering LDL cholesterol is good for you when something other than a statin does it is highly argued and debated, and so things are not always as clear as you want, even though it's perfectly clear that a high LDL cholesterol is bad for you. The question isn't that.

The epidemiology is clear enough, and maybe that's what's most relevant to tobacco, but whether a treatment has an effect is not always clear. And, of course, if we needed more reasons for doubt, recent drugs, everybody knows, epidemiologically, that a low HDL is bad for you; it leads to heart disease. Whether drugs to treat that are going to be effective is highly debated, and some very prominent failures have been conspicuous.

So what are the problems? One, sometimes the surrogate that people identify isn't really related to the outcome. I mean, there are classic cases. Everybody knows if you have an infection, you have a high white count and a high fever, but that's not the cause of the infection, and treating those things won't improve the infection; they improve the fever and things like that. So we know about those.

The other possibility, when you're talking about drugs, is

that drugs do things other than the effect you're interested in. They have off-target effects, and there are classic examples of that that have been very disturbing over the years.

So antiarrhythmic drugs have many effects other than decreasing the arrhythmia you're interested in, and it's always hard to predict whether what they do and what you can measure will have the effect you want to have. The classic example of that is the CAST study.

It was known that people who, after a heart attack, had a lot of ventricular premature beats, had increased mortality, considerably increased mortality.

There were some studies that tried to affect that, but they didn't show much because the drugs didn't have much effect on ventricular prematurity rate. Along came CAST, which studied encainide/flecainide, which had profound effects on the ventricular premature beats that were associated with the bad outcome. And, in fact, you couldn't get into the Cardiac Arrhythmia Suppression Trial unless the drug, in a prescreen, lowered the ventricular premature beat right by a least 70%. So it was a very good test of whether the thing that led to increased mortality, if you made it disappear, would improve survival. So the drugs wiped out the ventricular premature

beats and doubled mortality. So that took care of that for a while. But it reminds you that surrogates are not always right. It was very plausible, very plausible. The trial was enriched, and it didn't work at all.

In the area of heart failure, we've had -- we had a whole series of drugs that make the heart beat stronger, which ought to be the remedy for a lot of heart failure, you would think. And there were both biomarkers of improved function and early benefit in exercise tests and a whole series of drugs when tested for longer periods of time. Even though they had those favorable effects, they increased mortality. They all did.

And then there's interesting experience with erythropoietin. We know that can increase your hematocrit, and there were profound epidemiologic data that showed that people with renal disease who had better hematocrit did better, survived better. So there were efforts to use erythropoietin to push the hematocrit up into the range that was associated with better survival. Uniformly, those studies showed that using more erythropoietin to get a higher hematocrit increased mortality in various ways by increasing heart failure and things like that. Interestingly enough, within the subgroups of people who did and didn't get higher levels of

erythropoietin, a high hematocrit was associated with better survival. So they were right about the hematocrit, but the drug did something else that -- anyway. So we had -- we're very sober about presuming too much.

And I already talked about that.

Now, there are, at the same time, surrogate endpoints that are clearly the basis for approval of drugs. There aren't too many, but some of them are sort of obvious. And this slide has some errors in it as I go through it.

There's overwhelming data from randomized trials that lowering blood pressure is good for you, and it seems fairly clear that something in tobacco that raised blood pressure would be very suspect as being bad for you, so that's an endpoint that one might think about. We are very confident that LDL lowering by statins is overwhelmingly evidence of a good effect. There's considerable uncertainty about the role of triglycerides and HDL and stuff like that. In heart failure and arrhythmias, we have no surrogates at all. We have markers of effectiveness, but we don't really know what they do, and we always ask for outcome studies in those areas. It says 4b A1C, it means hemoglobin A1c, is widely used as a surrogate endpoint for any diabetic drugs and is pretty convincingly related to

improve microvascular outcomes.

We approve drugs because they raise or lower potassium. We know it's bad to have a high potassium, bad to have a low potassium; those are fine. You lower uric acid, you improve gout. In oncology, tumor responses are regularly used for at least accelerated approval. So some of these, we're quite confident.

It's also worth noting that we use biomarkers that we think predict outcome not as study endpoints but to make the trials more likely to succeed. So we pick people with markers of a high rate of progression in heart failure or anything else to put people in the study who are more likely to have events. So we use C-reactive protein to enrich a study that's trying to improve coronary artery disease because that's associated with a bad outcome. We don't use it as a biomarker for proven drugs, but it does signal risk, and that may be more relevant to the smoking area. And we sometimes use biomarkers to predict response, a different question. There are specific cystic fibrosis genetic markers that tell you whether a particular drug will work, so we use all those.

Of interest, we don't usually think of safety biomarkers as being surrogate endpoints, but there are a couple of cases

where you might think of that. One, it seems fairly obvious that where you believe a biomarker is a measure of drug effectiveness, like lowering blood pressure, that some element of tobacco or smoking that raises blood pressure, LDL cholesterol is likely to be a predictor of a bad outcome; that seems fairly easy. Increasing the risk of diabetes or pre-diabetes is clearly bad for outcomes; epidemiologically, that's clear.

And as others have mentioned, altering pulmonary function tests adversely is almost surely going to be a bad outcome, and that's, of course, what smoking did. If you raised uric acid or decreased kidney function, those would be bad. So there are things to look for that are very plausible safety biomarker/surrogates. They're all -- those markers are all things that we treat to lower risk. It seems fairly obvious that making them worse will have adverse consequences.

I want to mention two other safety biomarker/surrogates that we've used over the years and that have proved correct. We now, we believe, know how to identify drugs that are going to be toxic to the liver and be lethally toxic to the liver as distinguished from drugs that just, you know, damage the liver, and it's reversible, with an elaborate long history. If a drug

causes hepatic necrosis, which you know because the transaminases go up, there's always a question of whether that's going to be reversible or permanent, whether some people will die or need transplant and things like that.

Many, many, many, many years ago Hy Zimmerman, who was interested in lower toxicity, recognized that if a drug kills off enough liver to keep you from putting your -- putting out your bilirubin improperly, it probably will not -- the liver probably can no longer recover. Liver regrows; it's -- you can kill a lot of it, and you can still grow it back, but if you do more than a certain amount, it probably can't go back. And so we developed a policy that said if you have liver injury and it's bad enough to make your bilirubin go up, that probably tells you that a certain fraction of people are going to die. And that has been overwhelmingly convincingly shown year after year, sometimes after we approved the drug, despite such a finding. But more recently we've turned drugs down, ximelagatran and lumiracoxib, that had those properties.

They were marketed in Europe and withdrawn from the market subsequently because of liver toxicity. So we're pretty sure we know how to detect, using a surrogate marker, if you like, or a biomarker, surrogate marker, evidence of severe liver

injury.

A similar situation is we've learned that drugs that prolong the QT interval of the electrocardiogram more than a certain amount pose a risk of a potential fatal arrhythmia called Torsade's des Pointes, TdP. And in the past, we didn't know how to look for that, and so some drugs were approved, terfenadine, cisapride and a few others that caused Torsade, but we didn't know about it. We now do a classic study that always reveals how much the QT prolongation is, and I think it's fair to say we will never unknowingly approve a drug that causes Torsade. You might approve it because it's worth the risk, but that's another safety biomarker surrogate, and there could be things like that in tobacco if one looks for them.

Anyway, so what's needed most is good data on what the various products actually do to an underlying risk before approval, and information about the extent and persistence of those risks over time. That doesn't mean there can't be biomarkers that could be extremely useful because they have very strong predictive ability, and that's different from using them as a surrogate for approval. That's the epidemiology of disease, and there's a lot of room for discovering that.

Thanks.

(Applause.)

DR. TEMPLE: Are we sitting here?

DR. DRESLER: Yes. If you will, please. And Dr. Hatsukami and Dr. Frost-Pineda, if you will please come up?

(Pause.)

DR. DRESLER: Okay. Well, this seems like a pretty easy topic and straightforward to start off the morning with. A little sarcasm there perhaps. So do we have any questions from the audience or people, if you're online? Please send your questions in online. And if you wanted to ask questions, go ahead, step up to the microphone or raise your hand, and we'll get you a card or I'll bring by the microphone.

Let's start off with one of the questions that Dr. Chang alluded to before. What are some of the other terms that have been used for biomarkers of potential harm? So we've had it up on our slides, BOPH, you know?

DR. TEMPLE: Well, people call them risk factors and things like that, you know? If someone has decreased FEV_1 -- first of all, actually, we have our own internal debate about whether that's a biomarker or a clinical endpoint, but leaving that aside, all of those things have been called function tests and things like that. And whether they are clinical endpoints

or biomarkers could be debated and people can argue about it, but everybody knows it's bad. So if smoking leads to worsened lung function, whether you think it's a biomarker or a clinical endpoint, that's not good.

DR. DRESLER: Okay.

DR. TEMPLE: We try to lay out the terminology in the BEST program, and mostly I think that everybody knows they're biomarkers if they're not clinical endpoints, but sometimes they're called predictive and sometimes they're called other things, and we tried to lay through all that terminology. But clearly what everybody wants to know is are you doing something that is epidemiologically associated with a bad outcome. That's what you want to know.

DR. FROST-PINEDA: Another term that's used is biomarker of biological effect. That's often used in the literature as well.

DR. DRESLER: Thank you. Anyone else? Thank you.

So let's look at the differences between drug and tobacco context. So what are some of the differences when looking between -- looking at drug and tobacco context, and what implications does that have for the use of biomarkers?

DR. HATSUKAMI: Well, it seems like for drugs you really

are targeting a specific disease. When you're talking about biomarkers for tobacco, it's not just one disease that you need to consider. You need to consider, you know, all the various diseases. And so, you know, I would think that that would be one difference between biomarkers for intervention, like pharmacologic equations or assessing biomarkers for tobacco products.

DR. TEMPLE: Well, it's very important to distinguish between things you are worried about and that make you nervous and things that you use for approval. I was presenting a discussion of how we use surrogates to make us believe that a drug will have a desirable effect, but we get nervous about a million things about a drug.

For example, we get nervous about the animal data. If a drug is carcinogenic in an animal, we don't have human data to show that, but we're very nervous. And if the finding is striking and the disease to be treated has other treatments and there's nothing special about this, we might reject the drug because it was carcinogenic, or companies, as a matter of fact, won't even develop it if it's carcinogenic. So you worry about those things.

There are all kinds of interesting animal findings, you

know, sclerosis in some tissue or something like that, so you follow that up in the human data, but if you're nervous and you're not sure you have enough data, sometimes those things lead to rejection also, even though you don't, in any sense, really know what the consequence is for the human. So there's all kinds of things. Some drugs -- well, as I was saying, some drugs prolong the QT interval or they do something else that makes you nervous about the electrocardiogram; that would be worrisome. You don't like things that raise blood pressure; that makes you nervous. Sometimes drugs cause weight gain; that can make you nervous. It can provoke diabetes, it can provoke other consequences, so these are all things you worry about when a drug does them, and they relate to safety.

Only some of them lead to non-approval, but they certainly lead to warnings and monitor this and all kinds of stuff like that. Some drugs increase the rates of seizures. There's a million different things that drugs can do other than what the desired effect is. Some of them are overt, clinically manifest like seizures; some of them are things where you're not quite so sure what the consequence is, changing in lipids and things like that.

And we're developing more and more markers that we think

predict outcome, that you measure the blood and people can make those nervous. I mean, just a good example, C-reactive protein levels were used to enrich a trial of rosuvastatin to see if it could decrease cardiovascular outcomes in people with more or less normal LDL cholesterol, so they had to enrich the study to get people who would have events, okay. So they did that by making them have a high LDL. Now suppose a drug -- I don't mean LDL -- C-reactive protein. Now suppose a drug increased your C-reactive protein levels, some evidence of inflammation. What would we do with that? Could tobacco do that? I don't know.

But there's lots of -- as you learn more and more about epidemiology, there's more and more things to be nervous about. And I think those are all things everybody would worry about. And then you'd start to look and try to get epidemiologic data to see if the outcome really follows that or not, or how soon, and all of those kinds of questions. So there's lots to look for, infinite quantity of things to look for as we learn more.

DR. FROST-PINEDA: I think another major difference between looking at drugs and looking at tobacco products is, for drugs you're looking at safety and effectiveness of the drug; for tobacco products you may be looking does it reduce

exposure to specific smoke constituents, or does it reduce harm? And for people that have a long history of smoking, that may be difficult to demonstrate.

DR. DRESLER: You know, I wanted to follow up on that because I was thinking, Dr. Temple, you're talking about treating a disease, and in a tobacco product, looking at perhaps preventing it or not making it worse in a disease that has started. And I had noticed, you know, the age for the TES study, the average age was 41 and 43, and that's prior to most cancers and cardiovascular disease that's being treated, perhaps hypertension is in that age.

So how do we start looking at biomarkers of what age and what degree of disease and then what degree of not worsening of disease versus -- Dr. Temple, you're talking about treating the disease. I mean, it seems like they're quite different in what you're asking the biomarkers to do.

DR. TEMPLE: Well, they are. There's a difference between whether something predicts outcome that's an epidemiologic finding and knowing that reversing that abnormality will do good. There's two main reasons for that, I try to say. One is you're not always quite sure that you know the exact cause; is the thing you're finding on the causal pathway? So that's one

reason. But the other thing is that when it comes to drugs, they will do more than one thing. And you know, classic, wonderful, horrible example is we've just had some drugs that lower -- that raise HDL cholesterol. Well, low HDL cholesterol is pretty strongly associated with a bad cardiovascular outcome. And there have been drugs that raise it a lot. And if you'd ask me, I would have bet anything that they'd come out showing favorable results, but not only didn't they show favorable results, the first one, torcetrapib, actually made cardiovascular outcomes worse.

So here's a drug that raised HDL and actually lowered LDL a lot, and it made the outcome worse. That's very hard to understand and very unnerving. So in the drug area, you got to be very careful, and we tend to be nervous and cautious. But the evidence that -- but suppose some bad, some tobacco component lowered HDL, your going-in impression would be that that is not a good thing to do, and it's not the same, you know.

In fact, it could be that the tobacco component that lowered HDL also did something wonderful to some other aspect of your cardiovascular disease, but you wouldn't know that and you wouldn't think that and you'd have a high level of worry.

It's not the quite the same as intervening with a drug where there's always the possibility of doing a lot of things, some of them bad, some of them good. I think you'd be nervous just on epidemiologic grounds if you saw an effect on a marker that we know.

DR. DRESLER: Okay.

DR. HATSUKAMI: I think that, you know, basically addressing what you were asking, you really do need to consider the population that you're recruiting, and I think that's one of the things that I had mentioned that it has to be a population that's representative of different, you know, a spectrum of people that -- of different ages, potentially comorbidities, different genders, and so on, so forth.

So I think that's going to be really important for future studies, to -- for developing biomarkers of exposure. And you can also consider, as I had mentioned in my PowerPoint, actually recruiting people that are already at high risk for disease. So, for example, some of the studies that we have conducted are people that have cardiovascular disease, and so you know, what happens to biomarkers of potential exposure in that particular population? So I think those are things that you need to consider when you're trying to determine what

biomarkers should be used.

DR. FROST-PINEDA: I just want to mention, in the TES, the population was reflective of the U.S. population, so we did have age range from 21 to 88. Another thing I wanted to mention is that it may be to have a number of BOPHs included in the study versus just the single endpoint may be beneficial.

DR. NELSON: Paul Nelson, RAI Services. Dr. Temple's talk really kind of got at validation, and I'd like to ask kind of a question around validation. What should be done or taken to account to ensure that biomarkers of potential harm are effectively producing or predicting a reduction in risk rather than simply being related to changes in exposure?

DR. TEMPLE: Well, I'll start. But if I understand you, it's the usual, you're raising the usual question about whether an association, if -- whether modification of something that's associated with bad outcome will lead to benefit. And the only way you can do that rigorously, most rigorously, is to do a trial in which you take something that, I don't know, that tobacco did and intervene to reverse it. You know, let's say some component raises blood pressure. I guess you could say okay, I'm going to take some people who are smoking or taking this other thing, and I'm going to lower some of their blood

pressure and I'm going to leave the rest untreated. I know how to do that; we have lots of blood pressure drugs. Now, you could try to do that. The effect on blood pressure is so modest that you'd probably need a study of a few hundred thousand, and most people would say that's not worth doing, so the question is going to be do I know enough about blood pressure already to know that raising it a little is going to be bad, not necessarily measurably bad, but bad.

I could also think about whether there's a subset of people who have a really big change even if most people have a small change, and I could try to think about that. And I think on a matter like blood pressure, you'd bring your epidemiologic knowledge, and you wouldn't do a trial. It's a dubiously ethical trial anyway, and you'd probably just assume it.

But for markers whose outcome and effect and consequences are less clear, you become more tempted to try to do an intervention. I mean, something everybody in the room has to answer is when you're talking about bad things that tobacco does, what are the ethics of doing a trial at all? It's not easy to imagine deliberately exposing people to the adverse effect, so I'm -- you guys have to figure that out.

DR. HATSUKAMI: So I think that there are, as I mentioned,

case control studies that can be conducted because there are existing cohorts that do have bio-samples that have been collected prior to the incidence of the disease.

And I hearken back to the exposure workshop that we had where we presented data where the data was presented on the Shanghai Cohort, for example, where they had collected bio-specimens and then follow these people and saw, determine who developed cancer, for example, and who didn't. And you can use that to know, to determine what their biomarkers of exposure is predictive of the disease by analyzing those bio-samples. So that's one way.

But I think we can also rely on studies that have already been conducted, like the intervention studies, you know. If you find that intervention that lowers blood pressure has an improvement in terms of cardiovascular disease or mortality from a cardiovascular disease, then I think that we can use those types of studies to inform us in terms of what might happen, you know, if there is a lowering of some of those biomarkers of potential harm.

DR. FROST-PINEDA: First, I'm just going to mention there's also been a large number of studies that have looked at smoking cessation, so I think with tobacco products, if you can

get the BOPH to the level of people who have quit smoking, that that would be a positive outcome.

DR. NELSON: And I think that may be closer to kind of the crux of the question. A manufacturer wants to introduce a new product or produce a new product and -- or make a modified risk tobacco product claim around that product, you can look at say, for example, smokers and people are using that product and showing a change in a number of biomarkers of potential harm, but then the question comes back to is showing that change enough to support an application, or is there more information that needs to be known around that biomarker of potential harm so that you can effectively make a claim around the product?

DR. TEMPLE: I think what everybody's been saying is that it depends on how much you know about the marker. Taking an easy one, a no-brainer, if you like, if you show that your new product doesn't raise blood pressure and the other one didn't -- and the other one did, you'd be pretty comfortable. We know having a higher blood pressure is bad.

Similarly, if you had differential effects on some pulmonary function test, you'd be comfortable. I'm less sure about what differences in heart rate mean, you know, or something like that because we're not as sure. And the more

exotic you get, the more the marker is distant from what you've got data on, the more difficult it becomes to know what happened, and you resort to epidemiologic studies or case control studies where those exist, and you think about it.

You know you're not going to be as sure as you would be from a controlled trial, but it's not easy to think about how you can do controlled trials in these settings frankly.

DR. SARKAR: Hi. My name is Mohamadi Sarkar from Altria Client Services. I enjoyed the presentations by all the three speakers. My question is slightly shifting the paradigm of how we look at these biomarkers. So we know that, you know, smoking is the most harmful of the tobacco products, and that's the one that causes the most harm. And you got thousands of toxicants that are in cigarette smoke. So if you have a new tobacco product where people are switching from cigarettes to this new tobacco product, clearly you have eliminated or significantly reduced exposure to many of these harmful constituents. Now, if you accompany that with kind of a range of biomarkers that we're looking at today, biomarkers for oxidative stress, for chronic inflammation, when does it become essential to demonstrate statistically significant reductions in these biomarkers?

And we saw that there are many other confounders that could influence that versus converging lines of evidence that show that favorable changes in pretty much all of these biomarkers, accompanied with the fact that you are eliminating exposure to all these toxicants from cigarette smoke, how much of an evidence is considered sufficient to make the determination that, you know, you are getting there, given the fact that, you know, you get people to stop smoking.

DR. HATSUKAMI: I don't know if I can answer that question, yeah. Yeah, I think it's rather difficult. But let's take electronic cigarettes, you know, for example, and we know that there aren't the multiple constituents that you see with cigarette smoking. And let's say that with the electronic cigarettes you show reductions that you would find, reductions in certain biomarkers that are similar to nonsmokers or smokers that quit smoking, okay. And that occurs in the majority of -- maybe biomarkers that are associated with cancer. What you may not see, though, is a reduction in some biomarkers such as inflammation or oxidative stress.

And so, you know, I guess in that kind of situation, I think I would be concerned that, you know, the -- well, my main concern is making sure that some of those biomarkers has not

increased, but if you see a reduction in the majority of the biomarkers, I would think that something like that would be suitable for, you know, a reduced risk claim, for example, but that's just kind of my thing.

So one of the things that you have to make sure is the safety issue. I think you need to make sure you're not inducing more harm but then also demonstrating that there may be a reduction in harm in certain, potentially certain, diseases. I don't know if that's clear or not.

DR. TEMPLE: I mean, others know what form -- others in the room know much more about this than I do. I would have thought that concluding that various forms are less likely to be carcinogenic is not so hard. If there are no carcinogens in the delivery of this product, you're probably not worried about lung cancer anymore. I would have thought that's fairly straightforward. What I've never understood is why cigarettes increase cardiovascular risk. I mean, if somebody knows what the substances that do that are, then that's fine, but I don't think we really know.

And, you know, causing inflammation, that's fine; everybody sort of believes that's bad and that's what C-reactive protein sort of measures, but it's not at all clear

to me how obvious that is. I mean non-steroidal inflammatory drugs reduce inflammation, but they don't improve cardiovascular outcomes. Some people think they worsen them. I don't know if that's true, but they don't improve it.

So the cardiovascular area seems much harder, to me, to figure out what the relevant endpoints are, the relevant markers are, and I don't know how to do that. I think -- I guess -- I think you're going to have to resort to epidemiologic data because I don't think you can do the controlled trials that we're interested in, ethically and other ways. But that seems like the area of the more difficult problem, to me.

DR. FROST-PINEDA: I agree that favorable changes in biomarkers of potential harm and reductions in exposure are both good things, but I think it's going to be one of the challenges for the FDA to interpret all the information that they receive.

DR. DRESLER: How much interpersonal variability is typical for a biomarker? So interpersonal variability, how much is typical for a biomarker?

DR. FROST-PINEDA: I can't answer what's typical for an individual, but I know in TES we saw a huge range in any of the

different biomarkers, so you have like very low to very high and everywhere in between. And there's many things that depending on the time point when you were taking the sample that could impact the biomarker, for example, white blood cells, if it's allergy season or, you know, if there's infections, you know, flu season. So it can change over time even within a person.

DR. TEMPLE: Well, I mean, even you can easily imagine that some new substance would provoke changes in blood pressure that are highly variable from one person to another, and the bigger it is, the worse the problem is. It's also -- probably depends on what age you are and what your other risk factors are and all kinds of other things. But for some of them, it's pretty clear. Raising blood pressure even a little is bad. You may not be able to detect it, but it's going to be bad. We've got mountains of data on that. It's almost always bad. There are other factors. Some of the other ones, I mean, I don't know what a change in heart rate means ever, but I certainly don't know what a small change in heart -- I mean, you change it by 2, is that a big deal? I don't know how you know that. I think those are very hard.

Similarly, though, if you reduce FEV_1, I just know that's

bad, and I'm sure there are differences in response of one person to another, but it's sort of easy to figure out that you don't want to do that. You also want to -- you don't want to deliver known carcinogens to the lung, you know. Some things are fairly obvious, but a lot of them are not. That's the hard part.

DR. HATSUKAMI: I would imagine that -- I would think that Dr. Hecht would know the answer to this, but there's so many different ways going from exposure to actually cancer, so not to the extent to which an individual detoxifies the carcinogen, for example, you know, that they extend to which apoptosis occurs. And so there's just so many different individual variability in terms of how one goes from exposure to disease, and probably Dr. Hecht can elaborate on that a little bit more than I can.

DR. DRESLER: Well, one of the things that I heard you say, Dr. Temple, and it came up in one of the questions, so you're talking about a clinically significant difference versus a statistical significant difference. And you kept -- your clinical statistical difference is bad.

And so I'm just, you know, how do we decide to use statistical or clinically significant increases or decreases?

How do you quantify that if you're looking at heart rate? You can have a statistical significant difference, but is it clinically important?

DR. TEMPLE: Well, it depends on what you're talking about. I mean, biomarkers, one of the nice things about biomarkers is that they're pretty precise. It's very easy to tell whether a biomarker has changed, you know. It's not hard to know whether there's a statistical difference. You still have to find out what it means. And I was giving the example of blood pressure. We know epidemiologically that higher blood pressure translates to more strokes and stuff like that. So to me, if you -- if it's clear that some substance raises blood pressure and the statistics are clear that it does, it's not a chance occurrence and you believe it, I'd say clinically you already know that that's bad.

You may be uncertain about how bad it is if it's only an average of 2 mmHg, but we know from massive amounts of epidemiology that that will increase the risk in that population. So the statistic is just find out whether it's true or not, but then you have to figure out what it means. And some things are obvious, and a lot of things are not.

DR. DRESLER: And one last question. We actually had a

few minutes because we had shorter introductions at the start, so one last question before we go for break, and then we'll be back on time, on schedule.

Recognizing that tobacco products contain multiple toxins that could have diverse effects, is it possible that a reduced harm product could reduce some risk but increase others? Will a single biomarker ever be an acceptable measure for reduced harm product, and how many will be required? I think that's the hard question right there. I think I just answered that one for us. Please.

DR. FROST-PINEDA: I don't have an actual number, but I would say you need multiple biomarkers of exposure, multiple biomarkers of potential harm, and you have to look at all that information together to make a decision.

DR. TEMPLE: Yeah. But still, I mean, other people in the room may know, why do cigarettes increase cardiovascular risk? I mean, do we know?

DR. DRESLER: Next session, sir.

DR. TEMPLE: Yeah, okay.

(Laughter.)

DR. TEMPLE: I know why they cause cancer, but I don't think that's so obvious, so that makes it a little tricky to

know how to improve it.

DR. HATSUKAMI: I guess I would be really concerned if there was a particular tobacco product that did reduce the risk of some diseases but increased the risk of another. You know, I would be very concerned about that. And I think, you know, it's true, we have to use multiple -- there's never going to be a single biomarker that we can use to demonstrate whether a product has a potential to reduce risk.

DR. DRESLER: Okay. All right, thank you. Any other last questions?

(No response.)

DR. DRESLER: Thank you so very much for starting off this workshop. We have 15 minutes for break, so it is 10 after now, so let's come back at 25 after 10:00. The kiosk is right outside. Thank you.

(Applause.)

DR. DRESLER: Thank you.

(Off the record at 10:10 a.m.)

(On the record at 10:26 a.m.)

DR. DRESLER: I'm understanding it is 10:25, so shall we head back towards our seats in order to start? So, indeed, our next session is on cardiovascular disease, and our first

speaker will be Dr. Neal Benowitz from the University of California, San Francisco, speaking on Biomarkers of Potential Harm: Tobacco-induced Cardiovascular Disease.

DR. BENOWITZ: My challenge is to explain to Dr. Temple how cigarette smoking causes cardiovascular disease. And what I'd like to do is to talk about the various mechanisms, and then as I go through the various mechanisms, just sort of give some overview of biomarkers that are used to explore those mechanisms, which I think others will examine further. And then give some examples of sort of study designs that have been used to look at those biomarkers in various pathways.

So I'll start off by showing you the picture of the new Zuckerberg San Francisco General Hospital and Trauma Center, where I work. Funded in part by taxpayers of San Francisco, but then when they ran out of money, by the Zuckerberg family.

And here are my disclosures.

So first, just some brief comments about just what kind of cardiovascular diseases smoking causes, and I've broken this up into three categories which have a little bit different pathophysiology. So first category is acute vascular events. So we know acute myocardial infarction, a heart attack; sudden death; stroke; and then restenosis or reocclusion after

revascularization procedures of coronary arteries and other arteries.

Just make a note here, and we'll come back to this later, that there's a disproportionate increased risk of sudden death compared to the others, where the risk factors for the other events may be twofold, but sudden death is fourfold. So there's something about cigarette smoking that also makes you more likely to die suddenly.

The second category is accelerated atherosclerosis, and this is seen in virtually all vessels of the body: coronary vessels, more so in peripheral vessels and aorta and brain vessels.

And then there are other cardiovascular effects which are quite interesting. One is the aggravation of heart failure. Not entirely clear how this works, but we know that patients who quit smoking do improve as much as if they are given ACE inhibitors, which is a classic key treatment for heart failure.

Atrial fibrillation is the most common cardiac arrhythmia associated with increased risk of stroke and heart failure itself. And smoking is a strong risk factor for atrial fibrillation. And then, of course, impaired wound healing. So that's the backdrop.

You've heard Dorothy and others talk about dose response, and I will as well, but this figure is very important when we try to say what's the relationship between biomarkers and dose of exposure. So these are data based on synthesis of many epidemiological studies looking at the risk of ischemic heart disease events versus the number of cigarettes per day and going down into the passive smoking range. And you can see that is not linear at all, so that much of the risk occurs with very low-level exposure, up to five cigarettes per day. And then above five cigarettes per day, there is an increase as well, but much more gradual. And if you look at secondhand smoke exposure studies, you see very strong biomarker changes that maybe 50 or 75% of those that you see in active smokers. So it's a little bit tricky to do dose responses for cardiovascular disease and biomarkers. But there's a caveat. I'm going to show you some examples later of where this may be relevant.

So how does cigarette smoking cause cardiovascular disease? Well, it does a lot of things by multiple pathways that interact and overlap, and overlap with other medical diseases. I'm not going to read this slide here, but I'm going to go through these mechanisms in more detail later in the

talk. But virtually every cardiovascular risk factor that we know is worsened by cigarette smoking or induced by cigarette smoking. So we'll get back to that more later.

The next question would be what are the major constituents that cause cardiovascular disease? And to me, these are the six categories that are most important. So oxidizing chemicals are key in cardiovascular disease, as well as all the other diseases you'll be hearing about for cigarette smoking. Carbon monoxide clearly has effects. It reduces the capacity of blood to deliver oxygen to the heart and also does other things that are prothrombotic, which we'll talk about. There are thousands of volatile organic compounds. Acrolein is one we just talked about a lot that has substantial cardiovascular toxicity. And if you look at people who have done risk assessments of different constituents and looking at non-cancer risk, acrolein turns out to be a huge contributor to cardiovascular risk.

Particulates. Those work by other mechanisms; they cause oxidative stress, inflammatory responses. We know that air pollution is a substantial risk factor for cardiovascular disease. Cigarette smoking exposes you to much more particulates than air pollution, and interestingly, if you look at cardiovascular events versus particles on a dose-response

curve and you start with pollution, secondhand smoke, and active cigarette smoking, the curve is pretty linear, so it looks like there's a pretty good correlation between particles and cardiovascular risk.

Heavy metals. Cadmium has been talked about. Of course, nicotine, which clearly plays some role, but we think that it's not the major contributor.

I'm going to start with this scheme before I talk about specific mechanisms, just to sort of orient you. And so here I've shown some of the main constituents and then some of the pathways by which, that they work. And this is very simplistic. So say if we look at oxidant chemicals, particulates, and whatnot, so they all cause inflammation, and chronic inflammation is a known cardiovascular risk.

Specifically, the oxidant chemicals and particles, as well as inflammatory response, activate platelets and cause thrombosis. They also cause endothelial dysfunction, so endothelium, as we'll talk about later, are the blood cells that line blood vessels. It's one of the largest organs in the body, and it's critical for maintaining blood supply. So if you have thrombosis, if you have reduced blood flow, that reduces the supply of nutrients to the heart.

Carbon monoxide, as I said before, binds the hemoglobin, reduces the amount of oxygen that it can carry and the amount that gets released. And so on top of reduced blood flow, you also have less oxygen availability, so that aggravates things. So these factors end up reducing myocardial blood supply and blood flow and nutrient availability. Then you have factors -- endothelial dysfunction can be associated with coronary vasoconstriction.

Nicotine basically activates the sympathetic nervous system, that's its main function here, and it does increase heart rate, it increases blood pressure, increases myocardial contractility, not more than mild exercise, but it still does these things. So it can increase the myocardial demand for oxygen in blood flow, and then you have a situation where there's less blood flow, less oxygen, more demand, you have ischemia, myocardial infarction, you have thrombosis.

So this is just sort of -- and sudden death occurs usually in the context of ischemia plus, say, catecholamines, and nicotine causes catecholamine release, and so this may be why sudden death is so much more of an issue in smokers compared to nonsmokers with the same heart attack. So this is an overview. And now I'm going to just go through some of the mechanisms in

more detail and some of the biomarker questions.

So here's -- these are more of my thoughts. These have been discussed already by Dr. Hatsukami. So the kind of biomarkers that we'll be looking at are those that are physiological or pharmacologic based on mechanisms -- mechanism pathways of cardiovascular pathogenesis, so that's one type. Second type are those that are markers of organ dysfunction and injury, which is like the next step up. And the third, which I'll talk about at the end, are markers of cardiovascular disease, mostly for the purpose of this study, subclinical markers, like what things could we look at before someone has manifest cardiovascular disease.

Optimal biomarkers of cardiovascular harm. It would be nice if we had specificity; we don't. Normalization after cessation of tobacco use, actually, that's the case for many biomarkers. I'll show you some examples. But some are long-lasting abnormalities, and so biomarkers do not come back to normal quickly. We'd like these to be predictive of cardiovascular disease, but we know in general they are. We don't know specifically for the smoking-related changes how predictive they are. And, of course, in general, we'd like to see a dose response in relation to product epidemiology, but

for smoking, the epidemiology is not so clear in terms of dose response either so -- we'll get back to some of these points as I go on.

So first thing, oxidant stress. I'm sure you can't see that diagram in this auditorium, but oxidant stress is an issue for all smoking-related diseases. The oxidants in tobacco smoke include particles, nitrogen oxides, many aldehydes. There's a huge reactive oxygen species load in cigarette smoke.

Oxidants are pro-inflammatory. They reduce nitric oxide release. Nitric oxide is key, that is released from endothelial cells and dilates blood vessels to maintain blood flow. It has got antithrombotic effects to keep the blood from clotting. It's got anti-inflammatory effects, so when you reduce nitric oxide, you aggravate all the other risk factors.

Oxidants directly activate platelets and are prothrombotic. They cause endothelial injury and dysfunction and also oxidize lipids like LDL, which makes it more atherogenic. So clearly oxidative stress causes a lot of things that are well-known cardiovascular risk factors.

Some of the biomarkers. Others might talk about this as well. I'll just point out three. One, which is the much commonly used, are the urinary F2-isoprostanes, which are

basically lipid peroxidation products. Thiobarbituric acid oxidation. So in a sense, this is an early injury marker. And then the third one I'll just talk about is vitamin C levels. Vitamin C is an antioxidant, and when you have a high oxidant load, the vitamin C levels go down because it's being used up.

And the third [sic] one here, oxidized LDL, which is much more atherogenic than non-oxidized LDL, and so that plays an important role, we think, in atherogenesis.

And so a study I'll show here is an example of some of the biomarkers. It's a study where controlled nonsmokers were studied, and then smokers were studied before smoking and after smoking, so it gives us two -- we can look at smokers versus nonsmokers and what's the acute effect of smoking. And I'll just point out two effects.

So the first --

(Off microphone comment.)

DR. BENOWITZ: Where's the mouse? Okay, good. So if we look at the F2-isoprostanes, nonsmokers and smokers at baseline pre-cigarette, so this is how we can see -- and in this study, a huge difference in lipid peroxidation based on smoking status. You can also see that 1 hour after smoking a cigarette, there is also a substantial increase. So here's a

marker that's good both for chronic exposure and acute exposure. HETEs here, that's shown here, are also lipid peroxidation products, and it shows the same pattern. 80-Hydroxy-dG, you know, is interesting. That is an DNA oxidation product, and you can see here that smokers have higher -- than nonsmokers, but as you might expect, there's no acute effect of smoking in terms of DNA changes. So this just gives you some perspective of a design and what some of the magnitude of the effects might be for oxidant stress biomarkers.

The next major effect, which is key for cardiovascular disease, is endothelial injury and dysfunction. And as I said before, endothelium is really critical for maintaining blood flow to various organs, including the heart and the brain, et cetera. Oxidants destroy nitric oxide and reduce generation of nitric oxide. As shown in this cartoon, you can see that endothelial cells are damaged and begin to slough off as well, so there's endothelial injury. When you block nitric oxide, you impair the ability of blood vessels to dilate, especially in response to ischemic stress. You reduce the antithrombotic effect, so you see more platelet activation, more adhesion to blood vessels. And there could also be pathologic angiogenesis or increases in smooth muscle cells in areas where it's not

supposed to be.

So how much data -- I actually thought someone else was going to talk about this, but I'll just mention the kind of biomarkers that can be used to look at endothelial function. So the first one, which is widely used, is something called flow-mediated dilation. That is when you have increased flow and the blood vessel dilates. That's important for maintaining local blood flow, and that's a function of nitric oxide.

So there are ways, using ultrasound of the brachial artery, to look at brachial artery size, putting a blood pressure cuff on to occlude blood flow, and then you see increase in blood flow when it gets released, and normally the blood vessel dilates; in smokers, it does not dilate as much. And this is a very widely used marker. We can also look at biomarkers -- called circulating endothelial precursor cells, which are cells that actually are repaired endothelium. They come from bone marrow, and when you damage endothelium, then you have more repair cells, so they go up. You can look at circulating microvesicles, which are bits and pieces of damaged cells, including endothelial cells, pulmonary cells, and that's been a marker as well. And a variety of other markers have been used that I won't talk about.

Third effect, thrombogenic effect, which is really critical for especially acute cardiovascular events. And so here's a scheme showing a blood vessel that's a got a plaque. The plaque ruptures, and then platelets and other blood cells attach to it, and then the platelets activate clotting and you get a blood clot, and if the blood clot totally occludes the blood vessel, you have an infarction.

And thrombosis is really something that's a little bit different from cigarette smoking versus other cardiovascular disease. It's much more of a thrombotic burden, and I'll show an interesting slide in a minute. So there is enhanced acute thrombotic events, there's platelet activation, there's increased red blood cell mass, fibrinogen, and blood viscosity. So those make molecular blood clots. And then there's impaired fibrinolysis and fibrinolysis in the breakdown of clots, and so you see more clots and less effective breakdown.

And here's a scheme I just wanted to show you because I think that this is really key in terms of understanding one thing that smoking does on thrombosis. So carbon monoxide causes relative hypoxemia, and just like a high altitude, when you have a hypoxemia, your body adapts by increasing red blood cell mass. So if you see someone that's got hemoglobin at 55

or 60%, they're almost all smokers.

And that increases blood viscosity, makes it thicker. You know, it's like erythropoietin, makes blood thicker. Oxidant gases cause an inflammatory response, increases fibrinogen, which makes blood thicker. Oxidant gases and other things activate platelets so that the platelets are more likely to clot.

Nicotine plays a minor role -- there is evidence that it can increase factors that impair fibrinolysis, and so it could play a role as well, but generally, increased viscosity, increased platelet activation, and impaired fibrinolysis tremendously increase the risk of blood clots.

Here's a cardioangiogram of a patient of mine I want to show you because I think it makes the point nicely. So this was a 40-year-old man who was a smoker who came in with myocardial infarction, and the angiogram of his right coronary artery shows basically a sudden occlusion of flow, so he had a big blood clot that's blocking flow. He got thrombolysis, which basically is medication to dissolve blood clots, and you can see here is a normal coronary artery. Most people with heart attacks have got atherosclerosis throughout the blood vessel, so it's not smooth, you know; it's ratty, you know;

it's not large like this. This is a normal looking coronary artery.

So here's an example of a person who has relatively clean coronary arteries but had a myocardial infarction because of a thrombotic event. And this is something that's pretty characteristic for cigarette smoking as opposed to other causes of myocardial infarction and explains, at least in part, the smoker's paradox, which is if you have a myocardial infarction and you're a smoker and you quit, your prognosis is much better than someone who's not a smoker who has a myocardial infarction. It's because you have a major reversible risk factor.

So in terms of biomarkers for hypercoagulable state, here are some that are used. One, thromboxane A2 is released when platelets get activated, and you can look at thromboxane A2 metabolites in a year as a marker of in vivo platelet activity. Red blood cell mass, talked about. Fibrinogen, we talked about. And you can also look at fibrinolytic factors like tissue plasminogen activator.

And so here's an example of how biomarkers were used to investigate the issue of the prothrombotic effects of snuff or snus in Sweden. That's a study that was done looking at young

men before they had atherosclerotic disease being screened for the Swedish military and looked at non-tobacco users, cigarette smokers, and snus-only users.

And so you look at cotinine levels, you can see that smokers and snus users had very high cotinine levels and comparable, so nicotine exposure was similar for the two. But if you looked at Tx-M, which is a thromboxane A2 metabolite in a year, and you can see that cigarette smokers had an elevated level. The snus users and non-tobacco users had the same level. So this was used to argue that nicotine itself is not playing a significant role in terms of activating platelets. An example of that biomarker.

Inflammation, another mechanism which also plays a role in virtually all smoking-related diseases. Inflammation plays a role in accelerating atherosclerosis. If you have a coronary plaque, it causes plaque rupture and acute events. It also causes prothrombotic event. We've known for many, many years that smoking increases white blood cell, C-reactive protein, fibrinogen, and that these are all risk factors for future cardiovascular events in general.

Smoking -- and so inflammatory response activates inflammatory cells like macrophages and adhesion molecules, so

inflammatory cells get activated and adhere to blood vessel walls and cause inflammation of blood vessel walls. And then as I mentioned before, oxidized LDL is a pro-inflammatory.

Some examples of studies, of a study design to look at this, here's a study where never smokers and former smokers were compared to people who had smoked longer than 6 hours before the blood sample -- or less than 6 hours. So this is sort of looking at acute effects and chronic effects. And this slide shows differences on a log scale in percentages from the never smokers. In general, you can see that never smokers and former smokers look alike. If you look at specific findings, like isoprostanes, we talked about before for lipids, much higher in smokers but higher even if you smoked within 6 hours of that than not. ICAM, which is a marker of inflammatory response, you know, again, higher in smokers compared to nonsmokers and higher if you smoked more recently. Interleukin-6, another inflammatory marker, same thing.

Fibrinogen, the -- not same response. CRP, the same thing. So we see that smokers are higher than the nonsmokers, but there's also an acute effect if you smoke more recently. This is an example of looking at multiple inflammatory markers in that kind of study design.

Hemodynamic effects are really those effects on -- that specifically affect blood vessel tone, heart function, blood flow. And so we talked about things like increased heart rate and blood pressure, which are really pharmacologic effects of nicotine, and whether they are biomarkers of harm is an interesting question. Heart rate effect, which I'll show you later. Certainly, in animals, if you put animals on an atherogenic diet and you ablate the cardiac pacemaker and put an artificial pacemaker in, the higher the heart rate, the more atherogenesis that they develop. So we do think that heart rate is a risk factor for atherogenesis. Blood pressure clearly is a risk factor. Some of the other hemodynamic effects of cigarette smoking, cardiac output, so the amount of blood that the heart pumps out goes up; the amount of work the heart does goes up; the metabolic demand goes up.

Coronary blood flow increases whenever there's increased myocardial work, but if you compare smoker versus nonsmoker, it goes up less than it would in a nonsmoker, so there is some reduction in the reserve of blood flow in the heart. And then smoking can result in constriction of coronary blood vessels because of sympathetic effects. Endothelial effects are -- vasospastic angina, which is kind of a situation where one gets

constriction of blood vessels and gets chest pain spontaneously because of spasm of blood vessels, is higher in smokers.

So here's an example just of -- this is sort of an acute biomarker study. It's from some work we did years ago comparing the heart rate effects and blood pressure effects acutely of cigarette smoking, oral snuff, chewing tobacco, and nicotine gum. So acutely, heart rate increases about 10 beats a minute. Blood pressure increases 5 to 10 mmHg and is seen with all forms of nicotine. And these effects go back to normal within 90 minutes, in general. So this is like a classic acute effect.

If we look at sort of the dose-response questions and also circadian questions, here are data from a study we did many, many years ago looking at one of the earliest reduced nicotine content cigarettes. And we looked at -- on the left, we switched people to either a low-nicotine content cigarette or a high-nicotine content cigarette and then looked at blood nicotine levels, and you'll see here that with a high-nicotine cigarette, blood levels are at least four times higher than the low.

And then on this panel, we looked at heart rate throughout the entire day with ambulatory heart rate monitoring and blood

pressure monitoring as well. This is heart rates and beats per hour. And you can see, this is the time when people were not smoking, and this is when they were using either high- or low-nicotine content cigarettes.

So, one, you can see the heart rate does go up and stays up throughout the whole day at an average of seven beats per minute. And when that reaches a threshold for causing harm, we do not really know, but this is a very clear pharmacologic effect of nicotine. Blood pressure tended to increase a little bit, but the effects are small. And actually, most studies, when they looked at cigarette smokers, have not reported any relationship between cigarette smoking and hypertension.

But when you do circadian studies, you do see a small effect, a persistent effect, and there's more hypertensive heart disease in smokers. So we think that even though the effect is small for blood pressure, that there could be some effects. This might be a reasonable biomarker to look at.

For lipids, we know that lipid abnormalities, especially low HDL and high LDL and high triglycerides are cardiovascular risk factors. We know that oxidized LDL is harmful. We also think nicotine is involved here, at least in terms of fatty acids because you're increasing lipolysis and causing fatty

acid release.

And here is one of my favorite covers from *Time* magazine from many years ago where they had a whole article on cholesterol without talking about cigarette smoking except for the back cover.

And so here's an example of a biomarker study from Mike Fiore's group in Wisconsin, looking at a different model. What happens when you treat smokers with smoking cessation treatment? So this was a large study where they got about 36% of smokers to quit, and then it looked at blood lipids at baseline and 1 year later. And this shows the changes 1 year later, and if you just focus on the people who quit smoking, and this looks at HDL cholesterol, which is the most sensitive to cigarette smoking.

So this bar is total cholesterol, this is total HDL particles, and these are large particles. Those are three formations of HDL. But you can see that there's quite a substantial increase in normalization of HDL at least a year later when someone quit smoking, whereas the people who keep on smoking, there's no change. Here's an example of another design to look at biomarker changes.

Diabetes is a major risk factor for cardiovascular

disease. And cigarette smoking can increase risk of diabetes. We all know. It does this, we think, by impairing insulin sensitivity, and this slide shows you what insulin sensitivity is, so you get glucose early. That's what glucose blood curve looks like in smokers and nonsmokers, but if you look at the insulin that's required to do this, smokers require much more insulin because they're not as sensitive. And so biomarkers to look at this.

The most important one is probably hemoglobin A1c, which is glycated hemoglobin, so glucose combines with hemoglobin, stays around for a long time. It's a marker of long-term glucose control, very well correlated to complications. And you can see here there's a dose response between cigarettes per day and hemoglobin A1c. And also if you look at smokers versus former smokers versus current smokers, there's a difference.

So I need to finish up. Let me just talk about this, and then I'll stop.

So the last category of biomarkers has to do with arrhythmias. And again, we talked about sudden death, atrial fibrillation, and also people who have internal defibrillators get more shocks if they're cigarette smokers. And the biological plausibility here is mostly catecholamine effects,

although there may be some myocardial fibrosis effects and some ischemia effects.

Here are examples. Atrial fibrillation -- let me go back -- is shown here. Irregular heartbeat associated with risk of stroke and heart failure. Ventricular fibrillation, often fatal. And so we really don't have good biomarkers for this in terms of disease states, but I'll say we do -- we can look at catecholamine excretion.

And here's an example of a study we did years ago looking at smoking with nicotine patches and looking at urine epinephrine excretion, and showing that if you look at people on zero nicotine patch, if they were a smoker, they had about a 60% higher epinephrine excretion.

But you can also see that if they have patches on and nicotine levels are much higher compared to baseline, there's no difference. So the dose response is such that a small level of nicotine causes this effect and higher doses don't. But this could be a biomarker that could be used to examine arrhythmias.

So my time is up. I think I need to stop at that point. We can talk more later. Thank you.

(Applause.)

DR. DRESLER: I'm sorry. I think you could probably talk all day on the topic, too, though it is a complex one. So thank you very much, Dr. Benowitz.

Our next speaker is Dr. Bobbette Jones from RAI Services Company, and she will be speaking on Biomarkers of Potential Harm Among Users of Different Tobacco Products: Results of Two Studies Compared with Published Literature.

DR. JONES: Thank you, Dr. Dresler. And I'd like to thank the Center for Tobacco Products for allowing me to present today and to Dr. Benowitz for setting such a good stage for my biomarkers of potential harm that I'm going to be discussing.

This is my disclosure.

FDA CTP issued a series of questions to gather information about biomarkers of potential harm for the purpose of tobacco regulation. I chose Focus Question #9: What studies have been conducted using biomarkers of potential harm to compare potential health risks across different classes of tobacco products?

So in my presentation I am going to present the rationale for and the identification of biomarkers of potential harm, which we investigated in two clinical studies; at a very high level, present these two studies and consumers of different

classes of tobacco products; present statistically significant findings and how they compare to the literature; and provide some conclusions.

So between 2007 and 2009, R.J. Reynolds Tobacco Company conducted two clinical studies. One was a study randomizing smokers to either a tobacco-heating cigarette, snus, or an ultra-low machine yield tobacco-burning cigarette. The second was a cross-sectional study of smokers, moist snuff consumers, and non-tobacco consumers.

At the time we designed these two studies, we had several rationale for exploring these biomarkers of potential harm. First was to explore the Institute of Medicine's approaches, specifically, effect measures to assess potential harm reduction. A second was to evaluate those biomarkers that hold promise in future PREP assessment, and to identify biomarkers of potential harm that were potentially relevant to cardiovascular disease that could be used in future studies.

So what did we study? I have grouped these biomarkers sort of by pathway, as Dr. Benowitz has laid out, just to present them easier, remembering as several have said, these are multi-functional. But we looked at the cardiac risk markers in lipid metabolism. We looked at atherosclerosis

factors. One of the most common and consistently measured inflammation markers, WBCs. We looked at more inflammation mediators like interleukin, C-reactive protein. Cell adhesion molecules, for example, sICAM-1. Within the nitric oxide pathway, we looked at homocysteine. Endothelial function markers, for example, the von Willebrand factor. Some coagulation factors like fibrinogen. The hematologic markers. Markers for insulin resistance. And some miscellaneous markers. In the urine, we looked at several isoprostanes as markers of oxidative stress. And then some of the platelet activation factors, some of the thromboxanes.

So at a very high level, I do want to present the two studies. The details and the design of both of these studies have been published in the literature, so again, I'm going to make this high level. The first study was the randomized switching study where smokers were randomized to either a tobacco-heating cigarette, snus, or an ultra-low machine yield tobacco-burning cigarette.

For the purposes of this workshop, I'm going to focus on the biomarkers of potential harm that we compared between smokers and never smokers at baseline, and then the selected biomarkers of potential harm that we evaluated in the smokers

after they were switched to one of the three products.

This was a multi-center, randomized study of smokers who were switched to one of the three product groups for 24 weeks. Our goal was to enroll 150 smokers with 50 in each of the three cohorts and a comparison group of never smokers at baseline. After randomization, subjects were switched to one of these three study products, either the tobacco tobacco-heating cigarette, snus, or the tobacco-burning cigarette.

These were the products as they were packaged and sold at the time of the study, which was in 2007. Subjects, however, received these assigned study products in unbranded packaging, free from any commercial trademarks or product descriptors.

We enrolled generally healthy males and non-pregnant females between the ages of 28 and 55. Cigarette smokers needed to self-report smoking greater than or equal to 15 cigarettes a day for greater than 10 years with no intention to quit.

In addition to a screening visit for eligibility, we had three 24-hour overnight confinement visits, one at Week 0, Week 12, and Week 24. And our never smokers were confined at Week 0 only. We also had every 2-week product distribution visits in which we distributed their product and assessed product

compliance via a daily IVRS diary. Our assessments at Weeks 0, 12, and 24 included biomarkers of potential harm in both fasting blood and 24-hour urine collections.

So what did our sample look like? Across the smokers and the never smokers, you can see that we had a largely Caucasian population of around in their early forties. In the smokers we had a very nice gender split between the males and the females, and we had a few more females in the never smoker group.

The smokers had a mean use of approximately one pack of cigarettes per day. I present the intent-to-treat sample and the per-protocol sample here because it was the intent-to-treat sample in which we -- excuse me, I'm looking for the pointer. Whoop, sorry. Oh, there it is. Okay, thank you.

I present the intent-to-treat and the per-protocol sample because it was the intent-to-treat sample that we compared the never smokers to at baseline, and it was the per-protocol sample that we used for our Week 12 and Week 24 change from baseline with our biomarkers of potential harm.

Okay, moving on to the cross-sectional study of cardiovascular disease biomarkers in adult smokers and moist snuff consumers. Our primary objective here, in these exclusive use groups, was to assess biomarkers of potential

harm and to identify those endpoints related to CVD risk that differed among the three cohorts.

This was a single site, cross-sectional, overnight confinement study, and because of the nature of exclusive moist snuff consumers, this was an all-male population. Our goal was to enroll 180, 60 in each of the three cohorts, and we stratified ages within each cohort. The subjects' usual brands of cigarettes or moist snuff were their products during this study.

So our males were generally healthy, free of clinically significant disease, and for our exclusive smokers and moist snuff consumers, they had to use product for at least 3 years prior to the study at the amounts indicated in the slide with no intention to quit. Aside from the screening visits, we had one 24-hour overnight confinement period in which we assessed our clinical study endpoints.

And again, I want to focus on our biomarkers of potential harm in fasting blood and spot urine. So what did this study cohort look like? Again, being all males, you can see that we did successfully recruit our complement of smokers and non-tobacco consumers. We were short in our recruitment of moist snuff consumers because we had some difficulty with the upper

age strata. But this was -- within the cohorts, we can see that it was, as I said, all males. It was predominantly Caucasian. They were healthy by their BMI and their COPD status.

And I think the next most important thing to point out is their years of product use, that within ages irrespective of cohort, their years of product use were similar and that the mean use for the smokers was approximately a pack of cigarettes per day, and for the moist snuff consumers, four to four and a half cans per week.

So moving along to the study results, which is the most interesting, and I do want to set the stage just by saying that on the subsequent slides, I am only presenting statistically significant differences. Coral shading and red text indicates that the biomarker finding is consistent and the directionality is consistent with the reported literature. Gold shading indicates that our finding was not consistent with what had been reported. Blue text indicates I couldn't find any literature for a comparison. And I'll remind you of some of the abbreviations.

So to discuss the cross-sectional study first in which we measured some of the lipid metabolism markers, you can see that

we were consistent with the literature findings and that apolipoprotein A-1 and A-2 was significantly less in the smokers and the moist snuff consumers compared to never tobacco consumers.

Our findings were not consistent with the literature around oxidized LDL and triglycerides. We found no significant findings between the smokers and the never smokers or non-tobacco consumers. We did notice in the randomized switching study, however, that at Week 24 there was a change from baseline, and the tobacco-burning cigarette group had an increase in triglycerides by 32%.

Endothelial function marker circulating endothelial precursor cells in the randomized switching study, we found no consistency with the literature at baseline. We did see an increase at Week 24 from baseline in the tobacco-burning cigarette group, which was significant. The von Willebrand factor was very consistent with the literature, smokers having elevated levels compared to the non-tobacco consumers.

Nitric oxide pathway. Again, we saw consistency with the literature, smokers having elevated levels of homocysteine in the randomized switching study and folate being depressed in the smokers as compared to the non-tobacco consumers in the

cross-sectional study.

We had probably some of the most interesting findings to us in that if you look at the cross-sectional study first, you'll see that the most consistent and reported inflammation marker, which is WBCs, was very consistent with the literature, smokers being elevated in both the two studies. sICAM was elevated in both of the studies; sVCAM as well as the interleukin-8 and also the IL-p12.

What was interesting in the cross-sectional study is that we found no statistically significant difference between the non-tobacco consumers and the moist snuff consumers for WBCs, sICAM, or the IL-12(p70). If you look at the change from baseline for Week 12 and 24 in the randomized switching study, for sICAM, we found that all three product groups showed us a decrease in this marker in both Week 12 and 24. And the tobacco-heating cigarette groups showed a decrease at Weeks 12 and 24 with white blood cells, as well as the snus group at Week 12. We found no consistency with the reported literature around C-reactive protein, but we did see that at Week 12, that smokers switched to the tobacco-burning cigarette did see a reduction from baseline.

Fibrinogen and platelets were not consistent with

literature reports; however, in the randomized switching study, we did see a reduction from baseline in the tobacco-heating cigarettes at 12 and 24 weeks, and the tobacco-burning cigarette at Week 12. And very consistent with the literature was the finding that hemoglobin and hematocrits are elevated in smokers compared to non-tobacco consumers or never smokers.

The urine findings, we did see elevations in the smokers in the cross-sectional study around the isoprostanes and the thromboxane, and the non-tobacco consumers and never smokers looked fairly -- well, there was no statistical significance between those groups, so they looked similar. We saw the same with the randomized switching study in that smokers had elevated levels compared to the non-tobacco consumers.

We also saw that at Week 24, the tobacco-burning cigarette group had some reductions from baseline, as well as the snus group in some of the isoprostanes.

So, in summary, our findings in the cross-sectional study with dissimilar tobacco products, we found that smoker findings generally agreed with the reported literature, and the three biomarkers of potential harm that best differentiated the smokers from the two nonsmoking groups were sICAM and the two interleukins 8 and 12.

The biomarker data in this study and other published data show that smokers differ from non-smoking groups and suggest moist snuff consumers more closely resemble non-tobacco consumers.

In the randomized switching study, again, smoker findings generally agree with the reported literature. After product switching, we saw significant reductions in markers of inflammation and oxidative stress at both Weeks 12 and 24 and all three product groups. sICAM was reduced, and in those switched to the tobacco-heating cigarettes, white blood cells were reduced. And in the snus group, they were reduced only at Week 12. The most consistent and significant findings in biomarker of harm reductions were observed in smokers who were switched to the tobacco-heating cigarettes.

So, in conclusion, several biomarkers of potential harm that we studied across different classes of tobacco products appear to be useful in differentiating different product user groups. We believe further research is indicated on sICAM and interleukins 8 and 12 as may be potential screening metrics to assess CVD risk.

The biomarker data from users of different tobacco products may be useful in developing scientific consensus

regarding which biomarkers are relevant to changes in risk for tobacco-use-related diseases. And biomarkers of potential harm may have the potential to predict health risks across different classes of tobacco products in the absence of long-term epidemiological studies.

I'd like to acknowledge all of my colleagues who contributed either to the study execution and/or the interpretation of the data, those clinical research organizations and analytical laboratories who were instrumental in the success of these studies, and I have included my references that I cited in my presentation.

And thank you for your attention.

(Applause.)

DR. DRESLER: Thank you. Our next speaker will be Dr. Thomas Wang from Vanderbilt University Medical Center, and he will be speaking on Biomarkers of Cardiovascular Disease.

DR. WANG: Thanks a lot, terrific. I appreciate the invitation. So I'm going to step back and broaden the topic a little bit and focus, as the title indicates, on biomarkers of cardiovascular disease. A lot of the data that I'll show will come in both smokers and nonsmokers.

The question that I'd like to address with my slides is

the issue of both what makes a good cardiovascular biomarker, but also what are the biomarkers that, in the last decade or so of literature, we and others or many other groups have identified as being somewhat able to predict those who will go on and develop cardiovascular disease.

So -- sorry. These are my disclosures.

So the answer of whether biomarkers are useful for identifying cardiovascular disease obviously depends, in part, on how the biomarker is being used and what the purpose of the identification is. And what I mean by that is if you think of the different ways in which a cardiovascular biomarker may be used, one, as Dr. Benowitz in his talk highlighted nicely, was that it might give us some biological insight about processes or pathways that are perturbed on the path toward cardiovascular disease. The second is a clinical aim, obviously biomarkers in cardiovascular disease are frequently used to aid screening, diagnosis, and prognosis. And the last is something that was spoken about earlier this morning, which is to facilitate clinical trials. And the two categories of facilitation again, as mentioned this morning, are, one, to enrich a target population and, second, to serve as a surrogate endpoint.

So for this talk, I'm not going to talk about the first bullet, biological insight; I will deal a little bit with the clinical uses and a little bit with this issue of a target population.

So the one framework I'd like to use for organizing the talk is to consider the disease stage of cardiovascular disease. The biomarkers that we might consider are largely dependent on what stage of cardiovascular disease that we're interested in. There are some biomarkers that are useful because they reflect underlying genetic or environmental risk factors. And in my talk, when I mention risk factors, I'm not talking about biomarkers. I'm talking about actual etiologic risk factors. And so, as mentioned earlier, many biomarkers, in fact, I would contend the vast majority of biomarkers, have nothing to do with etiology; they may reflect something, but they, in and of themselves, are not etiologic. And the major exception to that, of course, would be something like LDL cholesterol.

Further along in the evolution of cardiovascular disease -- and these arrows cover, really, decades we know from autopsy study -- is the development of subclinical disease. And some of the biomarkers that we use in cardiology are really markers

of existing subclinical disease. A person hasn't had a heart attack yet, but they have atherosclerosis or they have cardiac abnormalities. And then, of course, the thing that we all care about is the actual overt cardiovascular event.

So just to start, biomarkers that reflect this earlier stage of disease, and this is obviously something that is pertinent to this discussion because tobacco is a risk factor for cardiovascular disease, and so many of the biomarkers that we're talking about are biomarkers at this early stage of disease. And this has been reviewed, so I won't review it, but there are many pathways that are perturbed in the context of smoking. Inflammation has been discussed. I'll talk about it briefly just to review some of the data.

This schematic shows what again has been discussed before, but the evolution that leads to an MI is a culmination of two processes. One is atherogenesis, but the other is that thrombotic event, and so biomarkers of either the atherogenetic inflammatory process or the thrombotic event are things that could potentially be interesting for looking at.

And so the prototypic biomarker -- I'm sorry about this bar at the bottom -- is CRP. So one of the earlier studies to look at the predictive value of CRP for predicting future

events -- and this is in a group of actually mostly nonsmokers. These were physicians. In this group, higher levels of CRP were associated with higher risk for cardiovascular disease, and this is a randomized trial, so it shows both placebo and aspirin. And actually, interestingly, the effect was mostly in individuals not taking aspirin.

There have now been -- I'm not sure why these slides aren't showing. This could be a major problem if these figures don't show, but I'll summarize. This is from a meta-analysis published in 2010 showing that -- and there probably have been 40 studies in the epidemiologic literature showing a similar association between CRP levels at baseline and future coronary heart disease risk. And the magnitude of the association is about one-and-a-half-fold, so individuals with CRP levels in the top third of the normal distribution tend to have about 50% increase in the risk of cardiovascular disease.

This can be done with other inflammatory biomarkers. We've heard about IL-6, which is upstream of CRP. And so IL-6 levels have a similar association in that high IL-6 levels are associated with about a 50% increase in the risk of coronary heart disease.

Is this showing up on someone else's screen, or is this

just --

(Pause.)

DR. WANG: Sorry about that.

(Pause.)

(Off microphone comment.)

DR. WANG: Yeah. Maybe it's time for lunch.

DR. DRESLER: Well, how far did you get? I'm sorry, it's --

DR. WANG: I normally check my slides, but I seem to be --

(Pause.)

DR. DRESLER: Technology, you know, in 2016. So what we'll do, we'll have Dr. Bhatnagar, who is the next speaker, give his slides, and then it will give those guys some time to work on Dr. Wang's slides.

So Dr. Bhatnagar is from the American Heart Association and the University of Louisville, and he will be speaking on Biomarkers of Cardiovascular Injury Due to Tobacco Product Use. And they'll work on getting your slides up there, okay, so --

DR. BHATNAGAR: Thank you for the invitation, and thank you for pre-pointing my presentation. I'm trying to put this in context. So what I was going to talk about is about a few things, which is the overall view of what cardiovascular

disease -- and the use of biomarkers and then specifically about tobacco.

So I've shown this slide many times. It's that cardiovascular disease is one of the leading causes of smoke-induced death, and this is not to show that the other diseases are less important, but just to sort of remind ourselves it's such a huge problem, and we know the least about the mechanisms of the underlying -- our understanding of cardiovascular disease. And this is the problem. The problem is that there are many forms of cardiovascular disease, and most of these forms are affected by tobacco smoking.

And as you can see, there are variable risks associated with each of these outcomes, so whether it could be MI or coronary artery disease or stroke or atrial fibrillation, there are different aspects of cardiovascular disease that are affected by smoking. So the question then becomes is what we are studying, what particular endpoints are we interested in and what particular biomarkers would be informative of that particular state? So there isn't a generalized biomarker that we can, in most cases, use for all of these different outcomes.

The other problem is -- and Dr. Benowitz showed this -- was that the nonlinear dose-response curve of smoking and

cardiovascular mortality, and this is both for particulate air pollution, but also for smoking. So most of the harm is at low concentrations, and so therefore the biomarkers, if they are following or tracking the harm, are likely to also be nonlinear and be difficult to estimate a dose-response curve if you don't look at the entire range of the changes. So how do we understand -- classically understand cardiovascular disease risk? And in terms of actual predictability, we have been talking about risk factors. In fact, the word "risk factor" was coined by the people or the Framingham investors who were looking at cardiovascular disease risk.

So there are a variety of risk factors that have been identified and very well validated in multiple epidemiological studies, and these are listed here, at least the ones in red have been very well established. And they go into calculation of what we call the Framingham Risk Score. And this risk score is predictive of events and has been, again, in very large populations.

The key point here, though, is that smoking increases the cardiovascular disease risk, and these effects are beyond the effects of other risk factors, and therefore it is an independent -- it's an independent risk factor for

cardiovascular disease. What it means is that if you calculate the association between smoking and cardiovascular disease risk, you cannot account for that association by correcting for, say, blood pressure or for lipids or for age, so it is by some other mechanism, which is not to say that part of the effects of smoking are not through these traditional risk factors, but it is independent of all of this. There's something about smoking that imparts a risk and is not mediated entirely by classical risk factors. We don't know what that may be.

So smoking also affects traditional risk factors, and so acutely, it could change things like blood pressure, and we talked about that, and Dr. Benowitz showed some data. The significant sort of thing I want to point out was that hypertension is particularly the most significant and important risk factor for cardiovascular disease. And sometimes in some studies, smokers may actually have low pressure or lower blood pressure, but it is acutely increased by smoking, and this effect could be attributable to nicotine. And maybe it could also be observed in newer devices, as e-cigarettes.

Then there's a traditional use of biomarkers, is heart rate variability, and it is reflective of autonomic balance

between the sympathetic and the parasympathetic nervous system, and that the lower the values of heart rate variability, the worse the outcome. So if you have low heart rate variability, then you have a much more sicker heart, but if you high rates of variability, it's supposedly a sign of good health. And so it's been shown that acutely, tobacco smoking or exposure to tobacco smoke can reduce heart rate variability, and that might be associated with the triggering of acute events and related to some changes in the electrical activity of the heart because of changes in the autonomic balance.

Heart rate. We talked about heart rate. It is sort of a relatively good predictor of mortality, and again, both nicotine and smoking increases heart rate. Dr. Benowitz mentioned that there was an increase in atherosclerosis and atherogenesis, and that's true as well.

The classically used biomarkers of inflammation, and we've talked about all of these also, is C-reactive proteins and fibrinogen, and the levels of C-reactive proteins are higher in cardiovascular disease risk patients and patients with unstable coronary disease and also are predictive of future coronary events. However, the changes in CRP are rather nonspecific and even after cessation seem to persist for many years. So it's

not clear how valid this is or whether this could be just used as for risk stratification and for additional -- an increase in additional predictive capability. And I'll show some data that we did in our studies.

And then there's fibrinogen, also, is like CRP, may be an indicator of inflammation. It's an established coronary disease, heart disease, biomarker. But if you add CRP and fibrinogen to all our risk prediction, it doesn't give you very much, maybe a better predictive capacity of a percent or half a percent. So although these biomarkers are important, useful, significant, and predictive, it's unclear to what level that they are predictors. So that's the reason that they're not currently used in clinical practice to improve risk prediction.

One of the other biomarkers, and this was identified in the Surgeon General's report, are the circulating angiogenic cells, what used to be called endothelial progenitor cells. And these cells are circulating cells that are in the blood, they come from the bone marrow, and they are supposed to be involved in repair and regeneration of vascular tissue. And low levels of these cells was really harmful, as that is an indication of compromised repair mechanisms. So this is an outgrowth assay, so take the blood and you grow out these stem

cells or progenitor cells, and you can see that nonsmokers have rather rich population, and light smokers have less, and heavy smokers have barely any, and this is associated -- the levels of these cells and we call them, used to call them EPCs, are associated with a number of risk factors. So they are predictive of risk and they are decreased in smokers.

Now, that could be used as a biomarker. The problem, though, is that not only tobacco smoke or smoking, but lots of other different insults and conditions can deplete the levels of these cells.

So one particular study that we did was to study whether one component of tobacco smoke, which is acrolein, could be responsible for decreasing the levels of these stem cells or circulating levels of these cells in the blood. And to measure the exposure to acrolein, we used a metabolite which is generated in the urine. And we found that there was an association with specific subpopulation of these cells with the levels of this metabolite in the urine, so therefore, it would somehow be important in associating with the level or suppression of the levels of these cells.

And the sort of question that we're grappling with, are there specific subsets of these cells that are responsible or

associated specifically for smoking exposure and not other types of exposure? Can we determine different types of exposure by using different types of subpopulations? There are about 18 or 20 populations that you can identify, and there are subpopulations that may be associated with particular different types of risk.

But the main sort of risks with smoking seems to be with thrombosis. I was mentioning that smoking is an independent risk factor, so if it's not entirely due to changes in blood pressure or in lipids, is it thrombosis? So there are some who believe that the main problem with smoking is it's prothrombotic.

And this is the dose-response curve, and it's been suggested that some of the initial effects are entirely due to platelet aggregation and that most of these effects are because smoking creates a heightened state of thrombosis in the body. And so although there isn't, by itself, sufficient -- this is not by itself sufficient, if you have an event or some sort of a secondary event that triggers plaque rupture -- which in most people happens many, many times before they're clinically evident -- that could lead to an exaggerated thrombotic response, and that's the reason why there are high levels of

mortality in smokers. So that's the view.

So there are many ways of measuring platelet activation and platelet aggregation. One of the -- sort of an important way is to measure in vivo how platelets aggregate. When they get activated, they get aggregates, and they form the aggregates' monocytes.

And so we measured that as a measure of platelet activation in vivo, and it seems to correlate well with other platelet -- markers of platelet activation. And we found that it's associated significantly with the levels of HPMA, which is a metabolite of acrolein. It is also associated with cotinine, so -- which means that there are higher levels of thrombosis in people who are exposed to tobacco smoke.

But to even increase our risk prediction, one of the best ways of looking is to look at the subclinical progression of the disease rather than just biomarkers that are predictive, which is -- and this is -- and now we get into actual, the disease process itself. And so several methods of subclinical disease progression have been followed. This is coronary artery calcification, and this is also in deposition of calcium in the coronary arteries, and if you increase or you add this score, you're going to increase your predictability by sort of

including this calcium score.

So we wanted to test whether that would be something that we could monitor to see the extent of injury by smoking, and for that we turned to this well-established cohort, which is known as MESA, or Multi-Ethnic Study of Atherosclerosis, and includes a rather large number of people of different diverse ethnic backgrounds, and there are very good rigorous clinical work done on these people. There's a large number of smokers in the group as well.

What we found, though, was that current and former smokers have higher levels of CRP and IL-6 compared to people who have not smoked, and only the current smokers in the highest quartile had a relative increase in fibrinogen, so fibrinogen seems to be rather, sort of, a blunt biomarker.

But if you look at the score as people quit smoking, you can see that there is an association with the calcium scores, as well as with a little bit less with CRP. But if you investigate the relationship between CRP and different levels of the coronary calcium, you get a much better prediction of risk. So which means that one biomarker by itself may not be sufficient, but you need to probably put in multiple biomarkers that together would be much better predictive instead of

looking at one solitary thing.

And this is the same idea, that if you look at the coronary calcification score by itself, you get greater predictability if you add to CRP. But just a point to note here is that if you look at baseline, this is people with zero CAC, so they actually have low risk, but as you increase the risk or have higher levels of calcification, you get higher levels of events.

So the next we wanted just to figure out if there was -- which of these biomarkers would be better in the sense which would be more sensitive or specific, more selective or others at higher doses. And so that's kind of the investigations that we are trying to get into. So some of this is unpublished, and what I wanted to show here was that we looked at a set of three biomarkers in this study: hs-CRP, IL-6, and fibrinogen, and as you can see, hs-CRP is very much up higher with the low levels of tobacco smoke exposure, and then it sort of declines there, some sort of immunosuppressive effects at very high levels of smoking. But most people are restricted to 20 cigarettes a day and not all day or so, but in any case we see that there is a much more steeper increase in CRP than it is with IL-6, and fibrinogen is relatively flat. And this is in relation to

cigarette smokes per day.

If you look at cotinine levels, the difference is even more dramatic, and you can see that there is a very strong and steep relationship with CRP, but others are not faring so well. So by doing such inter-biomarker comparisons, we can estimate the relative sensitivity of these biomarkers and then be able to select one that would be most appropriate and most sensitive to look at the effects of smoking.

So the strengths and weaknesses of current biomarkers could be understood in the context of what they're reflecting. We can look at some of the acute effects like heart rate variability, we can look at the calcification levels of the angiogenic cells, but I think the most important or useful information emerge from a combination of the biomarkers and how we could stratify risk by looking at different biomarker categories which are reflective of different mechanisms, because certainly smoking is not just a single insult and not mediated through one particular mechanism.

So another way of looking at it would be to look at different levels of exposure and mechanism base and mode of action-based understanding of the different chemicals that are present in tobacco smoke. And this is sort of an analysis of

the different levels of, say, formaldehyde in e-cigarettes.

So these are the reported levels, and as you can see, that at these levels you get different types of effect. So, for example, at very low levels, even 0.2-4 ppm, you get some eye irritation and lung irritation, but you go to suddenly higher levels, you get cardiac oxidative stress, you get bradycardia and so on. So the biomarker that you follow is dose response, dose-responsive, and therefore, you have to be careful of what biomarker we are measuring at which particular dose.

And the same is true for acrolein. These are the levels of acrolein that's been reported in e-cigarettes, and you can see that even at very, very low concentrations, you can see effects like proliferation of nasal epithelium, increase in blood coagulation. You see increase in arterial pressure at levels that are present in e-cigarettes. So what this analysis tells us is what biomarker to select at what level. This doesn't necessarily mean that e-cigarettes cause a lot of things, but they've been caused by formaldehyde in acrolein, so you have to be careful. And in designing studies, you have to take into account what are they actually measuring and what are we actually trying to say in terms of their relative toxicity.

An important point here, and I'm back to formaldehyde, but

the important point is that such studies have been done extensively in atmospheric sciences, in regulation of traffic commissions, indoor air pollutions, occupational exposures, and from that we know, that one of the lowest permissible limits of, say, formaldehyde is 0.025 ppm. So if we do decide that this is a permissible limit for indoor air exposure and occupational exposure, the same standards could be applied for e-cigarettes or other new tobacco products, and say in order for us to consider that this is safe, we need to meet these standards as we meet for other things.

And the same thing for acrolein. The problem is that these levels are very, very low and may be difficult to meet, but regardless, in our measurement of the biomarkers, the idea is that if we come to the lowest no-observed associated adverse effect, then we can say at this level we don't expect to see any change of the biomarkers. So -- but this is how we've been progressing so far, but that's one of the things that has been sort of a hangover from our past, is that we could measure one thing at a time, and we are fighting about which biomarker is better, which is not as good, which is sensitive or not sensitive.

Maybe with advanced technologies, we don't need to go into

these debates anymore. We can measure a very large number of biomarkers at the same time, and there may not be one particular biomarker that's changed but a signature effect, that a range of biomarkers are changed in a certain fashion, and if we can do appropriate pattern recognition of these changes, we can say much more than we have been able to say maybe even 5 years earlier from today.

Certainly, we can go and sequence the entire genome; we don't have to look at one particular susceptibility gene. We can see those other genomes for maybe $1500 at first, and then we might be able to have much more predictive power and sensitivity than we've had so far. So this is the new, brave new world of omics that we need to transition into. And there's some interesting sort of, some rays of hope here that if you look at this metabolism or metabolites in the blood, you can actually predict heart failure mortality or events. And this is not just one metabolite; this is sort of a principal component analysis of the entire range of metabolite change, and one of the components comprised of these four different components was predicted much more than one particular metabolite per se.

So this is where we need to be going in the future, and

especially with tobacco research, it's sort of obvious, traditionally, sort of lingered behind the rest of the developments. It's an important step forward, and we need to consider that seriously.

However, we cannot simplify the problem too much because if we start using in vitro high throughput analysis, perhaps we can do for cancer, but we cannot in heart disease, cannot do that because the main things that or the main factors that govern heart disease, such as blood pressure, insulin resistance, cholesterol, are emergent properties that are derived from the combination of different organs. Blood pressure is not determined by any one specific organ; there's blood pressures -- is cervical control, there is venal control, there is vessel control. These emergent properties cannot be simulated in vitro, and therefore, they're not very sort of amenable to high throughput analysis. So whatever we learn from those techniques, maybe we need to be careful, but we are getting more and more sophisticated, we can look at things in such complicated holistic manner, in an omics way, that we might probably make some real headway there.

So I'm going to end here, and thank you for very much for being part of this event.

(Applause.)

DR. DRESLER: Thank you. Thank you for stepping in for us. I think we're set for the slides? Try again? So again, Dr. Wang.

Thank you, Dr. Wang, for your patience.

(Pause.)

DR. WANG: Great. I'm just going to scroll ahead. Can I use the mouse to scroll? Okay. It's just me.

DR. DRESLER: So the -- our pointer/slider thing is -- now how about -- it's an unhappy changer.

DR. WANG: Is there a keyboard?

DR. DRESLER: No, the keyboard's not connected to it, so -- and you've got -- slides, huh? Would the mouse?

(Off microphone response.)

DR. DRESLER: Okay, so let's try that. Let's see. Try this one. Sometimes -- unhappy.

(Off microphone comment.)

DR. WANG: Should I just tell you to --

DR. DRESLER: Yeah, I was just going to say that's our next option.

DR. WANG: Yeah, why don't I do that? Many things are conspiring against me today. Better give up while I'm ahead.

So can you go to the next slide? Next slide. Next slide.

So -- okay, thanks. So I appreciate your patience. So again, picking up where I left off, so these early phase biomarkers of cardiovascular risk, CRP being an important biomarker of inflammation, this is a meta-analysis. A couple slides will have this format that shows a large array of epidemiologic studies and the association between elevated CRP levels and relative risk showing again, as I said, about a 1.5-fold relative risk of coronary heart disease and those with elevated CRP levels.

Can you go to the next slide?

Similar pattern for IL-6, actually almost identical, about a 1.6-fold risk of coronary heart disease, and those are the elevated IL-6 levels, another inflammatory biomarker.

Next slide.

Fibrinogen, we've heard about from some of our other speakers. So this is an inflammatory biomarker, it's an acute phase protein, but it's also a component of the human stasis thrombosis cascade. The interesting thing about fibrinogen is that it is associated with cardiovascular events. This shows cardiovascular deaths in the top half of the slide. But it's also about equally associated with the non-cardiovascular

deaths, and so emphasizing a point that I think was made earlier, that fibrinogen, while it's a good marker, is relatively nonspecific.

Next slide.

So this was also raised just in the prior talk. The statistical associations that we see and many of us report are not exactly what we're interested in. If you want to know whether a biomarker identifies correctly someone who's going to develop cardiovascular disease or not, we're interested in other characteristics of the test like is it sensitive, is it specific. The common way, as you all or many of you know, to aggregate the information on sensitivity and specificity for a test is the area under the ROC curve or the c-statistic. And one way to think about this is that you have a certain ability to predict risk just on the basis of conventional risk factors, knowing someone's age and gender. And so if you take that off the table and ask, when you add a biomarker, how much do you increase that ability, how much do you increase the area under the curve, you have at least one way of judging how much biomarkers add to your overall sense of someone's risk.

And so shown in this large study, the -- that's not working either. So the -- if you go from the rows, if you take

a non-lipid-based model, so just conventional risk factors, and then add various lipids, you can increase a c-statistic. And as you can see along the bottom, you can use these statistics by 0.01, 0.02, 0.03. The message here is that if you add an inflammatory marker like CRP or fibrinogen, the results are about the same.

Can you go to the next?

The incremental increase in c-statistic is very modest, 0.0039, and most studies have shown this, that despite the statistical association, the addition of an inflammatory marker like CRP adds actually very little to your overall ability to predict cardiovascular risk.

Can you go to the next slide?

And that's true if you look at a number of other biomarkers, too. So it turns out once you've measured CRP, it doesn't really help to measure other inflammatory markers in the same person. This just shows a couple other potential ones like serum amyloid A, homocysteine, Lp(a).

The next slide.

And so that has led cardiovascular investigators to ask, well, what if you start looking for biomarkers of a more advanced disease process? So closer to that subclinical

disease status that we talked about earlier. And one of the features about looking for biomarkers that reflect subclinical disease as opposed to early risk for disease is that now you're more likely also to be looking at biomarkers that are actually made by cardiovascular tissue.

Things like IL-6 and CRP are made by the liver or lipocytes, and so they're not actually secreted from the tissues of interest. On the other hand, these later stage biomarkers can actually be secreted by the heart, and so as one example -- next slide -- we, in Framingham and other cohorts, were interested in this biomarker or this set of biomarkers, the natriuretic peptides. The natriuretic peptides are a set of hormones that are made by the heart, and they're specifically made under conditions of increased wall stress or cardiac dysfunction.

So if you'll go to the next slide.

So if you look at cohort like Framingham, this is from a study that my colleagues and I did over 10 years ago now, looking at baseline levels of BNP and assessing the association of those baseline levels with risk over 10 years. As it turns out, having a high BNP level at baseline is associated with the risk of all sorts of things, including death, heart failure,

AF, stroke, also coronary heart disease.

And if you'll go to the next slide.

Subsequent studies have now been -- over 40 studies that have looked at BNP levels as a predictor of cardiovascular risk, just like in the previous iteration, studies that looked at inflammatory markers. There is, in fact, a very strong statistical association between baseline, BNP levels, and future cardiovascular risk up to threefold, and that's true of all sorts of cardiovascular disease and coronary heart disease.

Next slide.

This is just one of the few smoking slides I have, but you know, you might ask how is a cardiac stress marker like BNP actually related to an exposure like smoking, which doesn't -- isn't thought to have at least direct effects on the heart. And, in fact, if you look at smokers versus never smokers, they have a threefold likelihood of having elevated BNP levels, even adjusting for all other characteristics, suggesting that, in fact, smoking does, as mentioned on prior slides, potentially cause subclinical increases in cardiac stress or cardiac dysfunction.

Next slide.

I'm just going to talk about a couple other similar

markers of cardiovascular stress. One is one that many of us are familiar with in the clinical setting, the troponins. The troponins are used to identify people with acute myocardial infarction. These are the levels shown in the red at the very top of the curve. When you have myocardial necrosis, the heart release troponin. With now very sensitive assays, you can measure troponins, these cardiac death markers, in almost three-quarters or two-thirds of the general population, and so many of us walking around have these markers even though, you know, obviously very few of us are walking around with active coronary ischemia.

And so if you look -- next slide -- at epidemiologic cohorts and relate either the ability to detect troponin versus undetectable troponin, shown on the left, or various levels of troponin, shown on the right, you find that higher levels of these troponins in the blood are associated with an increased risk of cardiovascular death following individuals out from 5 to 10 years, and so another direct cardiac marker.

Next slide.

And now there are markers that integrate both inflammation and cardiac stress, and so one example is GDF15. This is a member of the TGF-beta superfamily that is secreted from all

sorts of cells in the body: heart cells, smooth muscle cells, endothelial cells, but also adipocytes and macrophages. GDF15 is an anti-inflammatory affecter within the body.

If you'll go to the next slide.

Another example is soluble ST2. Soluble ST2, ST2 is a receptor for interleukin-1, a cytokine, and like GDF15, ST2, when bound to its receptor or when bound with ligand, is thought to reduce cardiac remodeling.

And so if you go to the next slide.

These are data from Framingham again. Adding these markers of inflammation and hemodynamic stress, ST2 and GDF15, to more traditional markers provides even further information on cardiovascular risk, both all mortality and cardiovascular risk.

Next slide.

So I'm going to talk briefly, then, about the issue that I alluded to before, that statistical association is not the same as prognostic value. This is a study that we published subsequent to the earlier studies, looking at what happens if you combine lots of biomarkers. And so this looks at 10 biomarkers, again, using the Framingham cohort. Biomarkers of inflammation, hemostasis, neural hormonal activation.

And again, it shows that if you look at the area not under the curve as a metric, that you increase the area under the curve only very little even if you add 10 biomarkers to conventional risk factors. And so emphasizing that the strength of statistical association does not always imply that measuring the biomarker will enable you to better predict risk of having an event, which is very relevant when you think about using these biomarkers for targeting populations or trials or other functions.

Next slide.

It led us also to ask the question that hypothetically, what if you added more biomarkers, more biomarkers than we have currently have, and so if we measure 10 biomarkers and get a little bit of anything, what if we measured 50 biomarkers or 100 biomarkers? We obviously don't have that many informative biomarkers, but suppose that we did. As Dr. Bhatnagar mentioned, there are lots of new technologies that might get us there. It turns out if you look at the effect of measuring additional biomarkers, and this is from a simulation based on the Framingham data, if you look at the impact on the c-statistic, the increase in the c-statistic is largely dependent on how closely correlated those biomarkers are.

So if you'd go to the next.

This curve shows a scenario when the biomarkers that you're adding are moderately correlated to the biomarkers that you already have, so correlation coefficient of 0.04. I'm sorry, 0.4. So in this case, even adding 50 biomarkers only gets you partway to an increase of statistics that would be considered potentially meaningful.

If you'll hit the next two.

If you then go to biomarkers that are less and less correlated with the existing biomarkers that you have, so you look at the orange, which is an inter-biomarker correlation that's low of 0.05, then you can raise your area under the curve very, very quickly.

And so the challenge here, if you go to the next, is that many of the biomarkers that we measure, not just in cardiovascular disease but in other things, are by nature correlated with each other, and they're correlated because we think that pathways like inflammation are important, and so we measure lots of inflammatory biomarkers. But by definition, measuring biomarkers of the same process will lead to the assumption that many of them are likely to be correlated with each other. And so from the pure clinical standpoint of adding

biomarkers that are going to increase your ability to predict risk, what you really want is biomarkers that are not correlated to the biomarkers that you have.

So if you'd go to the next slide.

This is exactly what was discussed in the prior talk. One approach is to use new technologies like proteomics or metabolomics to look at large and global panels of biomarkers that are relatively agnostic to pathway. In other words, you're not measuring biomarkers because they're associated with a pathway, but you're measuring large numbers of biomarkers, and it's likely that the ones that are going to come out of this that actually truly raise your ability to predict risk are not biomarkers and pathways that you already have put biomarkers in, like CRP for inflammation.

If you'll go to the next.

So just to get near the end, I want to talk about the very advanced stage in cardiovascular disease when you have established subclinical disease. These are biomarkers, in fact, of the disease itself, and as alluded to earlier, one of the better ways of doing this is with imaging because you're directly imaging either the heart or the arteries.

Next slide.

And so coronary calcium was already alluded to. Coronary calcium is almost pathognomonic for atherosclerosis because there are very few other causes in the non-renal failure patient of having coronary calcification in your coronary arteries.

Next.

And so I won't go through this data slide because it's been talked about a little bit before, but this is also from the MESA cohort showing that, in fact, if you measure coronary calcium and add it to the conventional risk factors, you get a much bigger increase in the c-statistic than we saw earlier with measurement of biomarkers like CRP and other things.

And, in fact, many studies have now supported that of our available tests, either soluble biomarkers or imaging tests, coronary calcium is probably the best that we have for identifying people with future risk of cardiovascular disease. Again, it makes sense because they already have atherosclerosis in their coronary artery, so you're catching them much closer to the event.

Next.

So this is a very specific and sensitive test, at least compared to other cardiovascular screening tests. Obviously,

it is more costly and involves radiation exposure. And the role of further testing after you find coronary calcification is, of course, unclear and is not really well studied.

Next.

There are also some caveats, though, and so while I make the case that if your goal is to predict risk, you look very far in the disease process, you also then have to contend with the fact that if you get very far in the disease process, you may not get to a point where you can actually reverse the marker that you used to identify it.

And so this is the coronary calcium example. And so this is from a small randomized study showing the effect of low-dose atorvastatin versus high dose atorvastatin. They're the first two bars. I'm sorry, this is a baseline and a final. So the first two bars are the low dose, and the last two bars are the high dose.

Anyways, the point is whether you randomize the low dose or high dose, your coronary calcium didn't change at all. And this has been replicated numerous times even in placebo control trials. It's now well known that if you take statins and you have a lot of coronary calcium, your coronary calcium is not going to go down despite the fact that, of course, we know that

statins are extremely useful for reducing cardiovascular risk. I have one other example.

If you go to the next slide.

This was somewhat alluded to in Dr. Temple's talk. So this is from the trial's experience with ezetimibe, Zetia, which is an LDL-lowering drug. The interesting thing about this series of trials is that it has yielded data on a number of surrogate endpoints as well as clinical endpoints. So it allows us, at least in the context of this drug, to compare how the surrogates do compared to the actual events.

And so the first trial, ENHANCE, was published in 2008, shows the effect of this drug when added to simvastatin on LDL, in the left panel, as an appreciable and significant drop in LDL. On the right panel, though, it shows that carotid intima-media thickness, which is another one of these noninvasive imaging markers of clinical atherosclerosis, didn't change at all. And so this is, on this one trial, looking at two surrogate markers: one, LDL, which changed, and the other, IMT, which did not change.

If you then go to what the trial actually showed -- next slide -- this is from the IMPROVE-IT trial, there was, in fact, a modest reduction in risk when you added Zetia to simvastatin

therapy, arguing, at least from this example, that LDL is, in fact, a superior surrogate to IMT, which is something that many other trial experiences have supported.

Next. And next after that.

So I appreciate your attention through this interrupted talk, but I'm just -- and by summarizing, disease stage, I would argue, is an important consideration for looking at cardiovascular biomarkers. You may get biomarkers that are very informative about pathophysiology but conversely have modest prognostic value. And vice versa.

There are biomarkers that can have a lot of prognostic value, like coronary calcium, but may tell you relatively little about underlying pathophysiology because many things may lead to coronary calcium. I would argue, like the last speaker, that less biased approaches using novel technologies is a useful platform for identifying newer biomarkers. And the very last bullet point, something that I think we'll hear throughout this workshop, the clinical and regulatory applications of these biomarkers remain to be determined.

And if you go to the last slide.

These are just my acknowledgements from many collaborators that contributed to the studies shown in these slides.

Thanks very much.

(Applause.)

DR. DRESLER: Thank you, Dr. Wang. And thank you very much for your patience. So it is lunchtime now, and it's -- we'll come back here at 10 after 1:00, so that gives us one hour. So 10 after 1:00 we'll come back and start. And what we'll have is we'll have the last speaker in order to bring us back to the topic, and then we'll have the panel discussion. Thank you.

(Whereupon, at 12:07 p.m., a lunch recess was taken.)

A F T E R N O O N S E S S I O N

(1:11 p.m.)

DR. DRESLER: Okay, so I see you still standing in the back. If you'll move forward, please. We'll go ahead and start for our last speaker, and thank her so much for doing the postprandial presentation. So we're very grateful. Dr. Shari Targum is from the Center, FDA Center for Drug Evaluation and Research, and she will speaking on Tobacco and Biomarkers of Cardiovascular Harm: Many Questions Remain.

DR. TARGUM: Thank you very much. So ladies and gentlemen, my name is Shari Targum. I've been at the Food and Drug Administration for 16 years on the drug side, and I've worked with Dr. Temple for all that time, so much of what I'm going to say will sound familiar.

So here are my disclaimers. The views expressed are my own and do not necessarily represent an official FDA position.

So biomarkers, I think there have been many wonderful presentations this morning talking about the various uses of biomarkers. The way I'm going to think about biomarkers in this discussion is really biomarkers as an endpoint. But I do

recognize there are other uses, for example, enriching the population and predictions. So the potential of biomarkers in the tobacco sphere has to do with the disease where there could potentially be a long latency period between tobacco exposure and cardiovascular outcome. And wouldn't it be nice if there were validated biomarkers and/or surrogate endpoints that could potentially result in shorter trials and faster product approvals if, of course, the regulators were convinced that there was a reduction in harm?

But there are unresolved questions, and I raise these questions as someone who is not a tobacco regulator but a drug regulator, but also someone who is a cardiologist and who sees patients with heart disease. So I've heard some very interesting work about tobacco exposure and atherosclerosis, and I guess what I'm curious is what's the mechanism and the natural history of tobacco exposure and atherosclerosis?

And the reason I'm asking this is I'm wondering when you study the biomarkers, when along the causal pathway should we be looking at these biomarkers? Then I'm sure you've heard this question before: There are many, many chemicals in tobacco smoke. Which components drive what cardiovascular biomarkers and by what mechanism? This next bullet I would

probably amend because I think there are data available on how changes in smoking-related biomarkers exist, but how they translate into cardiovascular risk, I still have questions about. And then how to account for differences in individual susceptibility.

So Dr. Temple this morning mentioned blood pressure, and blood pressure is an endpoint that we've long accepted in hypertension trials. What we really care about, though, is not the blood pressure but the ultimate clinical outcome, so we approve drugs that lower blood pressure because we accept that lowering blood pressure is going to lower the risk of strokes and heart attacks.

But how we got to blood pressure as an endpoint was based on a lot of epidemiologic data, including Framingham, which showed a risk of elevated blood pressure, along with randomized double-blind placebo controlled outcome studies that showed that if you reduce -- if you lower blood pressure, you reduce the risk of those outcomes.

This is a slide I took from the Joint National Council on Blood Pressure. This is JNC 7. And the reason I like this slide is it shows me a nice linear relationship between systolic and diastolic blood pressure. The different lines

correspond to different age groups, and you can see that, for both systolic and diastolic blood pressure, which is on the x-axis, for every -- I didn't mean to do that. For increases in blood pressure, you increase the stroke mortality. And there's another slide in that document that also talks about myocardial infarction mortality, but I just wanted to highlight this because it's a nice model.

It's based on a meta-analysis for a million adults in 61 prospective studies, so it took a lot of data to get there. But it's a nice way of understanding risk, and I'm not sure that this applies to the tobacco arena, but I just raise it because it's an example of a different biomarker associated with risk and how we got there.

I also wanted to show you a slide of failure modes of biomarkers. And this comes from the American Heart Association, but it actually comes from interesting work done by Fleming and DeMets, two biostatisticians. So let's see, is this a pointer? I want to see if I can point. Okay, excellent. So "A" would be a successful biomarker, a surrogate endpoint where you have a single pathway, and the surrogate endpoint is going to capture the entire effect of the true clinical outcome. "B" through "E," you can see that there's

more than one causal pathway, and in "B" the surrogate endpoint is not predicting the true clinical outcome. And "D" and "E," you have surrogate endpoints and you have interventions, but you also have off-target effects.

So how does this relate to tobacco? Well, it seems to me, from hearing the discussions this morning, that "A" probably is not going to apply because at least with cardiovascular disease, there are multiple pathways. So then the question is can you find surrogate endpoints that either capture the various pathways -- so it would be more than one surrogate endpoint. And can you find the right surrogate endpoint that captures enough of the pathways that you can affect a surrogate endpoint and, by extension, affect the clinical outcome?

Dr. Temple mentioned some surrogate endpoints that missed, and I'm probably going to highlight some of the same surrogate endpoints. This is a common -- this is kind of a case study, if you're studying surrogate endpoints, this becomes kind of a case study of the surrogate endpoint that missed. So when I was in training, after a heart attack, the presence of frequent ventricular premature beats was associated with an increased risk of death. So we learn that. Ventricular premature beats after a myocardial infarction make the patient high risk. So

then we assumed that if we suppress these beats, we would reduce the risk. And that was the 1980s approach to post-myocardial infarction care.

So someone would come in, they'd have their myocardial infarction, we'd look for extra beats, and then we'd try to suppress them. And several antiarrhythmics, including encainide and flecainide, were approved for this use.

Then the CAST trial came along, Dr. Temple referred to it, it was in the '90s, and it was a placebo control trial in patients after a myocardial infarction with ventricular ectopy that was suppressed. So they took responders, and they randomized them. And then during the follow-up, there were more deaths on study drug than placebo. And so encainide and flecainide, the arms, were stopped. Moricizine, which was the third drug, was withdrawn. But this study was an outcome study that led to a paradigm shift, and we no longer treat post-myocardial infarction ventricular ectopy the same way we did in the '80s. There are a couple of other -- these are sort of cautionary tales when one thinks of surrogate endpoints and why we're cautious with surrogate endpoints.

The other disease that makes us cautious is congestive heart failure because patients with congestive heart failure

tend to have -- their heart is putting out less blood, they have a decreased cardiac output, they have a decreased exercise capacity, and they're at increased risk of death. And you might think that, gee, if these are all along the causal pathway, that you can increase exercise capacity, and they'll live longer, but it turns out that's not necessarily the case. We know of two drugs that actually increased exercise capacity but worsened the mortality, so that -- at least in that disease we're fairly cautious.

So the use of biomarkers to evaluate harm, and I quote Dr. Fleming, who stated, "A correlate does not a surrogate make." So we have accepted blood pressure and LDL cholesterol as surrogate endpoints. It was based on a wealth of data, including outcome trials, and I say that I agree that in the tobacco arena this model doesn't apply because you can't really do a prospective randomized placebo control study; it's not going to work. But it is useful to at least look at the lessons of the past because then hopefully we can learn from them. Some failures of surrogate endpoints may have resulted from additional effects of the intervention, so the off-target effects. So some intervention might be good at one thing and not so good at another thing, which lends support to the idea

that you might need to study multiple biomarkers.

So some concluding thoughts. There is a potential for developing biomarkers or a panel of biomarkers to assess cardiovascular harm. The idea, though, that you are now setting multiple endpoints raises a whole different set of questions. So which biomarkers do you pick? And if you're studying multiple biomarkers, how do you weight the biomarkers in a panel? What's -- how do you analyze?

It becomes the problem of perhaps a composite endpoint. How do you analyze and how do you deal with multiplicity, and what do you do when some biomarkers go in the right direction and some biomarkers go in the wrong direction? Many other questions remain, so the exposure reduction to harm reduction, the relation of the change in biomarkers to harm reduction, what does a small change mean? And reproducibility of results, can you trust one study, or do you need multiple studies? Finally, since there's the issue of susceptibility, you might need to study different subpopulations to look for consistency of results.

Thank you very much.

(Applause.)

DR. DRESLER: So could the presenters from the last panel

come on up to the front, please?

(Pause.)

DR. DRESLER: And so the same thing, you can come up to the microphone and ask a question, or raise your hand and get a card, or send in your questions to the website.

Let me start out with one question, and this was something, that a few of you had brought up. Many speakers talked about the importance of using multiple biomarkers, but what is that approach? For example, do markers need to be validated first as individual biomarkers and then combined in the composite variables for disease prediction? Or should there be an agnostic approach be considered such as running omics data and doing data reduction? So what are the pros and cons of each approach?

DR. BHATNAGAR: Do you want me to start? So there are several problems starting from somebody -- fundamental problems to more complex ones. The fundamental problem is that if you measure 5,000 things, you are going to have to have some probability of just having that by chance. So you have to correct your multiple -- correct for multiple sampling, and that creates a problem if you have a very large sample size to be able to afford to do that, so that's one very simple

problem.

People do, by using something like false discovery rates and what the metabolomics and proteomics people have been using, but statistically it's difficult to come to a rigorous conclusion after sampling 5,000 things that you found something truly significant.

The second thing is that a way around that may be to actually say that we're not looking for all, everything is not totally independent, but they're related to each other so you can search for patterns, and in that sense, you can say that we're only looking for one thing and not 5,000 things. There's a second thing. A third is that something needs to be -- some of these things can be validated individually, but if you go and start measuring things collectively, you have to see whether collectively they are predictive and they're valid markers of the disease. So there are multiple layers of problems with it.

DR. DRESLER: Dr. Wang and then Dr. Benowitz.

DR. WANG: Sure. I would just add that -- and it's a good question, but I want to add there's probably no single approach, and so there's the global approach and the candidate approach, and likely different groups are going to try both

approaches.

Whichever approach you start with, though, the follow-up work will probably go in the other direction, and so you start with a global approach, you end up with a couple candidates, and then you do have to do further work to understand how and why those candidates might be associated with the risk, whereas if you start with a candidate approach based on knowledge of underlying biology and you find something, then certainly at that point you do have to ask yourself how much does it add to the other stuff that you already had, and so you do want to add it on and turn it into a combination.

And I would submit, as an example, the experience in genomics, and so there are candidate gene approaches to looking for common variants associated with diseases, and there are GWAS, genome wide approaches, and you will see both, although lately it's been more the latter than the former.

DR. DRESLER: Okay. Dr. Benowitz.

DR. BENOWITZ: I just wanted to respond sort of to your -- the comment about validated biomarkers. So what if we can't validate the biomarkers? When we're dealing with multiple pathophysiological pathways that are all operating at the same time and we can't disconnect them, we may not be able to

validate specific biomarkers to specific disease risk, but say we know that smoking causes thrombotic events, no question about that, and we know we have biomarkers of hypercoagulable state.

Now, how much evidence does one need to say that this is a biomarker that we should pay attention to? So we got to get the question there, what is validation? I don't know of any studies that try to validate future events, but we certainly know what the implications are. And for products that have to be regulated, you know, what if we can't validate them, you know? What's the point in which we use pathophysiological pathway data? Maybe one good biomarker for each pathway and come up with like a probabilistic statement that we think this most likely reflects risk and just phrase that as a question, a counter to what you've presumed.

DR. DRESLER: It is, but I'm going to turn it right back to you. So, you know, I think we started off at the first part that we're not asking for advice or decision, but I think you've hit the nail on the head; that's exactly right. So when would you say enough evidence is enough, that it's -- you can't validate it. Who's going to say you can't validate it? How is that going to be scientifically valid, and when would you know,

expert in the field?

DR. BENOWITZ: Well, I think if we can't validate it, then it does make sense to identify key pathophysiological pathways and identify what we think are the best biomarkers in those pathways and say this is some sort of a composite risk. And if a product makes this composite worse, that's bad; and if it makes it better, you know, it's better or less bad. Now, how to weight the various biomarkers, that's a question that I don't know the answer to. But we may ultimately be stuck with this kind of analysis because I'm not sure that we can validate these, especially with the new products. I think we can validate smokeless tobacco because of the Swedish experience; people have used it for 30 or 40 years, and so we really can validate those, but for e-cigarettes, how can we validate that? We can't.

DR. DRESLER: Okay.

DR. WANG: I guess for biomarker, the issue of validation, which is obviously a challenge, you know, there is biological validation which means, you know, is this signal some random signal, or is there some biological thing underlying it, and that's how the validation may rely on completely different modes of investigation, experimental models, looking at

orthogonal data, like genetic data and other data.

There's the statistical validation that just doesn't replicate in multiple samples, and that we can obviously do. There's the clinical validation getting toward what you're getting at, is the biomarker actually going to be useful for at least guiding clinical trials, and that certainly can be challenging. It seems like it's not a simple answer, what you're going to do with any finding that you might get from these global approaches, but it will really depend on the context.

DR. BHATNAGAR: So doing it in, for example, going back to GWAS, you find that some stray gene associated with an outcome, you next go back and knock them out -- and see whether, what is the path that they're being affected and what role do they play? And then you go back again and look at it for disease models so it's a very complicated cumulative path to validation, but I think in order to really understand that this is what this biomarker is doing, we need to have some mechanistic understanding of what the biomarker is and what it is reporting.

DR. DRESLER: You know, when I started out the panel, I say if there's any questions, you know, you guys can ask each

other questions, too. So you're more than welcome to do that, too.

What approaches could distinguish effects of tobacco use from effects of other confounding factors? So I think, Dr. Bhatnagar, you had brought that up and others that, you know, there's many pathways to cardiovascular disease. How are you going to distinguish which ones are the effect from tobacco use versus other -- obesity?

DR. BHATNAGAR: That's been the bane of -- that everything that we see in terms of disease, some in terms of disease exacerbation is similar to lots of other processes. Are they uniquely tobacco-related outcomes that only happen in tobacco users is very rare. And because it would -- tobacco would enhance all other garden variety type of atherosclerosis disease. So that's been difficult. There are certain features of cardiovascular disease that are unique to tobacco users. For instance, young women who smoke would have plaque erosion and not rupture; they would just lose a single layer of endothelium, and then they have sudden cardiac death. That's one of the characteristic features of cardiac death because of smoking. But there isn't -- otherwise if we look at any pathology, cardiovascular pathology, you can't tell that was

just smoking or some other cause.

DR. DRESLER: Dr. Jones.

DR. JONES: Yes. I would think in some of our -- and I think we alluded to this in some of our studies, that as many potential confounding factors as we can gather information on. We talked about gender, BMI, you know, race, diet, exercise. Collecting those factors so that you can control, when you could, in your analysis for some of those.

DR. BHATNAGAR: The problem with that it's sometimes difficult to distinguish are -- those confounders or effect modifiers. So you don't know whether there's this magnifying effect of smoking because we don't know the mechanism, so we can't say -- we can't rule out that you can rule out a confounder because it's not in the disease mechanism. We don't have that evidence. So it's --

DR. BENOWITZ: So there are two confounders that are not directly related that we could look at. One is genetics, so one can do genetic screening first and then look at the effects of different risk factors in people with high and low-risk genetic profiles. And so that's a way that we could actually try to separate out that risk.

The second, which is something Dr. Wang and I talked about

over lunch, is age application. So when you're looking at risk across the whole range of ages, age is the strongest risk factor when you get old; no matter what, you know, it overwhelms everything.

(Laughter.)

DR. BENOWITZ: The odds ratio of smoking for, say, myocardial infarction is highest the younger you are because there are -- because most people don't have heart attacks in their forties, and if you do, most likely you're a smoker. So doing -- you know, examining biomarkers, sort of, by different age groups might be a more powerful way to at least deal with that confounder. So I think there are some approaches for some of them. Others like body weight for diabetes and lipids, those are much harder.

DR. DRESLER: Sir?

DR. PRASAD: G. L. Prasad, RAI Services. I have two questions. First question to Dr. Benowitz. You showed in your slides that there are some acute as well as long-term effects, particularly with oxidative stress -- that's a difference. Free radical biology. So what kind of weightage should one give when we are looking at a reduced-risk product or for any other regulatory purposes, what kind of weightage one needs to

put for acute or long-term effects, considering that smoking-related diseases are dose and time dependent? They take a lot of time. Do we focus exclusively on the acute effects, or do we look at the long-term effects? And what's the long term, especially with the new products?

DR. BENOWITZ: I think that is really an important question. It's hard to know because for most of these biomarkers, there are both acute effects and long-term effects, and so things get -- worsen acutely. I don't know how we can weigh them. Obviously, for cardiovascular events, it's sort of the integral of the insult over years, and whether that's the sum of acute events multiplied by the number of pack-years a person has or something like that, I mean, that makes sense to me as well. I don't know that we have very many good long-term biomarkers of harm for cardiovascular disease anyway, except if you start looking at some clinical disease, you know, carotid intima-media thickness or coronary calcium. You can look at those sorts of things, but that's already when you have disease. So the earlier questions, before you have disease, I don't really know any good long-term cardiovascular biomarkers. I don't know if the other panel does.

DR. WANG: Actually, the example you raise is exactly what

I would say, which is subclinical disease imaging, so the value of it is it integrates exposure, all sorts of risk, over periods of time, so something like coronary calcium generally -- it's a pretty good indicator like hemoglobin A1c, what your exposure to all cardiovascular risk has been.

You know, but again as I mentioned in my earlier remarks, that the disadvantage of it is that the expectations may not change quickly, if at all, when you alter one of those risk factors like tobacco exposure. And so, you know, it gets back to the question of, you know, what are you going to look at if you're trying to study the impact of various exposures and interventions. It seems like you would want to look at an array of complementary markers, markers that represent earlier stages in disease process or markers that represent things that may change relatively acutely as well as markers that may represent longer-term exposure to risk.

DR. BHATNAGAR: There are some validated markers of disease regression, and they're used in statin trials, and CAC was one of them. And you could use some other markers, you know; CRP goes down very, very slowly, but you can use other indices of inflammation, so there has been -- there is plaque regression, there are many years of getting to measure plaque,

and some of these potent anticholesterolemic drugs can cause plaque regression, so there can be some possibility if people are quitting, that they could see either stabilization or regression of plaque, and there are ways to get to that.

DR. WANG: Yeah, I guess I would argue, though, that -- you know, for those markers, again, there are plenty of examples where a beneficial drug didn't lower CAC or didn't reduce IMT but still lowered risk, so it may be a situation where if you see regression of subclinical disease, that's useful, but the lack of regression does not exclude the possibility of benefit.

DR. PRASAD: My second question is to Dr. Targum. We talk about several patterns of biomarkers, whether they come from various omics studies for known markers. Is there any guidance or any thought about -- like I know that there are some oncology patterns, marker, gene -- and so on and so forth that are being used. So are there any learnings that can apply for some of these tobacco-related biomarkers?

(Off microphone comment.)

DR. PRASAD: Oh.

DR. TARGUM: Could you repeat the question?

DR. PRASAD: Yeah, sure. There are known patterns of

biomarkers that are used in different areas like oncology, oncology patterns. So are there any learnings from those that we could take and apply to tobacco-related biomarkers for product evaluation or assessing risk or the harm?

DR. TARGUM: So if I understand your question, you're asking are there lessons from other fields such as oncology that one could take. That's a very good question. So you're asking from the realm of biomarkers?

DR. PRASAD: That's correct. From the realm of biomarkers.

DR. TARGUM: For regulation. Well, I guess that is going to be very disease dependent. I think in the realm of -- in the regulatory realm of cardiovascular disease, my approach has been rather cautious, and it's based on the lessons of, for example, the CAST trial. So we tend to take more of a cautious approach. But I think that's very much -- I think it's debatable. I have other colleagues in the Agency that may have different opinions, and I think it's very much based on the particular disease.

DR. DRESLER: Dr. Jones, a question for you. What were the compliance rates across the different groups of switchers? So the people in your trial who switched, how compliant were

they with staying on their new arm?

DR. JONES: That's a very good question. And that is one of the reasons we had our intent-to-treat sample as well as our per protocol sample. I didn't show the numbers, but I believe we didn't -- our per-protocol sample for the tobacco-heating cigarette was fairly high for compliance because we didn't lose many from the intent-to-treat to the per-protocol. Where we lost -- and the same for the tobacco-burning cigarette, where we lost our biggest -- and you might suspect this. Where we lost our biggest or had our biggest issues with compliance was with the snus group because we went from 45 in the snus group who were in the intent-to-treat sample down to 33 in the per-protocol.

And that meant that they had to have an overall mean compliance cumulative for the 24 weeks of at least 50%, so there was significant -- I wouldn't say significant. There was probably substantial dual use between cigarettes and snus in that snus group, and that may have been why we didn't see some of the changes that we might have expected to see.

DR. DRESLER: I think that was --

DR. JONES: Compliance is --

DR. DRESLER: -- a follow-up question.

DR. JONES: Yeah, compliance is tough and was tough in that group.

DR. DRESLER: Okay.

DR. BENOWITZ: So I just echo this problem with compliance, especially looking at cardiovascular biomarkers where a small amount of exposure can have a big effect. It's really difficult or impossible to do outpatient studies when you switch people to novel products when they can smoke regular cigarettes. And what we really need is situations where people are confined, and those studies, for financial reasons, are becoming impossible to do anymore. So we have a real economic roadblock to actually doing the studies we need to really understand.

DR. HATSUKAMI: So we're getting a better understanding about how complicated this field is, there are multiple pathways, and you know, we don't even know if we're targeting the right causal pathways sometimes. But what we do see is we do see a dose-response effect in terms of the number of cigarettes smoked and then the occurrence of cardiovascular disease or cancer. The slopes may be different. So I guess my question to you folks is in what situation would just biomarkers of exposure be sufficient?

DR. BENOWITZ: For what?

DR. HATSUKAMI: For cardiovascular disease, let's say or -- yeah, for cardiovascular disease.

(Off microphone comment.)

DR. HATSUKAMI: For risk prediction, yeah. Would there ever be a situation where just biomarkers of exposure would be sufficient? In evaluating tobacco products.

DR. BENOWITZ: For some areas more than others probably. The problem is disentangling the 7,000 or 9,000 chemicals; they're all highly correlated. So you have to be able to find a product that has less of one but has everything else, and there, there's a problem because some of the biological effects of different products overlap as well.

So I think it would be very difficult to isolate products and say that that these products have an effect, except for nicotine. Nicotine has got sort of unique effects, so you could look at nicotine. But aside from that, there are so many drugs that are involved in an inflammatory response or whatnot, so I think it would be very difficult for most. I don't know what others think, but I think it would be very challenging.

DR. BHATNAGAR: So from the -- I mean, I agree, and it's difficult. But to start off, if you do want to do risk

estimates -- and it's been done, where you take all the chemicals that are present in, say, even in tobacco and you estimate at what level they're present and what's the risk associated with that chemical and you create a hazard index. And that's the reason, that's how they came out with, you know, this acrolein, this sort of corresponding to 90% of the total risk. So if you knew -- and there are many of them, there are 7,000 chemicals there, but maybe only like two or three do any much of the harm. So if you do have a hazard index, you can calculate non-cancer and cancer risks just from knowing what the amounts are and looking at the dose-response curve. That's not completely valid and it's -- you know, it's fuzzy and it's loose, but that's a beginning.

DR. JONES: I think at the biomarker exposure workshop, one of our colleagues, Dr. Marano, was talking about quantitative risk assessment, and she's actually done some work around the HPHCs to try and get an idea of, you know, risk based on exposure markers.

DR. BENOWITZ: Well, you know, high-risk assessments, I think, are informative, but I'm really skeptical about how much you can extrapolate that to human disease because the events that are used in risk analyses are biochemical changes in mice

and rats and a bunch of things that are really far distant from human exposure. I think if you had epidemiology, and again, one example that's used a lot is nicotine comparing cardiovascular risk from smokeless tobacco and cigarettes. So there you might be able to parse out nicotine from other things by making that kind of comparison, but most of the risk assessments look at so many animal models that are so diverse and really have questionable relevance to human disease. So I think it's a good exercise and I like it, but I'm not sure how it's going to pan out for prediction.

DR. BHATNAGAR: It's a beginning, and the animal models may differ by, you know, double or half of what the human effects may be, but they're not orders of magnitude different. So you can get some place to start because without that we really have nowhere to go.

DR. SARKAR: Mohamadi from Altria Client Services. So I just want to build on something that Dr. Hatsukami pointed out. So I agree that, you know, maybe this risk assessment could be a theoretical construct, but if you have on top of it other lines of evidence that kind of help you build your -- you know, your strength of evidence, say, for example, nonclinical models or cell-based models or animal models, and then if you

accompany that with, you know, evidence from switching, because at the end of the day, and it goes back to the comment that I made earlier, that if you have people who switch from cigarettes to a new product, and the e-cigarettes was a good example that Dr. Hatsukami raised earlier this morning, so you know about the residual risk from these products, and then you have other forms of evidence that you can use to build your story. I mean, at the end of the day, would that be sufficient?

And if you also, then, on top of it -- so you have reduction in exposure and then accompanied by directional changes in a panel of biomarkers, you know, let's say it's chronic inflammation, oxidative stress, a lot of the biomarkers that we've seen today, that all of them have kind of common underlying themes for across all three diseases: cardiovascular, cancer, and COPD. Wouldn't it get you, you know, going in the same direction and allows you to build all the scientific evidence that one would need to get comfortable?

DR. BHATNAGAR: I agree, and it is a good and complicated question, but the problem -- we do not know much, how to -- this is theoretical constructs. We do not know much how to predict risk. But we do know that the claims that if you take

a product and you reduce something by 90% or 95% and claim there's a reduction in 95, 95% harm, that's wrong. Because we don't -- we know it's not linear. And so that, at least, we know is not as simplistic, and that's where the major problem is, that if I say -- if I take this reduced part of the product and I reduce everything by 90%, the harm is 90%, that's not true, absolutely. But we do not know exactly how much it is, and then it has to be done with each particular constituent that that product has before the claims could be substantiated.

I agree that only on the basis of the constituents you can get a fair enough idea about its harm, even though it may not be, you know, valid and it may need some more work, but that's what we can start off with. But we cannot directly extrapolate from concentration; we cannot confuse concentration with harm.

DR. BENOWITZ: I would just follow up. I think if -- we're talking about constituents, if you took a class of constituents like all organic combustion products being removed, we probably could conclude that that would essentially reduce risk. We know, in terms of qualitative use biomarkers, secondhand smoke produces between 50 to 75% of the signal of abnormal biomarkers as active smoking in a variety of studies because this dose response is really very steep at low levels

of exposure. But if there's no combustion, then most of those biomarkers go away. So I'm not sure we could look at particular constituents, but we certainly look at combustion products broadly.

DR. SARKAR: Yeah, I agree. I'm not talking about reduction in single constituents or even a class of constituents, but if you have, you know, a novel product that's so different than cigarette that you have drastically reduced exposure to pretty much all the constituents, all the class of constituents, and then if you -- I mean, if you think about in the broader context of regulatory evidence, you know, there is this whole different paradigm of population harm of all the unintended consequences that can result from changes in behavior or initiation and all the other dimensions of population effects that's separate.

But even if you just look at a product that has large reductions in exposure to pretty much everything, maybe that's one way of looking at it, as reductions in exposure accompanied by favorable changes in a panel of biomarkers of potential harm.

DR. BHATNAGAR: Somebody could do those calculations, not very difficult if somebody would attempt to start. Let's just

say if you reduce everything and you come to that and whatever residue is left, you can estimate its risk, and those are risk estimates that people, there's a whole profession of people who do that.

DR. DRESLER: So I have one last question that goes across all diseases, though, and not just cardiovascular. So not all smokers get disease, and people who smoke get different diseases, suggesting that the risk may differ among individuals. Does a collective hazard index that averages all together really reflect the reality?

So we've been talking about cardiovascular disease, but so I don't have the genes for cardiovascular disease, but maybe I do for COPD or cancer, so -- is there -- can you lump them all together to make a collective hazard ratio, or do you have to have individual disease hazard ratios?

DR. BENOWITZ: Just in the past, what's been published has been cancer, hazard risk, and non-cancer hazard risk. I haven't seen anything beyond that. I'm not sure if anyone else has, but --

DR. BHATNAGAR: Nobody has tried to put the things together, and sometimes they become mutually exclusive, attritional mortality, so people who develop cancer would not,

in the end, have heart disease, so we do not know if you completely cure cancer, that all smokers would end up having heart disease, or you completely cure COPD, then everybody would have, you know, cancer. So these things may be mutually directed, and it depends upon your genetic predisposition of which slot you end up in, but it's difficult to clump them all together and to estimate the risk in the absence of the other even together.

DR. WANG: Yeah, I would concur. I think it would be hard to envision an integrated adverse outcome, and it would be, I think, inadvisable to not look at the component diseases that you're interested in. The circumstance that this question describes is obviously true of all risk factors for disease. Between individuals, there can be significant variation in how risk is manifest when someone is exposed to something because of genetic factors and other things, and so I don't think you want to distill it down to a point where you can't assess those matters.

DR. BHATNAGAR: But I would add one thing, though, that you could estimate all-cause mortality, and that is independent of the cause. And so you do it -- now, we did that, and we found that smokers, on average, have 11 years of less life

expectancy than nonsmokers, so that's putting everything together and understanding only in terms of mortality, but that's the best we can do.

DR. DRESLER: Okay. All right, any other questions from online or in the audience?

(No response.)

DR. DRESLER: Then thank you, panel, very much for both, for the panel discussion and your presentations.

(Applause.)

DR. DRESLER: We'll now go on to Session 3, and Session 3 is Chronic Obstructive Pulmonary Disease (COPD). And our first speaker is Dr. Crapo from National Jewish Hospital in Denver, Colorado, and he will be speaking on Tobacco Use and COPD: Overview.

DR. CRAPO: Well, great. I will enjoy transitioning us to COPD, and there's a lot of relationships I'd like to go over. My conflicts of interest, I don't have any financial conflicts of interest. I would acknowledge that the COPDGene cohort I'm going to tell you a lot about is primarily funded by the National Heart, Lung, and Blood Institute with support by the COPD Foundation and a fairly broad industry advisory board.

So COPDGene is a project that's now ending its ninth year.

It's a very large cohort. It's a cohort of -- actually, it's a cohort of smokers. To get in this cohort, you had to smoke at least 10 pack-years, and that was basically the only requirement except for age. It turns out that we actually finished the recruitment. There's about 40% of the patients are considered to be control subjects by an FEV_1 evaluation, and 60% have various grades of COPD as measured by FEV_1.

Now, if you look at the smoking intensity, it turns out that almost nobody smokes 10 pack-years. Once they've smoked that much, they tended to smoke up into the 30, 40, and 50 pack-years, and you see that, going from the lower end to the higher end, we're going from the mid-30s up to close to 50 pack-years for each of the groups as defined by FEV_1, which is GOLD Grade down here.

Now, this is the classic distribution of a COPD on a curve where we show the FEV_1/FVC ratio on the y-axis and the FEV_1 on the x-axis. And you can see that the normal patients are those that have a normal ratio and a normal FEV_1 up in this quadrant. And GOLD 1, very -- say very early COPD, if you believe that is. And then GOLD 2 would be here, 3 about there, and 4 over here. We call -- we have a group we called GOLD U. It's really -- we also named it PRISm, but it's people that have a

normal ratio above 0.7 but have a low FEV_1. It's a unique group. I'm not going to spend a lot on talking about, but you can see it's part of this pattern.

Now, what I want to mention is FEV_1 is -- COPD has been graded by FEV_1 since -- for decades, and we recognize that's a strong biomarker. It's a strong correlation with severity of disease. But it's not a very sensitive indicator, and it's not very specific. But I would make one comment. It actually has a better -- if you have a low FEV_1, you have a higher probability of heart disease than you do with high cholesterol or with high blood pressure. FEV_1 is the best predictor of heart disease than I know of any of the biomarkers out there. So keep that in mind as we integrate our two fields.

Now, when I look at this cohort, just to define what it is, notice that this is -- red is the current smokers, and blue are former smokers. Notice that most of the people at younger age that came into our cohort are smokers, and as age goes on, they change into former smokers. And that's really the onset of disease. A primary reason why they stopped smoking is they had a heart attack or they developed some other disease. And you can literally see as the disease forms in the forties and fifties, people stop smoking. And we're talking about people

who didn't stop smoking when they were 20 or 30 years old because you had to be 45 years old to get in this cohort.

This is a very interesting diagram. It makes a very important point. And it actually came from a study done at the NCI in 2008. It was published in the *New York Times*; I'm giving the *New York Times* version of it. So they converted all the data into circles. The bigger the circle, the bigger the probability of that event. And you see various causes of death. This is smokers over here on the right and nonsmokers on the left.

And you can see that heart disease is a big cause of death, and actually, smokers develop more heart disease earlier and a little more extensively as they get -- the circles are a little larger. And the same is true for stroke underneath it. But the big difference is in lung cancer and in COPD -- this is men I'm looking at -- compared to the nonsmokers over here. Now, women have the same reaction. This is lung cancer and COPD, and this is the nonsmoker. Now, what I really put this up for is if you look at the middle group, which is the former smokers, people who stopped smoking, what's really interesting is that their incidence of cancer goes down, but their incidence of COPD does not. That's true for men and for women.

What I'm trying to tell you is if you develop the inflammatory predisposition and the patterns for COPD by the time you're 50 years old and you've been smoking for 50 pack-years, if you have COPD started, stopping smoking won't stop it. It's a progressive disease. And it's just as atherosclerosis is progressive. And so there are reasons to stop smoking, but the best reason is to stop very young. So stopping smoking is not sufficient.

And now, in COPDGene we're actually doing whole -- we're in the midst of doing whole genome analysis on the entire cohort. That's not done yet. We have done a GWAS analysis on it, and we've correlated all those findings with multiple other cohorts. This lists the nine primary regions that we defined within the COPD cohort that relate to development of emphysema or COPD. By combining our cohort with other cohorts that are available worldwide, there are now 24 genetic regions that have been identified. People are just in the process of figuring out what they mean and how they relate, but you can see their key inflammatory pathways, TGF-beta 2 is in there. The nicotine receptor genes are in the pattern. I'm not going to speak a lot about genetics today, but they are a way to help define risks, group people at risk. And we'll know more about

it as time goes forward.

I would like to say that one of the biggest problems with COPD is that we call it COPD. It's not one disease any more than an aortic aneurysm and myocardial atherosclerosis are the same disease. It only means that you have difficulty breathing out, and there are lots of different patterns of it.

And there are different diseases with different incidences and different biomarkers, which is really important; if you try to apply one biomarker to COPD, I think you'll generally fail because it's like applying one biomarker to a whole family of diseases where it doesn't have that specificity. Let me just illustrate that because we need -- we're not at the stage of defining the biomarkers by subtype, but it has to be done.

In COPD, there are many types of emphysema. I'm just going to illustrate two of them for you. This is centrilobular emphysema forming around the small airways and causing central breakdown which gradually enlarges. It's the most classic form that forms primarily in the upper lobes, and it's a progressive form of emphysema for this type of smoker. It's the most common type.

Take a look at this one, for example. That's paraseptal emphysema, completely different pattern. Lots of it in the

periphery with preservation of the internal part of the lung associated with a lot of airway wall inflammation because of different genetics. Our genetic work has shown that this thing -- genetic differences between this disease and the centrilobular emphysema. They're both strongly correlated with smoking.

Now, I mentioned the airway disease. I'd say if you go to airway disease, you can have involvement of the big airways, middle size airways, or small airways, and they're very different patterns, often overlapping but not necessarily. For example, this is -- whoops, I went the wrong way. This is an example of severe airway disease, very thickened airway walls. You can see in this section, going out like this. Now, I'm not sure -- the lights are probably too high, but this is actually paraseptal emphysema, if you look at the patterns of emphysema on the outside of the lung. But it's an example of very severe airway disease.

This is an example of airway disease, and once you see thickened airway walls, like that one right there, this one over here, the -- and/or ground-glass opacity is in the lung as you start to develop inflammatory lesion with these big thick airway walls right here.

This is another example of a person who would normally look at this lung and say it was totally normal, but there are actually very tiny densities. You can see one here and one here. And if you get it under a microscope, it looks like this. It's a small airway with a lot of small airway inflammation.

But it's so small, it's silent for most evaluations. And a large number of patients have just this. So it's a different disease with a different pattern and different biomarkers. I actually think that a lot of the biomarkers we were talking about in cardiology would correlate here because there -- inflammation of the airways are being mediated by many of the same markers of inflammation that we just talked about for atherosclerosis.

Now, the other interesting thing is if you look at -- this is a graphical description of the percent of our patients in our cohort who have airway disease but who do not have any significant emphysema. So this is airway disease without emphysema, and what's interesting, if you look at the -- GOLD U is this group that was upper left-hand corner that I showed you. GOLD 1 and GOLD 2.

Look at that, 50 to almost 60% of the subjects have airway

disease and not emphysema. Most people are in these lower cohorts. The number of people in the GOLD 3 and GOLD 4 group is small, so most people with COPD are actually in this category right here.

It's airway disease that's driving it, and it's certainly early, and airway disease is the biggest number in it. Now, emphysema is really bad. I'm not saying that. I'm just trying to tell you the percentages. Most people think COPD and emphysema are synonymous terms, and I'm telling you they're not. The biggest expression of COPD is actually airway inflammation.

So let's look at what happens -- how we measure follow-up. And our cohort is now just finishing a 5-year follow-up, and we'll be starting hopefully in another year or two on a 10-year follow-up. If you look at the FEV_1 change, and this is the GOLD 1-2 subjects, we're looking at the percent of loss on a yearly basis of FEV_1.

Remember, the FEV_1 is a measure that everyone thinks you measure COPD with, and it's about the only parameter besides exacerbations and hospital admissions or death that the FDA will use to evaluate a drug for efficacy. We're dealing with 50 to 100 ml of loss of FEV_1 per year, and that's the

predominant one; that's this group right here and this group right here.

And people who stop smoking, who are in these groups -- now, remember they had high smoking to start with, but if they stop smoking, it's the red bar, they still have these losses. They're losing the FEV_1. But if you're going to follow FEV_1 and get changes of biomarker, you're talking about following the patients for 5 or maybe 10 years to get that. It's not a short-term indication, and it's not the one that you want to use for doing the kinds of biomarkers you're talking about unless you're going to look at lifetimes.

So looking for other parameters. CT is the big one we're using. And in CT, we started using now what I'm calling -- which was called when it was first introduced as parametric response mapping, probably best illustrated as a kind of micromapping of the lung. You take the base -- actually, the CT that we take at baseline, and then we register all of the chest wall and mediastinal structures as to where they are in it and then use a computer program to find the same pixel on the follow-up CT and then look to see how that pixel changes.

So you're doing pixel-by-pixel comparisons across multiple years and -- or months, in this case. And let me show what you

can get out of it. If we took -- of our first 2,300 patients that we studied in that fashion, we found that about 40 -- well, 37% progressed and 63 did not progress in terms of this pixel-by-pixel analysis of a chest CT.

But we're talking about close to 40% of people having progression in this, and of those, 6% progressed with emphysema; 24% progressed with air trapping, which was probably -- as you develop that small airway inflammation, the first thing you do is start trapping gas. And then as it evolves into more inflammation, it breaks down and forms emphysema. So this would be the early one, and then, of course, there's about 7% had both emphysema and air trapping. I'm going to physically illustrate this on a couple of chest CTs for you, if you can see it.

This is -- it's much better on the bright screen, I'd say. But the baseline scan, if I look at this scan over here, the red that you can see along the center are the areas that were emphysema at 5 years earlier back here. The yellow are the regions that were gas trapping back here but are now emphysema. And the green were normal here and are now emphysema. And you can see actual progression, and you can see it in multiple areas, and you can count the number of areas and the number of

pixels that changed, that became emphysematous, either started as normal or is gas trapping and then became emphysematous.

This is another one. Was another one anyway. Let's see if I can go -- okay. This is another, this is a lady that was 66 years old and a GOLD 3, was fairly advanced emphysema, and again, you can see -- this is her -- after 5 years, this is her consolidated CT evaluation where red is the areas that were emphysema 5 years earlier; yellow is the areas that were gas trapping 5 years earlier but now have become emphysema; and the green are the areas that were normal 5 years earlier and have now become emphysema. So you can literally, physically see and count the number of pixels that are changing from normal or from gas trapping into emphysema.

And it's -- I think it's -- this should be a biomarker that we should consider going forward for following this disease. I'm doing a 5-year interval, but the question is can we get it down and get it to 6 months or a year and pick up change.

I'll skip that one for now.

Early diagnosis of COPD. I think it's absolutely critical to understand -- oh, wow. What am I doing? Am I pushing the wrong button?

DR. DRESLER: If you push it forward, the lights --

DR. CRAPO: I've got to be careful, okay. I'm too enthusiastic here.

So the -- my point is that if you want to understand this disease, if you want biomarkers for smoking to understand what it's doing, you need to find them here at this age, 45 or even earlier, but in my group the age started at 45. But that's where you want to make the diagnosis. If you wait for out here, it's too late; you've got massively destroyed lung, and the opportunity for modification of disease is minimal. You're doing symptom alleviation at that point. But we want to go early. And the blue on this graph are people that are said to be normal by our standard definition of COPD, which if you have a normal FEV_1, you're normal, you don't have any lung disease. I want to argue that's not true.

I'm looking back here now and thinking about biomarkers that you might use for smoking, but these people, remember, they smoked 30 to 50 pack-years. So we're going to go back into this age group and look at what goes on.

If I consider other things, are these people that are so-called GOLD 0, meaning a normal FEV_1, are they really normal? Well, I look at dyspnea, exercise capacity, the CT evidence of

emphysema, CT evidence of gas trapping, number of exacerbations or number of time they go to the hospital or get antibiotics, or significant comorbidities, like cardiovascular disease that we've talked about.

And if I do that, and I'll give you a couple examples -- this is just the CT changes. If I look at people that are said to be normal by FEV_1, 15% have airway disease. You can see thickened airway walls. And about 20% had various grades of emphysema is indicated in the red. So we've got, you know, almost a quarter of the patients, these have CT evidence of disease even though they have a normal FEV_1, so to speak.

If we look at gas trapping, this is people with gas trapping. Greater than 15% is on this side of the green line going up. And you can see that it's a large fraction of the people, I think 18% in this category and 7% in that category, have gas trapping. And the gas trapping is as high as 50 to 70% gas trapping. This is people with a normal FEV_1, so to speak.

If I look at dyspnea, now dyspnea score of 2 is really significant. It only goes to 3. And this means -- it means you can't do -- you can move around your house but you can't run or you can't walk any significant distance. Now, people

with GOLD 2, about half of them have a dyspnea score of 2. And our GOLD 0 people that are supposedly normal, I have about 15% that have Grade 2 dyspnea. If I look now -- I told you, I'm just saying if they had a normal FEV_1. This is their histogram of their FEV_1's, and above 80% is normal, so these are all normal values according to a population statistic. But look at where it peaks. It peaks at 90%, predicted. That, it should peak there. That's where -- if I do a histogram of nonsmokers, the shape of the histogram will be like this. It will go up like that and peak right there and come down.

But these people have all got obstruction, but it's moved, it just shifted the curve to the left but hasn't shifted it far enough to be abnormal by a population standard. So I'm trying to tell you there's a lot of disease there, and I'm just going through all -- everything that I just mentioned could be a biomarker for disease.

Let's go on and just talk about high-risk axis. We did this first, I think we used principal components analysis to see if we could put this together to determine risk of various groups. Well, in doing this, we fed into it all the CT data you see here and all of the PFT variables, and then we did principal components analysis by -- this is a group at the

university called -- and looked to see for clustering groups. And they found one cluster that really clustered strongly with the presence of emphysema, and they call it the emphysema disease axis. And look right here, the percent emphysema in upper-lower levels, very high correlation coefficients. But very low correlation coefficients for things like airway wall thickening down here, right, segmental wall thickening.

So this is the emphysema axis. Now, using the same numbers on -- they found another cluster that looked quite different, using all this data. They call this the airway disease axis because now the big correlation was with the airway wall thickness right here, and the correlation with emphysema is quite weak or poor. You see these emphysema numbers?

So we have two axes done by statistical analysis using principal components. And we put them on a curve here. What's interesting, firstly, mortality. This is what we had as of the first 5 years in the cohort. Actually, it's a little more than 5 years. We've got mortality going out now close to 9 years. Notice that if you're in the top quintile, the top 20%, your mortality increases a little more than twofold, especially in the last 10%. You see it curve at the end right there? This

is if you have -- if you're on the emphysema axis. And you could -- by the way, this could be a biomarker because you could take all those numbers I just said and create a biomarker, give each person a specific score. If you do a CT and a PFT on them, every single person will have, will fall, will get a number for each of these axes, and you can just literally read it off the axis. But the -- if I -- this is the emphysema disease axis for mortality.

This is the airway disease axis for mortality. And look at the mark of the increase in mortality in the top quintile here.

So if I graph these, back on that first graph I showed you, the gray is the rest of the cohort. Blue are the people on the high risk; the top quintile of the emphysema disease axis is blue. Red is the top quintile of the airway disease axis, and you can see that they literally separate by the function. And yellow is where the person's number falls on both axes simultaneously, which is the most risk.

And if I look at mortality in that group, this is the mortality numbers. If you don't follow any one of those high degrees, high-risk axes, your mortality is 5%. In the emphysema axis alone, you're at -- what's that? Ten percent.

And 18% in the airway disease axis and on both axes simultaneously, it's 34%. And it's not just mortality that's related to this.

This is the same data for dyspnea score. Remember, 2 is really bad. If you're on the emphysema axis, it's 1.5. Airway axis is 2.3, and combined axis is 2.9.

If you're in a 6-minute walk, how far you can walk in 6 minutes, this is -- it's 1,456 versus 1,352, dropping to 1,122 and then down to 971.

If you look at quality of life or SGRQ index, a bigger score is worse, worse quality of life. So you're seeing a change that follows a same pattern with a poorer quality of life on the emphysema axis and even worse quality of life if you're on the airway axis. And very bad if you're on both.

So those are indicating you can use these combined data from CT and from pulmonary function to gather some really powerful data about relative risk, and ultimately it will be disease progression because all these things I'm giving you are endpoints, which -- the most severe of which is mortality.

These are correlated with some genetic and biomarker findings. For example, the RAGE -- I'll show you that -- correlates with the emphysema axis. And nicotine addiction

correlates with the airway disease axis very strongly.

Let me just -- I'm going to end by showing you a couple more things, biomarkers. We've been doing plasma biomarkers on this group, and one of the ones that's really come out -- and you can actually find, maybe -- I think Graham will talk about some of these others in the next talk, but you can find many of the same biomarkers that you find in atherosclerosis, but RAGE is come out, is a very strong one, and sRAGE is our biomarker for emphysema.

And this is an example of the fall in sRAGE and the population as you go from none to mild to moderate to severe emphysema. And it actually correlates with the genetic score, so if you have a CC genotype, it would be that axis right there, and if you have the TT genotype, it starts out lower, has less fall.

But there's genetic correlations, can use both genotype and these plasma sRAGE levels to predict emphysema and do this independent of FEV_1. Because the fall is there, you can see the change. It correlates in emphysema, but it's an independent biomarker.

So where would I land? I would say that there are -- this is my final slide -- there are many potential biomarkers out of

this to correlate disease. And I think we should consider using imaging. It's time for CT to become a standard imaging technique to give us critical biomarkers to follow this disease. And you can do micromapping of emphysema, and you can do micromapping of expiratory gas trapping.

What we have yet to learn is how sensitive it is for short-term changes. But the changes in expiratory gas trapping should be responsive over relatively short intervals of time. That fully needs to be tested to make this -- to know the timing of the biomarker because the data I've given you is a 5-year interval, and we'd like to know what 6 months or a year would tell you. And then there are plasma biomarkers, which I just put forward, sRAGE, but I think that the next talk can go into a much larger family.

So thank you very much.

(Applause.)

DR. DRESLER: Whoever is near that light switch back there, if you can pop that back up, please? Unless you need it down?

(Off microphone response.)

DR. DRESLER: So leave it down. We'll leave them down, please. All right. So thank you. So our next speaker is

Dr. Graham Barr from Columbia University Medical Center, and he will be speaking on Imaging Biomarkers of Potential Harm: CT and MRI.

DR. BARR: Don't push the big button there, okay. Great. Thank you very much for inviting me. It's a pleasure to be here. And I'm going to speak maybe a slightly different song from what James just gave but a bit more of an overview on imaging biomarkers of potential harm using CT and MRI.

But I would point out this work is mostly funded by NHLBI. This is drawing in part from work in the MESA study, which was referred to earlier, the cardiologists, where we have a lung study on top, and partly from SPIROMICS, which is a subphenotyping and intima-media markers study in COPD, which has been designed with a fair amount of help and assistance from FDA, so thank you for that.

So I'm going to quickly mention a couple of the advantages and disadvantages of CT and MRI measures; say a few words about the background of COPD, because it's not necessarily a disease that all of us talk about all the time; and then focus on biologically based imaging biomarkers, which I think is a key advantage here to try to unify some of the disparate biology we've been talking about with intermediary imaging biomarkers

that match the biology; and then briefly mention test characteristics and gaps in knowledge as we think about applying these both in the short and the long term. I would say these are also a little bit more from the general population perspective and how much we can -- if we can move into populations who may be at risk in addition to just related to harm reduction.

So some of the, I think, major advantages of imaging biomarkers is that, first of all, they're quite translatable. We can do many of the same measures in mice and animal models very well, move into histology, and then fall into in vivo. We mentioned humans, using very analogous approaches of CT and to some degree MRI. And I'll give some examples of that, but we -- and sort of direct translatability.

And I would say that we found, in our own studies, that it's -- many of the animal studies of emphysema or mice models of emphysema, which really are not studying COPD because it's hard for mice to do spirometry, but rather measuring emphysema. We can then look at longitudinal change in emphysema in humans, and we seem to see a much stronger relationship and better translation. As has been alluded to earlier, they can be helpful in defining early disease, and whether we call that

subclinical disease or preclinical disease can be discussed, but these can be done very early on and including all the way down into adolescence and childhood.

I think more specific to some of the pathobiology and similar to -- certainly compared to the FEV_1, they're much more helpful and in many ways define some of the subphenotypes that COPDGene and SPIROMICS and other studies are elucidating. And then, of course, there's a whole world of molecular imaging particularly related to PET. I'm not going to talk about that in detail here, but just to -- since the topic was on CT and MRI, I mention those. And, of course, they're noninvasive and reasonably easily repeatable compared to invasive studies.

Some of the disadvantages. Some are not fully validated; I'll mention that at the end. The reproducibility of some of the measures is still being established, and certainly, cost is an issue when we get into larger-scale studies, although not necessarily. Radiation exposure for CT can be limiting in younger populations but less so in older. And I'll mention some contrast agents that have -- there are some concerns about but are generally very well tolerated. Probably of more -- worth more discussion is some of the paradoxical acute effects, and there's been references to short- and long-term effects

earlier in the session.

And indeed, some of the biomarkers, these imaging biomarkers, actually sort of go in the wrong direction temporarily, and you need to kind of establish a study related to tobacco exposure, and they need to be -- sort of a steady state has to be defined.

So in terms of background for COPD, you've probably all seen this slide just showing mortality rates in the U.S. approximately over my lifetime. So you don't need to read this from the back, but the lines that are dropping precipitously are heart disease and stroke. This is in men at the top and women at the bottom. Cancer is similar, dropping. And the line that is shooting up on the other side, particularly in women, also in men, and now the third leading cause of death is COPD, which is part of the reason I think we need to be talking about it now, and this is in the context of a massive reduction in current smoking in the U.S. population over the last 50 years. So this sort of emphasizes the point that the smoking risk for COPD is not really acute at all; it's really chronic in a lifetime exposure, in exposure to tobacco products in the teens through thirties are showing up as harmful as the baby boomer generation ages and is showing up not dying of

cardiovascular, instead dying of COPD.

But a lot of the definitions are a little bit confusing maybe, so I'll just take a word to say this, and this pertains directly to some of the imaging biomarkers. So the sort of WHO and CDC preferred term is chronic lower respiratory disease for capturing death rates, what was just shown in the last slide.

And this is -- I want to make sure I get the right button here, sorry -- is defined, includes chronic obstructive pulmonary disease, which is defined on spirometry as chronic airflow obstruction, and so this was the inclusion criteria for many participants in COPDGene and SPIROMICS. But other -- there is also asthma, which is intermittent airflow obstruction which typically shows up earlier in life and is not specifically smoking related. Chronic bronchitis, which is -- you know, has historically been defined by symptoms of a chronic productive cough but is being, I think, redefined currently, partly in COPDGene and partly in SPIROMICS, as symptomatic smokers and is probably a lot more important than we previously thought. And then finally emphysema, as James mentioned, is defined by a loss of lung tissue, which you can't measure on -- with a spirometer, but you can measure very well, as James mentioned, on CT scan, which of course now we can do

broadly in populations.

When we talk about chronic lung disease, I would just throw out that there's also interstitial lung disease related to fibrosis in the lung. And really, most of these are strongly smoking related. And many of them are directly influence measurable using imaging, specifically emphysema, as I mentioned, and to some degree, interstitial lung disease is defined by imaging, and chronic bronchitis is strong.

So what we find is interesting, too, though is that this then ties into the pathobiology, and even on sort of a human level CT scan, we find fairly strong differences between different CT metrics and the phenotypes I just laid out. So this is adapted from the MESA lung study where -- and other studies where we see a distinct, as James mentioned, too, loss of airway, small airways, but more specifically related to COPD.

In contrast, the pathophysiology of emphysema, particularly in this large group of people here who have emphysema in the absence of COPD, one strong hypothesis, microvascular compromise, which brings in much of the cardiovascular risk that was described in the morning session, and we've done a fair amount of work to suggest there's a

strong link there. And then when we talk about symptomatic smokers and chronic bronchitis, there seems to be much more distinct airway wall thickening.

And then on -- with early fibrosis, there's interstitial lung abnormalities, so all of these are measures that we can pick up noninvasively that seem to target different phenotypes sort of in the morbid and comorbid state.

So this is relevant, I think, to tobacco smoking because obviously cigarette smoke and tobacco have multiple effects, as was mentioned earlier, but what we can do is start to lay out -- and this is somewhat based on the data and somewhat hypothesis generating -- is different pathways toward different phenotypes. So we know that tobacco smoke can cause oxidative stress, inflammation, nitrosative stress, and this may probably contribute to small airways disease and a subsequent reduction in the FEV_1/FVC ratio that defines COPD. And sorry, the "D" got cut off there.

In contrast, when we think about the pathophysiology of emphysema, there are clear signals of abnormal endothelial function, endothelial apoptosis, and microvascular damage and dysfunction, which may be important in and of itself, and we think it may contribute to emphysema pathogenesis, and that's

very different from what is going with chronic bronchitis in terms of mucous hypersecretion, inflammation, airway wall thickening, and chronic bronchitis. And then really, in very different ways, smoking contributing to inflammation, the 5B pathway, interstitial lung abnormalities, and interstitial lung disease.

So as we think about different biological pathways that may be relevant to different products within tobacco, we might want to isolate down one of these, and specifically, for example, nicotine may be more important than did the endothelial emphysema axis, where some particulate matters may be much more relevant to small airways disease, lung function, and so forth.

So to start with the first of these, this is an example from Jim Hogg's lab where -- showing a micrograph of terminal bronchioles. This is down about the 18th to 20th generation in the normal lung here where -- if I can get a pointer -- these are alveoli, and what we just saw moving through was a normal terminal bronchiole.

When you look in patients who have emphysema and COPD, these terminal bronchioles are just completely not present, and he did a very elegant study to show complete loss of these

terminal bronchioles with increasing degrees of emphysema and increasing levels of disease. And I should have put another marker over here, but on the far left of the screen, there could be a mouse model showing very similar things using micro CT.

So you can move from a micro CT in mice to -- this is micro CT on cut sections of explanted lung on the left. And then you notice that the sample size is very small up here, four subjects that are controls and small numbers over here, but we can quickly move and look at a similar phenotype on regular CT scans in humans where, again, if I can pull out -- we can look at 2 mm airways which are now around, let's say, seventh generation, not all the way out at 18, but you might be able to see it right there; it's about a 2 mm airway, the scale is here, when we blow it up here. We can measure that, and then we can do much larger numbers of subjects, for example, in all of MESA.

This is, again, from a smaller group from New England. Again, see the similar thing of this loss of these small airways as you get more severe COPD. So here's an imaging biomarker that you can do in mice, you can do on sections in human, and you can do in vivo in living humans on a large

scale.

So there are -- there's sort of more refined ways to look at this, and this shows that functional small airways, sort of, is a type of that disease which can split out, and James sort of alluded to this, can split out what is really normal lung here from small airways collapsing or a functional measure of small airways disease in the lung to emphysema here.

So that works on inspiratory/expiratory CT, but you can get a similar measure using hyperpolarized gas on MRI. This is an example using hyperpolarized helium, but you can do the same thing with hyperpolarized xenon and fluorine, where on the top row here is a subject with a fairly normal lung. The red is the amount of emphysema, as was previously described, and relatively true elements here, and the green here is the ventilation of the gas into the lung on inspiration, and as you can see, it was a pretty normal ventilation, as you'd expect there.

But this patient has quite multiple loss here and a complete loss of ventilation in the upper lobes of the lung, on here. So this is a measure that takes seconds to perform, you can actually do this in children, they've done it down to about age 5, and it gives you a very direct measure of a non-

asthmatic patient of really small airways disease in a more sensitive way even than CT.

So if we turn and look at a different axis of how tobacco smoking may contribute to, in this case, pulmonary vascular damage -- the literature on acrolein and formaldehyde in the lung goes back about a decade and a half in most models showing that acrolein in tobacco smoke contributes to pulmonary endothelial apoptosis in addition to systemic pulmonary apoptosis and contributing possibly in mice to microvascular damage and dysfunction. So this is something that we've been interested in looking at in humans, and of course, the pulmonary microvasculature is the one that is first hit by anything you inhale into the lungs. So this has been tricky to access, but we and others have developed approaches using gadolinium-enhanced MRI where the big advantage of the lungs is it's totally black on MRI without gadolinium, and then you can measure a first pass of the blush through the microvasculature.

And if you look at this kind of edge of the lung, you can get a very clear signal from the signal intensity increase and calculate a directory of pulmonary microvasculature blood flow, volumes, and mean transit times, again, noninvasively. And we are now doing two of these measures in one session in about a

45-minute MRI so that we can do an intervention pre and post in the same intervention, and we're hoping somebody in our group is about to start doing this with e-cigarettes.

So the point, though, is that this is -- this measure is reduced, and I wouldn't say it's absolutely wiped out in COPD, but there's about a 50% reduction in pulmonary microvascular blood flow in people with very, very mild COPD, so really almost preclinical COPD, and then about a two-thirds reduction in pulmonary microvascular blood flow in moderate and severe COPD. And so this is, we think, important in the pathogenesis of emphysema but certainly has very direct effects in terms of cardiac function also.

A collaborator, Eric Hoffman, has done a similar thing with dual source CT. This is a little bit more complicated because it gives an even more precise measure, and it obviously involves radiation and iodinated contrast, but this shows the heat maps here of perfused blood volume where smokers without emphysema, red is deficits here. So smokers with mild emphysema have really marked perfusion defects compared to smokers without emphysema, and interestingly, some of this, although not all of it, is reversible with a pulmonary vasodilator.

We can also assess the pulmonary microvasculature on non-contrast CT. Now, this is looking at the arterials in larger vessels. We lose vessels below about 1 mm. And this, in SPIROMICS, is predicting a decline in lung function and progression of emphysema, as we might expect based on the pathophysiology. And so we think, again, for anything you're inhaling into the lung, this will have a direct effect, and we know it changes reasonably quickly.

And just one final word there on xenon imaging, has also -- this is sort of a fancy version of diffusing capacity, if you like, in that you can measure the air spaces and sort of the size of the air space, which is a measure of emphysema; I'll mention more in a minute. You can measure the lung parenchymal plasma volume and the amount in red cells, so this gives you a very localized measure of diffusion of gas into the blood.

So if you're interested in particularly regional changes in inhalation or regional toxicity from a novel smoking product, this actually gives you a direct measure. So as I mentioned, these microvascular changes, we hypothesize our link to emphysema, although there's obviously many other axes and RAGEs related to this, I should add, that may contribute to

emphysema.

So this is to point out that this measure of percent emphysema or percent attenuation areas is defined by a threshold on non-contrast CTs that shows the amount of emphysema in this lung. Again, this is a relatively simple measure to do if you acquire the CT properly, and it has been done at this point in tens and tens of thousands of subjects, both with disease such as in COPDGene and SPIROMICS and in healthy populations like the MESA study and also Framingham, and is highly prognostic both in COPD for increased mortality and in the general population, even in patients without COPD.

And just quickly measure all -- so this is sort of, if you like, the heavy or the most studied measure, alpha or D is a fractal measure that was described by Mishima a little while ago, and it's a very interesting measure of puff size to be related to early emphysema. And we actually found that in patients at age 60, this measure, who had never smoked cigarettes, this measure was strongly related to a self-reported history of environmental tobacco smoke as a kid, which is -- ties into a mechanical hypothesis of emphysema.

(Off microphone comment.)

DR. BARR: Okay, I'll stop. I'm almost done. And so you

can do a similar measure using Brownian motion on MRI, which is based on gas particles bouncing around in the alveolus here. And this shows this apparent diffusion capacity measure in the same subjects I showed earlier, again, with kind of a normal amount of measure. The yellow here is more emphysema that is sort of seen with better sensitivity than on the CT up here, and marked down here with a wipeout of gas at the top here. Interestingly, the Nottingham group in the UK showed that kids at age 5 exposed to environmental tobacco smoke had increases in this measure at age 5, were able to -- so very consistent with what we saw in the other study.

So I'm going to finish, sort of move a little more quickly, just to mention chronic bronchitis is sort of the standard surrogate measure, is airway wall thickening. This is showing, this is the airway blown up here, and you get -- can measure the wall thickness and the lumen diameter and then put these two together.

The difficult part here is one has a very large number of airways in the lung to deal with, and so averaging or identifying localizing studies in that is a little tricky and is something we've been working on in detail in SPIROMICS.

And just quickly, to touch on fibrosis, which overlaps in

a nontrivial proportion of COPD patients, this, again, is fairly identifiable on CT here, this sort of increased patchiness down here is consistent with fibrosis, and this can be done on visual read, and these groups have done a lot of work on this, or by quantitative measures referred to as HAA.

So there's a large range of measures. One of the -- some of these have been -- I think, have reasonably good reproducibility. There's much less work has been done in the newer ones compared to the older ones and certainly compared to things like spirometry. And part of the point of -- particularly in SPIROMICS we're looking at this in detail.

So if we look -- you know, people sort of complain about the FEV_1, but the FEV_1 has some very good test characteristics; intra-class correlation coefficient in a general population study like MESA lung is about 0.99. In other words, 1% of the variants in the test is due to test error, and 99% of the variants is due to population variation. So this is -- the FEV_1 is a very good test, it's just sort of an end. It represents multiple pathways.

So there's a lot of -- if we look at the fSAD, which is that small airways measure, we're about to -- Meilan Han is about to get data out on this where, in SPIROMICS we've

rescanned people over a month time, and this is going to look pretty good. Ventilation is a little tougher over that time, and perfusion is coming shortly. And I apologize, I don't have those for you right now. ADC is very tight, and actually percent emphysema is really quite tight, too, whereas the airway measures are a little bit harder to deal with.

Similarly for validation and prognosis, this is the pathology. Validation is also validation against microtubules. And there's a large amount of clinical data in terms of change of FEV_1 events and particularly for percent emphysema and the ILAs, mortality events, and the FEV_1.

I think I sort of pointed out the gaps in knowledge there, so we'll just finish up. There are multiple imaging biomarkers of potential harm. I think they're much more specific to the pathobiology and subphenotypes of disease, and improvements in technology and particularly the next generation of CT scanners will facilitate wider use.

Thank you.

(Applause.)

DR. DRESLER: Thank you. And what you heard towards the end of your talk was getting Dr. Rennard on the telephone. He will be joining us remotely from the UK, I believe London. And

so Dr. Steve Rennard will be speaking on Biological Biomarkers of Tobacco-Related Harm in the Lung.

So, Steve, are you there?

(No response.)

DR. DRESLER: Steve, are you on mute?

(Laughter.)

DR. DRESLER: So I heard the testing earlier. Steve, just double-check that you're not on mute. We're working in the room, too, but I'm not hearing you.

(Feedback.)

(Pause.)

DR. DRESLER: Great, thank you. Actually, I think we want it the other way around, though. It lights up for you guys and -- here we go, thank you.

For those of you who have been in our workshops before, we've had a few people present distally, and know that this room is going renovation, and starting after the first of the year, we will have upgraded systems that I've been told this will be seamless in 2017. Steve, can you hear me?

DR. RENNARD: Hello?

DR. DRESLER: Steve, yes.

DR. RENNARD: Hello?

DR. DRESLER: Good, good. Hi, Steve. This is Carolyn Dresler and --

(Feedback.)

DR. DRESLER: So, Steve, if you'll mute your webcast and then -- yeah, so that I don't hear myself -- good -- coming through. And then, Steve, if you'll say hello, then we know that we can hear you.

DR. RENNARD: Carolyn, can you hear me?

DR. DRESLER: Hear you very well, so thank you. And you're muted, and I don't have feedback.

DR. RENNARD: Can you hear me?

DR. DRESLER: Yes, you're great. It's great. So I've introduced you, that you will be speaking on Biological Biomarkers of --

DR. RENNARD: Hello, hello. Hello?

DR. DRESLER: Yes. I can hear you, Steve. And I think what the problem is, is that when you mute your webcast, you won't hear me.

DR. RENNARD: Hello, hello.

DR. DRESLER: So I'm going to ask -- I'm going to --

DR. RENNARD: Hello?

DR. DRESLER: I'm going to -- Steve, I can hear you.

DR. RENNARD: Hello?

DR. DRESLER: Ooh. You guys, how are you going to tell him that I can hear him fine and --

(Pause.)

(Off microphone comments.)

DR. RENNARD: Yeah. And first of all, I was delighted to be able to participate in this meeting. I'm glad the audiovisuals have worked. I am in Cambridge in the UK -- people in Cambridge here are very proud of that.

Since August of last year -- I got my disclosures on the next slide -- I've been employed by AstraZeneca. I still have my professorship at University of Nebraska Medical Center. And in the years before that, I have a number of disclosures which are indicated here. I will point out that the opinions I give are not that of my current or actually my former employer.

If you go to the next slide, please.

And as you heard from heart disease, there are no simple consequences with smoking, and this is true for the lung as well. Some of the lung diseases, in addition to COPD and interstitial lung disease, were mentioned in the previous talks by Graham and by James, but there's actually a number of diseases where the relationship is very well established,

including lung cancer. But there are other diseases where the relationship is suspected but as not as well studied.

If you go to the next slide.

This suggests that there's going to be lots of relationships between what goes on in the lung and what -- and the toxicities caused by cigarette smoke. And there's a number of biological fluids that potentially could be sampled in order to assess biomarkers to try to tease out those complex relationships. You know, I will touch -- I will focus primarily on the lung and in blood, but within the lung there's actually a number of different measures. There's exhaled breath measures, there's measures in sputum, either spontaneous or induced, and there's bronchoscopic measures.

And here's the time I'll focus on bronchoscopic measures here for what I think are the key points. I will talk somewhat about blood because I think that those are probably the most practical ones or for widespread use, particularly in areas such as assessing harm related to tobacco and related products. And as it's been mentioned already, biomarkers can also be measured in urine.

Go to the next slide, please.

Now, this slide shows a photograph of the inventor of the

flexible fiber optic bronchoscope, Shigeto Ikeda, performing a bronchoscopy on a patient, so people who had never done this or had it done, too -- but it's a fairly easy procedure. The patient is awake; the patient's actually holding his own tongue. He's actually assisting with the procedure. This is an old picture. With modern techniques, Ikeda should be wearing both gloves and a mask, but that probably was routine practice at the time the procedure was first started.

Now, the next slide shows what happens when the bronchoscope is advanced into the -- in through the mouth or the nose, then into the lower respiratory tract. The arrow there indicates where the bronchoscope is, in the trachea, aiding the division between the right and left lung, what's called the carina. And you can see that bifurcation there on the left with the cartilaginous rings. This is normal.

And in the next picture you can see -- next slide, please. You can see the bronchoscope's been advanced into the left lung, and you can see the branch point, again, dividing into the left lower lobe and then the left upper lobe, which includes both the lingula and the left upper lobe proper.

Now, the next slide shows what's happening in a patient with chronic bronchitis. Now, its name is right because

there's inflammation, and I think people can readily see that this airway is red, there's vasodilatation, there's actually edema. This actually is exactly the same features that people would recognize as inflammation if you were looking at the skin. It's the same processes. The -- is described. It's an inflammatory process, and it can be scored, visually, through the bronchoscope, but that wasn't my remit to describe here.

The next slide, please.

The bronchoscope, because it can actually access the lower respiratory tract, provides a way of accessing biological fluids from the lung. The lung is lined by fluids on the epithelial surface, and this fluid can be recovered. And the technique that's most commonly used to do this is called bronchoalveolar lavage, where the bronchoscope is advanced into the airway very gently but as far as it will go and achieves what we call a wedged position, so it's kind of like sitting like a cork in the airway. Sterile saline can then be passed through the hollow sampling channel of the bronchoscope. It won't come proximally because the bronchoscope is there like a cork, so it goes down deeper into the lung. So when the very first part of that fluid reaches the airway, it's kind of like a pump-priming effect. As you aspirate that fluid, you can

travel the airways. If you -- and infuse more fluid which you can get down into the alveoli spaces, and you can recover fluid, you can measure the amount of cells, you can characterize their number, their cellular features, and you can also measure biological components in the bronchoalveolar lavage fluid.

The next slide just shows one measure from bronchoscopy from a study by Thompson, a fairly old study, and you've heard, in several of the previous talks, the importance of peripheral blood neutrophils as risk factors. They're risk factors for heart disease; they're also risk factors for lung disease.

And I won't show -- but this shows data from Thompson showing that people with chronic bronchitis have, in the black bar there, far more neutrophils in their bronchial lavage than do asymptomatic smokers, but asymptomatic smokers have more than do normal nonsmokers. So as you might expect, bronchial neutrophils are present in people with chronic bronchitis, and this therefore serves as a measure of disease. I won't show the data as it relates to clinical features such as coughs, sputum production, and exacerbation, but I will show -- on the next slide addresses one of the questions that came up, which was the inter-subject variability in these biomarkers. So

these are exactly the same data, but it's individual data rather than group data, and so what you can see is that for the chronic bronchitis patients, on average, there's a marked increase in bronchial neutrophils.

But a fair number of individual patients had bronchial neutrophils that are completely within the normal range. And for asymptomatic smokers, you see a similar relationship, although maybe a more consistent mild elevation.

What this means -- if we go to the next slide -- is that you can measure biomarkers that are associated with disease, and you can measure them in the tissue of interest, and they will certainly predict disease risk; that is, higher bronchial neutrophils are associated with chronic bronchitis and the clinical features that go with it. But this is a statistical association; it's not a diagnostic. And Dr. Hatsukami, in her first remarks, said a perfect biomarker would be one that has a one-to-one relationship with a clinical outcome. That's certainly not true for even neutrophils measured within the lung, and it wouldn't be true for neutrophils measured in other biological fluids either.

Next slide, please.

So the measurement in a target organ is very logical, and

being a pulmonologist, it makes sense, to me, to measure things in the lung if you're interested in lung diseases, and there's no doubt that it can be very helpful in understanding things. But it's obviously not always practical, particularly in studies that will require large numbers of individuals, and therefore, a measure that could be performed more easily, like a blood test, is highly desirable.

So next slide, please.

So the next slide shows the relationships between smoking and lung function in COPD in data taken from the ECLIPSE study, which is a 3-year observational study of COPD patients in control funded by GlaxoSmithKline. Now, what can be seen here is that there is -- there's a certain number of measures that are associated both with baseline FEV_1, which are the numbers in the middle, and on the rate at which FEV_1 is changing. Now, I think that we're more interested in the activity of a disease rather than its severity; that is, we're interested in what's changing in the patient and whether those changes can be mitigated or reversed.

So we highlight -- now, the next slide.

We can see that the smoking status actually is very strongly related to both of these, and in fact, the current

smoking status in the ECLIPSE study was the strongest predictor of decline in lung function, that is, the progressive nature of the disease. In fact, in the ECLIPSE study you can see that the current smoking is a very, very strong predictor of disease progression, but the overall smoking history, that is the total number of pack-years accumulated, was not.

Next slide, please.

Now, a number of blood biomarkers were measured in ECLIPSE, and they, as you can see, show fairly weak relationships either with the baseline FEV_1 or with the rate of FEV_1 change, that is, disease activity. There is one relationship that achieves the clinical significance, and that's with the CC-16 with a p-value of 0.04, but I'll point out that these are uncorrected for multiple comparisons, and it's a weak relationship in any case.

Next slide, please.

Now, blood biomarkers can be assessed in more sophisticated ways, and we heard from James that, in fact, emphysema is a subtype of patients with COPD. COPD is defined in terms of anything that reduces FEV_1, but emphysema is present in only some of those individuals. And as you can see here, the blood biomarkers in the ECLIPSE patient are much more

strongly related to emphysema both in terms of baseline and in terms of the rate of change. In fact, the most striking of these, if we go to the next slide, is sRAGE.

Next slide, please.

Is sRAGE, which James also mentioned. Sure enough, actually, a highly statistically significant and a modestly strong effect in predicting the rate of change of FEV_1 in the ECLIPSE population, a relationship you would not see with the FEV_1, which would include obviously more patients than just those with emphysema.

Next slide, please.

Now, biomarkers can also provide important insight, and so these are data from the COPD Biomarker Qualification Consortium, which is pooled data from a variety of observational and interventional clinical trials in order to submit biomarkers for qualification for drug studies with the FDA. The first biomarker that was so qualified was plasma fibrinogen, which last year was qualified as a biomarker for stratifying patients at risk or exacerbations for either severe or moderate exacerbations.

Could we go back to the previous slide, please? Thank you.

Or -- and as shown in this slide, for mortality. And so what you can see here is the patients were divided into two groups of high and low fibrinogen, and the mortality is considerably higher in COPD patients with high in fibrinogen. So as we commented earlier, fibrinogen is a biomarker that provides some insight for cardiac disease patients; it does for COPD patients, as well, perhaps because of shared pathophysiologic mechanisms.

Next slide, please.

So to summarize at this point, biomarkers that can be assessed for COPD can be related not only to disease severity but importantly with disease progression. I showed some data with regard to FEV_1 and emphysema. Mortality is a kind of progression, and I didn't show the data with respect to exacerbations, but a number of biomarkers will predict the risk of future exacerbations. Now, progression of disease, however, that is progression of the existing COPD, is not really the same thing as assessing the risk of getting the disease if you're well. And I suspect that in the interest of regulating tobacco products, it's that group that will attract a lot of interest; that is, if somebody isn't sick, will they get sick, or what is their risk of getting sick if they use one kind of

product or another?

And so this really means is when do we want to measure the harm? Do we want to measure it in healthy people who are exposed, and I think we certainly do, and then we really want to measure it in comparison to something. We want to compare it, for example, to cigarettes. And so the question is if people use one product versus another, are risks mitigated? And these are two rather different questions, and does the biomarker predict either disease severity or progression of an established disease?

Next slide, please.

So we're trying to get a handle on this. I'm presenting data from an interventional study using a nicotine inhaler. What was done to -- in rural individuals who were willing to try to reduce their smoking but who were uninterested in quitting. They then used a nicotine inhaler with the target of trying to reduce their smoking by 50%. We then measured a number of different biomarkers, six of which are shown here, and also -- states several things, one of which is the importance of having control groups in clinical trials.

And what you can see is that people that did not achieve reduction are shown in -- and they reduced their number of

cigarettes by less than 50% are in yellow, and the people who reduced by more than 50% are in blue. After a couple of white blood counts, total lipocytes, which is a risk factor, it was down in both groups.

Now, why it went down is, of course, a matter of -- we don't understand, but it could be due to something else that happened in the clinical trial, but there was certainly no effect from reduction, at least by this analysis, in that biomarker. Now, for HDL, the results are rather different. There was a numerical increase in HDL in the people who did not achieve reduction, but there was a much more marked increase in the people who did achieve reduction, although they weren't exactly the same by randomization. But the difference in their changes is statistically significant. Similarly, there was no change in CRP in the people who did not achieve 50% reduction. There was a reduction in CRP in the people who reduced by 50% even over just a 4-month time frame, and that difference was statistically significant. And you can see that for fibrinogen and LDL, while there were changes with time, there were no differences between the reducers and the non-reducers.

Next slide, please.

So what this suggests, I think has been -- there are

multiple lung diseases. They relate to different features within the lung. They will undoubtedly have different pathobiology, and even what we classify as a single disease, at least nominally, like COPD, will be multiple sub-diseases.

We can assess disease risk with biomarkers, and we can assess disease progression, although these are not really diagnostics; they'll be useful in populations but not in individuals, at least not in any of the biologic, not any of the biomarkers that we have to date. But before I conclude, I'd like to make just some other points about biomarkers going forward. I think we'll expand a bit on what's been said before.

Next slide, please.

So as has been suggested, as I showed earlier, there's a lot of overlap in biomarkers measuring across groups. So these are data again from ECLIPSE in a paper -- showing the data for six blood biomarkers: white cell, CRP, interleukin-6, TNF-alpha, fibrinogen, and interleukin-8, and you can see that there are lot of significant differences between COPD patient smokers and nonsmokers.

Within these box and whisker plots, you can see that there's tremendous overlap across these populations, and the

statistical significance only comes because the populations are very, very large. As individual biomarkers go, none of these showed a very strong prediction with any of the specific parameters that we were interested in.

The next slide, please.

In order to try to refine that analysis, we did several things, and I'll show just two of them. First, what we did was we tried to make a score, if you will, of inflammation, with not just one biomarker, but if an individual had more than one biomarker, that is two or more biomarkers, we called that inflamed. And at the time of recruitment, we can see on the red bar on the left, that about 28% of the patients had two or more of those six biomarkers; it didn't matter which of those two were elevated. You can see that about 40% -- 43% actually had no elevated biomarkers. We then looked at those individuals. There was no strong association of those groups with outcomes at recruitment. So we followed them over the next year, and we repeated the analyses.

You can see that the people that had two biomarkers elevated, there were 28% of them, more than half of that group had two biomarkers that were elevated 1 year later. They weren't always the same two biomarkers, but they qualified for

having inflammation after a year, and we called those persistently inflamed individuals, and there were 16% of those.

Conversely, of the 43% that had no biomarkers, 70% of them still had no biomarkers, and so there were 30% of the COPD patients in ECLIPSE were persistently non-inflamed. Then when we looked at a variety of outcomes, we saw significant relationships, and I will just summarize, too, from all of our papers.

If you can go to the next slide, please. Next, please.

The exacerbation rate -- well, now we got both of them, but the exacerbation in white is one and a half exacerbations per year in those individuals who were persistently inflamed and only 0.9 per year in those that were persistently not inflamed. And the mortality was 13% versus 2% in those two groups, now a truly striking difference, much larger than we saw with the use of any single biomarker, suggesting that biomarkers used together, in fact, can provide tremendously improved prognostic information.

In addition, the relationship among the biomarkers may also be important. And the next slide summarizes the relationship among these six biomarkers for the groups included in ECLIPSE. Now, each of the balls represents one of the

biomarkers. The size of the ball indicates the number of patients that have elevations in those biomarkers. And then the size of the line indicates the strength of the associations between the biomarkers within a group.

And so you can see that for the normal smokers, there was a lot of the red, the black, and the yellow, but fairly weak association, whereas for the COPD patients, there's now a lot of the blue and a lot of the purple, and now we see very strong associations. The purple and the red, for example, are strongly associated, whereas the red and the yellow are very weakly associated. And actually what may be one of the stronger associations in the normal smokers? Well, I think that this creates a hypothesis that the relationship among the biomarkers has been narrowed, as well as their persistence over time may be a key feature in helping to sort out the biological significance of this type of biomarker.

And the next slide, then.

To summarize and to conclude, there's no doubt that measurement of biologic biomarkers provides useful information. They provide information, at least in groups, about prognosis, but they're generally weak predictors. They can reflect the effects of intervention, but these will raise clinical study

issues.

And I think that when prospective studies are done, they're going to have to have very careful clinical study design as we would do for any other clinical intervention study. Also, this is an extremely rapidly advancing field. The number of measures are increasing probably faster than exponentially; tomorrow we'll hear about the application of omics technology and other kinds of multiplex methods increase the number of measures we can make, from a few to hundreds or even thousands or tens of thousands. And I think that we'll not only have increased complexity by the number of measures that we get, but we need to understand their interactions, their interactions over time and their interactions among each other.

I have no doubt that this will advance our understanding of the various diseases that are caused by smoking. Understanding the pathophysiological mechanisms, of course, will provide some validation for the biomarkers when they're used. But I have no doubt that including these in studies assessing harm will be instructive.

Thank you very much.

DR. DRESLER: Thank you, Steve. And if you want to go on

mute, we will have a break, and then we'll have two speakers, and then we'll have the session, the panel session. So we will be coming back to you. Okay? All right.

So it is time for a break. We're scheduled for a 10-minute break. We are a few minutes behind, but let's still take 10 minutes break, and so let's be back here at 25 after, please. Thank you.

(Off the record at 3:16 p.m.)

(On the record at 3:29 p.m.)

DR. DRESLER: So it looks like people will still be wandering back in. So we have two more speakers, then we'll have the panel.

The next speaker is Dr. Robert Tarran from the University of North Carolina at Chapel Hill, the UNC School of Medicine and the TCORS program there. His topic will be Tobacco Exposure-Induced Biomarkers of Harm in Human Airway Epithelia.

Dr. Tarran.

DR. TARRAN: Okay, so thanks for giving me the chance to talk today. I'm very happy to do that and very glad to be here.

So here are my disclosures real quick. I'm going to start with my acknowledgements essentially. So I'm basically

representing the UNC School of Medicine TCORS. This is the title of our TCORS. We're very focused on the lung in my lab, airways -- but we do look at other cell types. So just to say. And I want to thank people for slides. So Carla, who works with me, gave me some slides. Mehmet and David Hill, Ilona Jaspers. And then also Neil Alexis, who runs the sample acquisition cohort. So these slides are from a bunch of different PIs.

So there's already been some great introductions on COPD, so I think we can go through this pretty quickly. COPD is important. It kills a lot of people. A major risk factor is tobacco smoke exposure. So I'm going to look at the -- so that's why we got interested in COPD as opposed to other types of disease.

So the lung and the airways or the respiratory system are the first point of contact for inhaled tobacco smoke. And for those of you who are from cardiovascular disease, here is a representation of the lung. It's the first point of exposure. So broadly speaking, the respiratory system can be divided into the upper airways, and we'll talk about those separately, and also the lower airways. And this will come up later.

So in my lab and others, we like to think about the airway

epithelia, so typically ciliated cells, shown here; also goblet cells, and they secrete mucins and mucus. They have cilia, which will clear the mucus. They also play a role in the immune response. So we have the epithelia, which acts as a physical barrier. They're basically a woven moist net down to the alveolar region for gas exchange. They can secrete cytokines. You know, they'll clear inhaled pathogens, clear inhaled particles, you know, clear cigarette smoke. And there's a lot of things in the A cell, as we'll talk about.

So people like myself who like to look at fluid transport, we think of the airways very clearly. So you have these submucosal glands, which can secrete fluid and mucus. And as I mentioned, there's goblet cells and cilia, which can produce mucus and kind of clear it. And so mucus clearance is going on in your lung all the time. So stuff is being secreted, it comes up the airways, and you swallow it. This is an important part of the natural innate defense mechanism.

Other people in our group like to think about things a little differently. So, for example, the biochemists think about these meshes of proteins which are present in the mucus. And so there's typically about a thousand proteins which are present in airway surface liquid.

We're starting to find -- Mehmet Kesimer and his people are starting to find mucus interactions and mucus proteomics. And I'll hope to convince you that these proteomics are very important for lung health, also potentially important for biomarkers of harm.

Also for mucins. I mean, having different types of mucins expressed in different places. So there are glandular mucins, mucins actually on the cilia, and secreted mucins. Again, they're all important for facilitating this clearance. And this can become deranged in many diseases such as COPD, also cystic fibrosis.

So we've already heard good definitions with the different COPD phenotypes, so I don't have to go too much over this. But I want to stress that I am really focusing on the chronic bronchitis phenotype, which this is a from a paper from Jim Hogg and who showed there's really mucus accumulation in the small airways. And this really has been shown -- the mucus accumulation has been shown to be a very good predictor of death. So we think it's important, so that's why we like to study it.

So like I've already said, the cells we study, the epithelia or the ciliated cells and the goblet cells, also the

alveolar macrophages. And if you think about early biomarkers of harm, so when a healthy person first starts smoking, these are probably two of the major cell types which could be driving the phenotype and are good major sources for biomarkers. And then as you start to develop lung disease, you're going to get, for chemotactic factors, other cell types becoming more important, such as neutrophils. So we already heard there typically aren't very many neutrophils in a normal lung. In a COPD lung with chronic neutrophilia, neutrophils go up. So if you're studying kind of like healthy smokers, maybe neutrophilia is less important than if you're studying biomarkers in COPD patients; neutrophilia may become more of a big deal.

So the overview of my talk. So I'm going to talk about biomarkers of harm in the upper airways, from the nasal regions. Again, in the tracheobronchial regions. We'll talk about sampling various leukocytes such as alveolar macrophages. And then we'll look at some in vitro corollaries of these.

So, first of all, nasal epithelia. So the nose is actually quite a good site for obtaining biomarkers. So, you know, the nose has the olfactory parts of the nose, but it's also other parts of the nose are lined ciliated cells, have

glands, have mucus-producing cells such as the lower airways. So it's representative. You know, it's very easy and very accessible with minimal training, so you can get samples from the nose without needing a big bronchoscopy suite or a CT scanner. So it works well in that way. And it's also relatively noninvasive, so you can do this on people of a variety of ages, a variety of diseases. And it's a target for inhaled agents such as toxins and pathogens, and it's been generally thought as being a decent model for studying exposure.

So just to show, here's a couple of pictures. I mean, if you breathe in tobacco smoke, you can breathe it out through the mouth or you can actually breathe it out through the nose. And so for most smokers, the nose does get quite a large dose of exposure.

So one of the things we like to study is CFTR. So this is the cystic fibrosis transmembrane conductance regulator. It's a chloride channel that's expressed in the epithelia in the nose, in the airways, and other cell types in the body. So you can say this is already a validated biomarker.

So mutations in CFTR cause cystic fibrosis. So deletion of phenylalanine here causes very severe lung disease. So we

already have this validation that CFTR is interesting. Over the last few years, it's become known that tobacco exposure actually inhibits CFTR function. So it's -- whereas with CF, it typically tends to be very severe, causing very acute lung disease. You get decent CFTR inhibition with tobacco exposure; so then potentially inhibition of CFTR can be used as a biomarker of harm.

It's also been known that Garry Cutting at Johns Hopkins showed that for CF patients who have mild mutations, which still have some residual function, for those patients, growing up in a smoking environment actually worsened their lung disease, whereas people with severe CFTR mutations growing up in a smoking environment had no additional effects, suggesting that any residual CFTR function you have is worth keeping, and kind of losing it makes it worse.

So in the airway epithelia, fluid balance is really important for keeping mucus moving. So you typically have chloride moving out of the cell through CFTR. So if you've got a runny nose and acute rhinitis, that's acute activation of CFTR generating fluid secretion into your lung. And this is balanced typically by setting absorption in the other direction. We can sometimes think of this as driving with your

foot on the accelerator and the brake; you get fluid moving into the lung and fluid moving out at the same time, and this allows for the fine tuning of the liquid balance. A normal person typically has about a teaspoon of salty water that lines the entire airways, which is maintained by these ion channels. And then we think with tobacco exposure you get less CFTR. When you get continued fluid absorption, you tend to get this dehydration, and we'll show you some data for this in a moment.

So one of the first things we did was use the nasal PD technique. So we have an exploring electrode. This is noninvasive, and it's placed under the turbinates in the nose, it's hooked up to a voltmeter, and you have a reference electrode to the skin. So then when CFTR gets activated, you get chloride ions, which are negatively charged, moving into the nose lumen, and you can pick up that change as a voltage on the voltmeter.

And this is some of the data here. So if we had -- so if you measure baseline PDs when you're first in with the electrode -- and then we can measure the amiloride. So amiloride is a drug that's approved to add to the nose. So to measure the change in voltage or PD with amiloride is an indicator of ENaC. And then we'll add isoproterenol to

raise cyclic AMP and activate CFTR.

And so between healthy nonsmokers -- so between healthy controls or healthy smokers, there was no difference in ENaC activity. But you can see that CFTR is down a lot, and these smokers had about 10 pack-years of smoking history but no major lung problems, suggesting that CFTR can be used as a marker of harm, because typically most people would say that lacking CFTR is predictive of impending lung disease.

So we also got IRB approval, at this time, to actually do acute -- so this is chronic cigarette smoke exposure. We also got IRB approval to do acute cigarette smoke exposure. This comes with pictures of one of the first test subjects. So what we had the person do is blow through a resistor, and this closes the soft palette, and it's previously been shown that if you do that, having smoked through the nose, the smoke gets down to the lungs. So that was a safety feature.

And so we have these -- they were called olives, these nasal olives, and the person is putting smoke, generated using ISO standards, through the nose. And then we did 10 puffs of smoke exposure and then measured the nasal PD using the voltmeter setup. And so measuring nasal PD is a very quick thing to do.

So here are some of the actual data. So this is the baseline PD. If we add amiloride, you get a decrease in voltage. And then here we have the isoproterenol to activate CFTR, and you get this hyperpolarization as the chloride ions go through CFTR into the lumen and the voltage electrode senses that. And you can see that just this is the same subject before and after just 10 puffs of cigarette smoke, and you can see that the CFTR voltage actually decreased very quickly after smoke.

This is some of the mean data, so I'll draw your attention down here to the control data. So like there's a basal CFTR response, and these basal responses actually match quite nicely the historical averages. And then just a little bit of cigarette smoke, so 10 puffs, and smoke went down.

And we measured at about 3 hours later, and you actually do see a recovery. It was not quite fully to baseline. So it suggests that a little bit of smoke will inhibit CFTR; a lot of smoke will inhibit it more. And then the recovery times. Some data suggests that people with COPD who have given up smoking, they don't get CFTR function back.

I should also point out that this nasal PD technique is actually quite widely used around the country. For example,

the CF Foundation have actually set up a network of trained operators to do it. So it's very reproducible from lab to lab, and it's actually fairly inexpensive to set up, so as a potential biomarker of exposure, it can be done. Other labs, if you go in for a bronchoscope, you can actually measure it in the lower airways, so in the tracheobronchial region. And people have looked into smokers, COPD is in the lower airways, and see a similar thing, this inhibition of CFTR.

So you can also acquire samples from the nose. So rather than just measure with electrode, you can do these nasal biopsies, and these are done -- you can buy these little kind of plastic -- just to kind of scrape the nose, and you can use that to do a lot of samples.

Ilona Jaspers, as part of our TCORS, has been doing gene arrays and DNA methylation arrays. We've also been doing proteomics and looking at the nasal lavage.

This is an example from Ilona's work looking at DNA methylation in the nasal biopsies from the nose. So I'll start with something very specific. Now you can see something that's very broad. And really what she's seeing is there's quite a difference in gene methylation between smokers and nonsmokers, which is shown here.

So in addition to doing more specific techniques, we can also go into the nose and do kind of like larger data techniques and see differences. And as well as doing DNA methylation, we're also doing gene arrays, like I said, and proteomics.

So as an example of the proteomics, Rebecca Fry and Ilona Jaspers have been looking at the effects of e-cig smoking on gene arrays in the nose. Rather than do a full gene array, they used NanoString technology, which is you pick like a panel of genes, so it's a little quicker.

So, for example, what they found is when they compared differentially expressed genes in just regular tobacco smokers versus e-cig users -- they actually compared to nonsmoker controls -- they actually found more genes would change in e-cig smokers than in smokers. And most of these changes were actually due to down-regulation, so which were indicative of immunosuppression, so again suggesting that you can get very interesting biomarkers from the nose. And like I said, a lot of these genes were unique to e-cigarette users.

So I'm not going to go into this, but you can also -- as you start to collect these genes which have changed, you can then start to do pathway analysis. So, you know, if one gene

changes, you're like, well, is it important? But you can see a whole bunch of genes, and the categories start to change. You can then start to make larger inferences.

Now I'll talk about the lower airways. So I've mentioned that if CFTR is inhibited, this tends to dehydrate the airways. So one of the other things that we've noticed is when CFTR isn't present, mucins actually fail to expand into their like normal function. So as well as having dehydrated mucins, you get mucins that are kind of just abnormally formed.

And this is data from Mehmet Kesimer's lab, so where he does EMs on mucins. So mucins are actually the biggest proteins in the body. One mucin could be like a micron in length because they're just so big. And so this is a scanning EM of kind of one mucin or a series of mucins down here, and you can see that if you inhibit CFTR -- and here he pharmacologically inhibits it -- these mucins fail to unfold, and they stay very, kind of, grouped up. So this is like kind of a functional consequence of there being, you know, lack of CFTR activity. So he's actually trying to go into the secretions.

So in addition to doing nasal secretions, we're doing induced sputum and also bronchoalveolar lavage using

bronchoscopies. And so we can actually get the samples and study the mucin characteristics. So, for example, if you see a failure for mucins to unfold after tobacco exposure or any other type of product, you'd say that's a potential biomarker of harm because those mucins, they can't be transported normally.

Additionally, our group and others have found that the mucus dehydration correlates quite well with disease exposure. So normally mucus is about 2% solid, so it's 98% water and 2% solid, which is 0.9% saline, approximately like saline chloride, then a whole bunch of these proteins, including mucins. And any dehydration tends to impair mucus clearance. So, for example, COPD patients, the mucus -- so they can puff up to about 6% solids. In CF, it's about 10%. And smokers are typically somewhere between normal and COPD.

Our group and others have also shown that the degree of mucus dehydration also correlates adversely with the degree of mucus clearance. So the thicker the mucus, the harder it gets out of the lung, which sounds obvious, but it's nice to show it.

This is some data from Wayne Anderson's recent paper in a Blue journal, showing that as mucus gets more dehydrated, it

doesn't clear as well.

So as we start to collect samples from the lower airways, such as the mucus, as well as looking at the biochemistry of the mucus, we can also look at its biophysical properties. So in my mind, it's like if smoking or other diseases actually cause mucus dehydration, that, to me, would be a biomarker of harm because you know that mucus is going to sit around and want to form plugs in the lower airways rather than actually get cleared.

Also, Wayne Anderson recently showed -- and this is data they generated from a previous NIH-funded double C score grant. The mucus dehydration actually correlates with a decrease in FEV_1 in COPD patients. So the more mucus dehydration you have, probably more mucus is going to sit and plug your airways. And so the more -- you know, the lower decline in FEV_1.

Well, I will actually agree with the previous speakers that FEV_1 is not a great marker; it's like a marker of -- I think of it as more of a marker of the large airways. And the incidence of disease starts in the smaller airways and like mucus plugging starts much, much earlier on. I think FEV_1 isn't sensitive enough to pick up none of the early disease.

So why? Because the COPD, I think for, you know,

assessing exposure of harm in healthy smokers, I don't think FEV_1 is good enough. So probably since the spirometry isn't sensitive enough, I think imaging probably is the way to go if you want a whole human corollary of the biochemistry.

So like I've mentioned, we've also been doing a lot of proteomics. So we're doing this both on the nose, the lower airways, on sputum BAL, and also other samples. And we can also do it in vitro. But we take these samples, and we reduce them and alkalize them. We do a trypsin digest, and then we send the tryptic peptides through the mass spec and look for coverage.

This is some data we have from the TCORS, which is ongoing, where we've compared -- so these are all healthy smokers using from different tobacco groups, and we looked at the proteomic change. So we've identified about a thousand proteins and then the ones that have good peptide coverage where we can pick up multiple sequences to be analyzed. And between groups of different types of tobacco product, we can actually start to see different proteomic profiles of the -- you know, several hundred proteins we see in the sputum. You know, different proteins have changed in different groups. And then we can start to interpret that to see what these proteins

do.

So, for example, if -- you know, the ASL and the sputum contains a whole bunch of proteins and peptides which are involved in bacterial killing. So if all of those proteins were suppressed after a hypothetical tobacco exposure, then you'd probably predict that that was bad.

So since we do get into sputum and bronchoalveolar lavage, we kind of also look for leukocytes. And typically, there's dendritic cells, neutrophils, monocytes and macrophages, and lymphocytes.

Oh, just as an example, Neil Alexis has been looking at the relative amount of M1 macrophages, which are pro-inflammatory, and M2 macrophages, which are anti-inflammatory, between different smoking groups. And again, this is based on the samples from healthy smokers of different tobacco products that were collected for TCORS.

So nonsmokers are shown here on the right, and they typically have the anti-inflammatory M2-type macrophages, which just kind of hang out in the lung as kind of sentinel cells, if you will. And you can see that in the different smoking groups, we're seeing a change in the distribution as more macrophages come in. And again, this is a good marker of, you

know, the potential for harm.

The other thing we can do with these macrophages is looking at alteration in biochemical pathways. So if you get a lot of information, typically the unfolded protein responses can be up-regulated, so your cells start making more protein. The ER starts getting crowded with more protein, and you get this unfolded response, which can have a lot of effect. It can drive -- you know, excessive UPR can drive cells towards apoptosis and other things.

There's a whole bunch of genes involved in regulating this UPR, such as increased spliced X-box protein 1, initiation of ATF-alpha and CHOP, et cetera, et cetera. So we can look for those.

And here we can see, compared to nonsmokers and smokers of different tobacco products, mRNA from different UPR proteins are shown to be up-regulated, again suggesting that you can pick these, you know, for inflammation cells, so the M2 macrophages, and then pick different changes. We're planning on going back and doing gene array analyses on these samples as well.

So just finally, so in vitro corollaries with this. So we've been using the BAT design, the tobacco smoke exposure

system. And then you can actually hook this up to a cigarette smoking machine to do kind of formal smoking, and we found this actually works. Well, we tried a few different systems, and I'm going to say I'm a big fan of it.

So we take the primary airway epithelia from a lung. So when we get lungs which aren't suitable for a lung transplant, for whatever reason, we can take and grow the cells for months. So they become nicely ciliated. So they have ciliated cells and goblet cells, and we place them in this culture system.

And then we can expose them to smoke using ISO standard or the Montreal TEMS standard. And we can do this with a whole bunch of different tobacco products and measure a whole bunch of parameters in vitro.

I think what's really important about the system is the way they designed it is they have media flowing on the serosal side. So the airway epithelia, I think, have very interesting properties. So they polarize. So the apical, the air-facing side, has different chemicals, different proteins, different properties, to the blood-facing side.

And the lumen-facing side is actually quite tough. You can get into whole different chemicals, and they'll see those chemicals, and they'll respond with changes in inflammation or

changes in innate defense, but they don't die, whereas the same concentrations of chemicals in the blood-facing side will kill the cells. And I think that's something that has been recognized by some groups but not by all groups. So doing these exposures, having media flow on the blood side is really important just to stop -- the metabolites get -- you know, building up.

And so using the system, we can get a bunch of measures. So here, so this red is confocal microscopy of the fluid layer above the airway surface. So we mentioned in vivo, the mucus is dehydrated, and that dehydration can be seen as a decrease in that fluid layer.

So we found that again, with using 10 puffs of smoke -- I should mention that normal airway epithelia can actually sense the volume of this fluid layer, and they can actually add fluid back or take it away as needed just to maintain that fluid. And we've shown here that over 24 hours, these cultures maintained the 7 micron liquid height. And then we found that just 10 puffs of smoke, which turns off CFTR, you then see there's some dehydration, which takes a few hours to recover.

So this is shown after acute exposure, but using this in vitro system, we can actually expose the cells daily for 2 or 3

weeks, if you want to, with different types of tobacco products. We've had good luck hooking this up to regular cigarettes, little cigars, e-cigarettes.

So we can expose these human cultures in vitro to a broad range of tobacco products and then see how it matches up to what we're seeing in vivo. And the hope is, you know, in vivo, sometimes we're limited, like we can only test a few tobacco products, whereas in vitro we can then expand this and test a wide range of tobacco products using this system.

So just for example, so here is Kentucky control cigarettes or three other types of tobacco product, which are kind of real tobacco products, which are not regulated that people smoke, and you can see that we see increased cytotoxicity using the same doses compared to Kentucky cigarettes. And the same thing to IL-8 is the pro-inflammatory cytokine that induces neutrophil influx into the lung. The cells, using these same three tobacco products, produce more IL-8 than Kentucky cigarettes would. So using the system, we can actually do, kind of, in vitro dosimetry and potential biomarkers of harm.

We also took the airway surface liquid from these cultures and Mehmet did proteomics on it, again using Kentucky or the

other four different types of tobacco product. And so this is the -- these are all a list of proteins, and you can see there's different expression in Kentucky versus some of the other tobacco products, again suggesting they're both very specifically using things like IL-8, cytotoxicity, ASL HdG you can find changes. But then when you go broad and start doing proteomics, you can also use in vitro to see some of the changes, which is also obviously in vivo.

So summary. So direct functional measures (e.g. mucus rheology, ion transport) as well as broader measures such as gene arrays and proteomics can be used as biomarkers of harm, I think, both in vivo and in vitro. And I think that the airways, the in vivo and the in vitro, do seem to match up, and I think this is good news because it means we can start to test a lot of -- more products in vitro than we can in vivo.

For biomarkers related to COPD, the relationship between the in vivo -- actually, I'm just reading what I just said, aren't I? I'll stop.

So I think genomics and proteomics show promise for determining potential biomarker of harm in new and emerging tobacco products. But I think the approach for our TCORS is going to be, especially for the reading of things like

e-cigarettes where we don't know what they do, is to have genomic and proteomic approaches, you know, find a bunch of things and then go back in and start to look for specific biomarkers to come and validate that.

So if you see like 50 genes are different e-cigarette users, we don't necessarily know what that means. But then you could start to do certain things and test it.

So one thing Ilona is doing is, she found the gene signature was one of immunosuppression, but she has IRB approval to -- so the live attenuated influenza virus, the FluMist, which is a virus which is replication deficient, you can add it to the nose and look at the degree of infection. So one thing she does is, in smokers, she basically gives them the FluMist kind of flu shot and looks at how much infection there is. And typically, with smokers, you get better infection of the virus in the nose than you would do in nonsmokers. I mean, this is a product that's available out there. It's not bad; it's just giving the same flu shot everybody else gets.

So, for example, with the e-cig patients, since they have that potential signature of immunosuppression, you can then go in with the FluMist and see if you actually do get increased viral infectability.

So I'll stop there. Thanks.

(Applause.)

DR. DRESLER: Thank you.

Our next speaker is Dr. Erika Torjun --

DR. TORJUSEN: Torjusen.

DR. DRESLER: I practiced, too. Thank you. From the FDA Center for Drug Evaluation and Research. She will be speaking on Forced Expiratory Volume in 1 Second: a Multifunctional Biomarker of Lung Function.

DR. TORJUSEN: Good afternoon. My name is Erika Torjusen. I am an allergist/immunologist and a medical officer in the Division of Pulmonary Allergy and Rheumatology Products at the FDA. Thank you for inviting me to speak here today.

The issue of endpoints for the development of a tobacco product is complex, which is why this workshop was designed. The purpose of my presentation is to review how my division in CDER uses the biomarker and surrogate endpoint, forced expiratory volume in 1 second, for regulatory purposes in the context of pulmonary drug development.

I have no conflicts of interest to disclose, and all opinions expressed in this presentation are my own. The materials presented are available in the public domain.

During this presentation, I will provide a brief overview of spirometry and the measurement of forced expiratory volume in 1 second, or FEV_1. I will then discuss how FEV_1 is a relevant and clinically important endpoint to evaluate both acute and chronic changes in pulmonary function. I will close with a discussion of the pros and cons of utilizing FEV_1 as an endpoint in clinical studies.

Spirometry is a simple test performed to assess lung function. Lungs are elastic and can inhale and exhale a certain volume of air at a rate appropriate for a subject's age and sex. Spirometry measures the volume and flow rate of gas breathed in and out of the lungs under the specific condition of maximal effort. This information is compared against established criterion standards which can aid in the diagnosis of specific lung disorders, such as asthma or COPD, and can be used to assess the response to treatment.

While full pulmonary function tests measure multiple lung volumes and flows, noted graphically above, spirometry specifically focuses on the forced vital capacity, denoted by the red oval. The forced vital capacity is the maximum volume of air that can be exhaled from the lungs after maximal inhalation.

Spirometry is a well-standardized and reproducible test in adequately coached subjects, with consensus documents and protocols published jointly by the American Thoracic Society and European Respiratory Society.

FEV_1 is one of the measurements obtained from spirometry. It is the volume of air that can be forced out in 1 second after maximal inhalation, called forced exhalation. FEV_1 is the measure of pulmonary function that may reflect the extent of airway obstruction and is considered an important measurement of lung health.

The graphs on this slide show time on the x-axis and lung volume on the y-axis. As seen in the graph on the left, most of the forced vital capacity, or FVC, can be forcibly exhaled in 1 second. The graph on the right shows how FEV_1 is affected by varying degrees of obstruction. FEV_1 is denoted by the intercept of the dashed lines for patients with normal lung function on the top curve, moderate obstruction in the middle curve, and severe obstruction on the bottom curve. It shows that as the degree of obstruction worsens, the amount of air forcibly exhaled in 1 second decreases.

I will now discuss the use of FEV_1 to measure acute and chronic changes in lung function.

FEV_1 is a biomarker, a characteristic that is measured as an indicator of normal biologic processes, pathogenic processes, or responses to an exposure or intervention, including therapeutic interventions.

Changes in FEV_1 have been shown to correlate with clinical characteristics, such as how a patient functions, feels, or survives that allow it to be used as an important clinical endpoint for regulatory purposes.

As such, it is a validated biomarker used as a surrogate endpoint, meaning that it is a measurement or physical sign that is used as a substitute for a clinically meaningful endpoint.

FEV_1 can be used to measure acute changes in lung function, such as an increase in FEV_1 in response to an inhaled bronchodilator. Bronchodilator drugs, such as beta-2 agonists and antimuscarinics, improve airflow obstruction and are beneficial in patients with asthma and COPD. A 10 to 15% increase in FEV_1 enables patients to breathe easier and feel better.

FEV_1 can also be used to test for airway hyperreactivity after inhalation of bronchoconstrictor agents such as mannitol and methacholine chloride. An acute decrease of 15 to 20% in

FEV_1 is indicative of airway hyperreactivity.

FEV_1 can also be used to measure chronic changes in lung function. The Environmental Protection Agency, or EPA, has used FEV_1 as a tool to help determine the health risk associated with environmental ozone exposure. The EPA has used modeling to estimate the percent of the population experiencing 10, 15, and 20% decrements in FEV_1 based on exposure levels to ozone.

Chronic changes in FEV_1 can also be used to assess disease progression, which may be the most relevant application of FEV_1 to our discussion today. FEV_1 can be used to assess if a specific intervention, such as a drug, is able to alter disease progression of a pulmonary disease such as COPD. Repeated measurements of FEV_1 over time could be used to demonstrate that the FEV_1 decline slopes will diverge, showing that airflow is better preserved in one group compared to another.

The change in FEV_1 over time is a clinically meaningful endpoint in obstructive lung disease. As seen on this slide, decreasing FEV_1 is associated with increased exacerbations, hospitalizations, and mortality in COPD patients, a smoking-related pulmonary disease.

This slide shows the natural history of lung function

decline. Years are on the x-axis and FEV_1 expresses the percent of value at age 25 on the y-axis. This is commonly referred to as the Fletcher curve and is adapted from the article published by Fletcher and Peto in 1977. The data was obtained in a prospective epidemiological study conducted in 1961, from a random sample of men age 30 to 59 working in West London. Subjects were followed and evaluated with spirometry every 6 months for 8 years. The final sample contributing data to the analysis included 792 subjects.

This slide illustrates a few key points. The first point is that lung function declines naturally with age. After age 25, lung function declines in nonsmokers an average of 20 to 40 mL per year. This is shown on the green curve at the top of the graph.

The rate of decline is more precipitous in regular smokers, who are susceptible to its effects, denoted by the red curve at the bottom of the graph.

Interestingly, susceptible smokers who quit demonstrate a slope of decline in FEV_1 that closely approximates a nonsmoker, even in advanced disease. This is demonstrated by the blue and yellow curves in the middle of the graph.

Finally, you can see in this figure that changes in FEV_1

occur gradually over time and that changes are less pronounced in healthy younger subjects.

As a follow-up to the Fletcher curve on the previous slide, it is clear that studies intended to assess disease alteration may be quite long. Multiple variables can affect the study duration for this type of study, such as the magnitude of effect. The largest effect one could observe may be approximated with the FEV_1 decline curves for smokers and nonsmokers, seen on the previous slide in the Fletcher curve. The smaller the magnitude of effect, the longer the study would need to be to detect a difference.

The size of the population study. The larger the population, the easier it would be to detect a difference. Accordingly, a small sample size would require a longer study.

There are also patient characteristics to consider, such as disease status and severity. Healthy subjects have a slow rate of decline in $FEV_{1,}$ and as such, this population would require a longer study duration to detect a difference.

Age is another important characteristic. Patients younger than 25 do not experience the same annual rate of decline in lung function, and this would also need to be considered in the study design.

I will close with a discussion of the pros and cons of using FEV_1 as an endpoint in a clinical trial.

The pros for using FEV_1 as an endpoint in a clinical study are that it is consistent and yields reproducible results; spirometry is readily available and accessible; FEV_1 is an established endpoint in obstructive lung disease; and FEV_1 decline is related to COPD disease status severity, morbidity, and mortality.

The most significant con for using FEV_1 as a clinical endpoint is the time required to detect a difference. Studies meant to assess for disease alteration may require many years depending on the study design, effectiveness of intervention, size of the study, and patient characteristics.

Thank you. This concludes my presentation.

(Applause.)

DR. DRESLER: Could I ask the speakers to come up? And we're working on getting Steve back on the phone. We'll ask Steve the hard question right off.

(Laughter.)

DR. DRESLER: Steve, you can hear us in the room, and I understand you've got the mechanism for how to speak to us.

So the first question I have --

DR. RENNARD: I think so. Can you hear me?

DR. DRESLER: Yes, we can hear you well. Thank you. And Steve, I promise that I will periodically turn to ask you a question. So the first question is to Dr. Tarran, though. In order to predict in vivo effect, have you tried to model in vitro/in vivo correlations for any biomarker that you have studied in vitro? So have you tried to model in vitro/in vivo correlations for any biomarker that you have studied in vitro?

DR. TARRAN: So by model, do they mean what? Mathematical modeling or predictive modeling or just comparisons?

DR. DRESLER: I'm sorry, the question didn't specify that.

DR. TARRAN: Yes, I figured they didn't.

DR. DRESLER: In order to predict in vivo effect.

DR. TARRAN: So no, we -- I guess for CFTR and mucus -- ASL dehydration, we've made, we've looked quite a lot, you know, at the comparisons. So yes, we have. And I think it's generally -- we've also looked at other disease, you know, diseases. So, for example, with cystic fibrosis, the less CFTR function you have, the quicker you acquire lung disease. So you could make the assumption that the less CFTR functioning you have after tobacco exposure, you know, the worse lung disease you're going to get.

DR. DRESLER: Okay. So the question that I have for you is that I heard some discrepancy in opinion about FEV_1 and when to use it. So opinions on that.

Dr. Crapo, did you want to start?

DR. CRAPO: I'll start because I think that FEV_1 is a key parameter to use. You noticed that I started with a COPDGene cohort. We first characterized everybody by FEV_1. It's the standard GOLD criteria. And I think you need to know that, and it's one of the key parameters we use for all of our risk analysis to get the emphysema and the airway disease access. But it's not -- it's very good for reactive airway disease, like if you're taking a bronchodilator and you've got bronchoconstriction, it will measure a change in a matter of seconds. But for chronic lung disease, if it was progressing, it's neither very sensitive for change nor very specific. And that's one of the cons that was brought up; it takes a long time, years to do the study. Maybe decades. And it's not very sensitive. My experience is that if you use population statistics to measure people, you'll miss half the cases of COPD by using FEV_1 as your only criteria for diagnosing.

And I don't think we should use -- well, we have to use population statistics, but in reality, the best way to use it

would be to follow some of its own PFTs so you can see their change. But unfortunately, we don't usually have PFTs before the disease starts.

DR. BARR: Yeah, I would agree. I mean, it's an incredibly important sort of GOLD standard test that is going to be used -- want to be used in most settings for these types of studies. And it has very good test characteristics, as I pointed out, and it's probably as good or better than most, almost all of the imaging biomarkers and probably true for cellular, also.

However, it's very -- you know, it's a fairly known specific at some level. And then as James pointed out, there's a large number of people who have significant emphysema, for example, in the general population who have normal spirometry yet increased mortality. The same is true for the symptomatic smokers and so forth.

DR. TORJUSEN: And so obviously, I think, from a regulatory perspective, you know, we use it more in the context of approving pulmonary drug products. And so, as we've said before, I think the bar that you require is going to be very different for a new drug that's going to be indicated for, for instance, COPD versus a new tobacco product that would be lower

risk.

So I think one of my colleagues from the cardiovascular division at the FDA made the same point that, you know, what we do from a regulatory perspective in terms of drug product approval would really maybe not necessarily directly apply as it's in the context of developing a new tobacco product. And I think, as we've seen here, that really in terms of detecting very early disease and predicting future things, I think that it's very difficult to use FEV_1 for that.

And as you saw from my presentation, that really -- you're really looking at the disease progression over time in a spectrum of years, whereas some of these other presenters, Dr. Crapo and Dr. Barr, presented how you can actually detect these small changes quickly with different modalities. So I think those are some of the advantages. But obviously, from a regulatory perspective, you know, we've used FEV_1; it's been validated, and that's really why we use it for drug approval.

DR. CRAPO: Can I just add one thing? I wanted to say that the biggest problem with FEV_1, in my opinion, is it created blindness on our side. We started this in the '70s and '80s and '90s, it developed and used it, and people stopped thinking about disease, and we literally missed a lot of these

and misunderstand it because we used it as the only criteria. It was our problem, not the patient's problem and not the test's problem.

DR. DRESLER: So before I go on to follow up, though, because to give Dr. Tarran a response, because from what I was hearing -- and tell me if I'm wrong on this. Your upper left-hand side of your curve, which were -- it's going to be that one?

DR. CRAPO: Yeah, that would be normal FEV_1 and normal FEV_1 ratio.

DR. DRESLER: Right. And what was their CFTR?

DR. CRAPO: What was their --

DR. DRESLER: CFTR. So that's what I'm wondering.

DR. TARRAN: Sure. CFTR hasn't been measured in a large population probably of COPD patients. It probably should be. Most of the studies have been small, 10-20 patients. So it needs to be done.

DR. DRESLER: What do you think it would be? You know, because that's what I was hearing him say is that maybe the FEV_1 is a grosser measurement and the CFTR is an earlier measurement, and that's what I'm wondering, if you guys would address that.

DR. TARRAN: I would predict that CFTR is probably currently with small airway disease much more so than with emphysema.

DR. CRAPO: You notice in our COPD study, when we started using other parameters, clinical things like dyspnea or quality of life or CT, we found a lot of disease in the people in the upper left corner. We showed that. So if you ask me would I expect CFTR to be abnormal -- normal in that? We're talking about heavy smokers that have a normal FEV_1 by population statistics. I would be willing to guess that maybe half of them are going to have an abnormal CFTR.

DR. BARR: So I think just one maybe final comment on this. It's important to realize that in multiple studies, it's not that one precedes the other necessarily. I mean, if we look at, for example, the Venn diagram I put up, which is approximately based on MESA, which is a population-based study, at an average of 70, the majority of people who have symptoms of chronic bronchitis have normal lung function. And the majority of patients with emphysema on CT scan have normal lung function. And that's been shown in several different studies. So people are moving along trajectories as they age, and it's not because you get a bit of emphysema; you're necessarily

going to develop COPD with an airflow obstruction, you know, at age 130. It's rather that these are somewhat overlapping but also independently existing diseases. And the biology, I think, is entirely different for one or the other.

DR. DRESLER: Okay. And then I promised Dr. Rennard that I wouldn't forget him, okay? So Dr. Rennard, did you want to say anything on this topic?

DR. RENNARD: Yes. Can you hear me?

DR. DRESLER: Yes, we can hear you well.

DR. RENNARD: Great, thanks. I think I would like to ask the other members of the panel. We've all talked about a lot of biomarkers that can measure effect, but I don't think that any of us really addressed the challenge that Dr. Temple gave us earlier today, that these biomarkers show an association with disease, but they're really not disease.

And, in fact, do we need to validate these biomarkers by showing that a change in the biomarker in either one direction or another really predicts the kinds of outcomes that we want to see, either for the better or for the worse? Showing the association probably will be insufficient to convince doubters, particularly those with experience of biomarkers where the associations and the plausibility and even clinical practice

all end up falling afoul of the data when the data were finally collected. So I would like to ask the other members of the panel -- I think it's a hard question -- how they think these various promising measures that seem to show correlation and plausibility can be validated.

DR. BARR: So I'll take that on. It's a little bit of a setup from Steve, because I think in many ways the SPIROMICS study, which Steve had a key role in building and setting up and continuing, is looking at some of these measures specifically. And I think for the -- it's really the ones that are further along, such as -- and have more data behind them, where percent emphysema or however you do the metric on the CT is probably getting closer, where we now know it is an independent predictor of mortality, faster decline in lung function, clinical events, otherwise, and we now have clinical trials showing biologically relevant improvement in those that are moving towards better symptom reduction and so forth. So I'm not sure it's quite there yet, and maybe that's in the eye of the beholder, but we're making progress, and I think with studies like SPIROMICS and COPDGene, we'll get there.

DR. TARRAN: Can I just pass a comment? Do we actually need that degree of stringency? Shouldn't the burden of proof

be on the tobacco industry to prove that it doesn't? So just to flip it on its head, rather than say are biomarkers good enough, rather say, well, maybe you should show that you don't see changes in biomarkers. I mean, just to go back to the drug things. If we made a drug for pulmonary disease which inhibited CFTR and affected mucus, modulated the immune response in healthy people, it wouldn't get approved. And so there are products which do the same thing. I mean, that's the thing is I think the bar is too high, to be honest. But that's just my opinion.

DR. CRAPO: Let me add that I think that we -- I think we actually do have good data showing that there are strong indicators of disease. And we haven't really done it for a lot of the individual parameters. But if you'll think about the PCA analysis that I showed you that came from chest CT and pulmonary function, it was a composite scale.

But on that composite scale, there was a dramatic correlation with morbidity and mortality, both. And now I think maybe, if you're going to just use one test, you need to do additional validation of that, and I suspect that the real value is going to come from a composite test. But I think we have information that says that these things we're looking at

as biomarkers are good predictors of disease. And by that, I mean morbidity and mortality.

DR. DRESLER: Dr. Bhatnagar.

DR. BHATNAGAR: So realizing that there are many different points for the lung injury and many different biomarkers, would you have any feel of what the dose-response relationship would be in terms of loss of lung function, for instance? And there are two sort of sub-questions in this. One is that there's a general understanding that it takes a long time for COPD to develop, and it's like very high levels of exposure.

The data that you were showing had to do with people in the '60s and '70s smoking two packs a day, and now it's like 10 to 12. So how relevant is that data? And then how do we understand this in terms of harm reduction? Say, if there's a linear relationship, we could safely assume that it would follow. But what do you think the dose-response relationship is, and is it like cardiovascular, which is nonlinear? Or is it like cancer, which is linear?

DR. CRAPO: Do you mean dose response for smoking?

DR. BHATNAGAR: Dose response of smoking with lung outcome. Dose response.

DR. CRAPO: Okay. Well, this is a very complex question,

but it's clearly a complex answer because we know that the people we're looking at, that I was studying, were all smoking 30 to 50 pack-years, and that's a very long scale. I have seen very little of this in people -- let's say a person who started smoking as a teenager and quits within a few years doesn't really show up on our scale. But in between that, I don't have any data to give me a good dose-response curve on it.

I would say that we need to consider sensitive populations, and I think that we're just beginning to learn that, the genetics. And there are unique aspects for both race and gender that affect these. I think that we may not want to use our dose-response curve on just general population, but we don't really know how to apply it yet. But we have good data that there are sensitive populations. And for example, in lung disease, the alpha-1 trypsin is a classic example of a highly sensitive population for which smoking has a much steeper dose-response curve.

DR. BARR: Again, it depends on the metric a little bit and the acute versus chronic effect. The chronic effects are obviously years and years. But I'm sure the cellular and certainly some of the imaging markers are highly responsive to low doses quickly. And I don't think there's any published

data really answering your question, but that should be fully obtainable.

DR. DRESLER: Steve? Any comments, Steve?

(No audible response.)

DR. DRESLER: Okay. Well, then, sort of let me follow that up because FEV_1, the curve, the Fletcher curve that you showed with the decrement -- and you were talking about 30-50 pack-year history of smoking. Okay, so let's pick somebody who has a 30 pack-year history of smoking, and they're going to a reduced-harm cigarette. Okay, so they're on the Fletcher curve, and how are you going to be able to tell that reduced-harm cigarette, how much did it reduce their change in trajectory of their FEV_1?

DR. CRAPO: Your first problem is you really have to have that person measured sequentially themselves before they change cigarettes because you need to know what their rate of change was, not what the population norm is. But you're assuming that it's on that curve. If you assume that they, as an individual, are on that Fletcher curve, then we would predict, if you go to a no-harm cigarette, that it would go back to a normal loss, which would be what? Twenty years. So CC here instead of 50 to 100 that I was measuring.

(Off microphone comment.)

DR. CRAPO: And we don't really have numbers for any other -- FEV_1 was the only one people who were actually given numbers for that kind of change. But I would think that the other parameters could be done and would be -- it's going to take huge population studies to get that kind of data, though. So it's kind of a vague answer. We're a long ways away from knowing the answer, but the FEV_1 data really supports that there would be a change.

DR. DRESLER: Um-hum. Well, okay, how about for the CT scan, then? So 30 pack-year history of smoking and let's say -- let's use the premise that e-cigarettes don't cause as much pulmonary damage. Let's use that as a premise, okay? So the 30 pack-year history of smoking, and they switch over to an e-cigarette. Will the CT scan be able to discriminate that?

DR. CRAPO: Remember, though, I showed you the CT scan on our population of people, and I looked at a 5-year, roughly a 5-year change. Now, some are a little longer than that, and some are a little shorter because they didn't all come in at Day 1.

But you're looking at a 5-year change, and we only had 6% of those that had a progression on the most sensitive CT

parameter for measuring emphysema, and about 20 to 30% that had a change for gas trapping. So there's a chance that gas trapping will pick it up, and that's a 5-year interval. This is a slowly progressive disease, and I think we're being unrealistic if we expect to get answers in 6 months or a year for someone that changes to a low-toxicity product, let's say. I just don't think that -- I think the disease is slower than that. So other people can respond.

DR. TARRAN: But then it becomes tough from a regulatory point of view because I think that population doesn't exist. So for e-cig smokers, the patients we've been capturing, they've switched. They're typically maybe 30s, 40s, 50s, and they switch for a couple of years, you know, because it's still new and it's just taking off. So what do we do?

Do we wait 30 years and then study them and say, oh, well, actually they were better or actually they were worse or no different? You know, you guys -- you know, the CTP is wanting answers from us now, but you know, do those official measurements really prove they were better or worse for disease? You have to wait for the disease progression.

DR. BARR: So I think, I mean, it really does get into a question of what's an acute or short -- and then short-term

effect versus long-term effect. And when we're talking about COPD, we're unfortunately talking about long-term exposures. And I'm not sure that we really solidly have a short-term surrogate endpoint to predict the longer ones. But I mean, in terms of trying to -- you know, in a more pragmatic point of view, trying to design a study, certainly on an imaging basis, I would think more responsive measures such as some of the blood flow measures that I showed or hyperpolarized gas ones where they're much more sensitive; you can see small-term defects that could show over, sort of, in some cases, acutely and within an hour, and some in the short term, sort of over a month or two.

But I do think it's important to think about both is there a harm reduction -- I mean, it's a different scientific question and equally challenging as to whether there's harm reduction in a current cigarette smoker switching to a "lower-harm" device or potentially lower-harm device versus taking a young adult who's never smoked in their life and then thinking about exposing them to the same topic and thinking about the true potential harm there.

DR. CRAPO: I think I was aware of one thing. I think it's a very important question because we feel it's very hard

and you're not going to be able to really get the real outcome. But I think that we need to address the question, and we're probably going to have to use surrogates like, for example, look at short-term changes in CFTR or sRAGE and a variety of other biomarkers like that and then make a best estimate. But it's going to take you decades to figure out, to prove that it actually changed the outcome.

DR. TORJUSEN: I also think that if you find a short-term outcome, that may have some sort of an effect and you feel somewhat confident -- I think you also want to make sure that that effect is actually long lasting. And so even if you find something in the short term that you actually are pretty confident with, I think that you'd really want to make sure that that effect is maintained.

I mean, for instance, you can see some sort of transient change in FEV_1, but that may not be maintained for, let's say, a year. That may be completely diminished by that point. So I think that's one important thing.

And I think also another interesting point about this discussion is really that I think it's keeping an open mind with things because of the fact that really, what we don't know is that this lower-risk potential tobacco product may actually

have multiple other adverse effects that we're not even discussing today because we're talking about the known tobacco products and their associated risks. So I think it's an interesting discussion that we have before us.

DR. DRESLER: Sir.

DR. NELSON: Paul Nelson, RAI Services.

Really, a question almost as a follow-on to the previous question to Dr. Crapo. When you looked at the differential CT scans, did you see a difference in either the progression severity or progression types? Looking at people who continued smoking and/or looking at progression in ex-smokers.

DR. CRAPO: I really can't rigorously answer your question yet because the 5-year data of progression only has a fairly modest progression. We really need 10 years to get good data to see how the curves are separating, and at this point in time, you know, we have all the expectations that stopping smoking is going to decrease progression. But I also showed you some data that COPD ends up with the same mortality. So it's a great question, and I wish I had a better answer.

Graham, you've been looking at more cohorts than I have. Can you answer it better?

DR. BARR: Yeah, we have data. We haven't published it

yet, but from the MESA lung study, where we're now at about 10 years of follow-up using the 10% emphysema measures on CT scan, and there, current smoking is definitely associated with increased progression of emphysema, whereas formerly it's not and obviously never smoking is not. You know, there's always sort of a healthy quitter effect and so forth. One must interpret the data a little bit cautiously, but there is the expected association that you see for current smoking and progression.

DR. NELSON: And I suppose that perhaps the logical follow-up is it takes time to get this sort of data. If someone were to switch from smoking to a different type of tobacco product, how long do you think it would take to actually be able to measure differences in effects or say yes, there is a clear difference in switching from one product to another?

DR. BARR: Well, I think there's -- I mean, there's data in for smoking cessation, switching from traditional cigarettes to nothing, showing changes in CT measures within a month. However, it's somewhat paradoxical, and how this would be interpreted for the newer product, I'm not sure.

DR. CRAPO: I think a few changes, like you just

mentioned, smokers actually have a higher density CT than nonsmokers do, if they change, and that's probably because the inflammatory cells leave the lung. But you also may have changes in other key parameters like the amount of cough and sputum production. So chronic bronchitis could change. There are a number of clinical parameters that could change that relate to the acute event, acute effects of acute smoking. The chronic change leading to the really chronic outcomes are likely to take a long time to resolve. I would say it would take at least, you know, thousands of people being measured and doing it over 5 or 10 years to figure that out. But the acute changes, like cough and sputum and dyspnea and walking distance and some of the biomarkers, these things could change pretty rapidly, and we already know most of them do change with smoking cessation. And so I'm comparing a new product that's more -- I'm estimating it based on assuming it might be similar to smoking cessation.

DR. DRESLER: Hold on those. Let me just ask Dr. Rennard a question. Then you.

So Dr. Rennard, did you want to respond?

DR. RENNARD: Yes. Carolyn, can you hear me?

DR. DRESLER: Yes.

DR. RENNARD: Can you hear me?

DR. DRESLER: Yes.

DR. RENNARD: Hello?

DR. DRESLER: Yes, we can hear you.

DR. RENNARD: Can you wave your hand if you can hear me? Because I have to turn off the sound when I speak.

DR. DRESLER: No, you're good.

DR. RENNARD: Okay, great. Yes, thank you. Okay. Yes, I'd like to just add to what James said, is that we know that with smoking cessation and smoking reduction, a number of biological measures change and pretty relatively quickly. So that and -- that would show with smoking cessation, that using bronchoalveolar lavage, alveolar macrophages, which appear to characteristically assimilate in very large numbers in a smoker -- it takes several months for that to happen.

Those results have been confirmed now in several different laboratories, and there are results from smoking reduction showing similar effect. Now, we don't yet have a tertiary blood biomarker that reliably tracks alveolar macrophage burden. But if there were, that could potentially be a very tractable measure of that specific biological effect.

Clearly, changes in just smoking reduction are associated

with changes in airway epithelial cell, goblet cell metaplasia, for example, and I suspect that many of the other biological effects in airway epithelium in vivo would also change and has the potential to change with the product. Now, assessing them by bronchoscopy may not be the easiest thing and the most tractable clinical outcome, but I think that we could look at a number of measures that are likely to be much more rapidly responsive. Certainly the FEV_1, but probably also more responsive in terms of trying the numbers, the imaging modalities as well.

DR. CRAPO: I would agree with Steve. I think that there are things like reduction of the inflammatory elements and mucus and airway cells. There's going to be a lot of changes of that nature. It's interesting. I think if you want to make that comparison, you really have to have a smoking cessation group, a change in smoking and the people who continue to smoke, to see what -- I mean, you really have an opportunity if someone does this, if patients are willing to do the study.

DR. DRESLER: Dr. Bhatnagar.

DR. BHATNAGAR: In cardiovascular literature, there is very well-documented risk about how the risk decreases within a couple of years after smoking. It comes out to 80% from a

smoker to almost a nonsmoker. We also know that, like, we had the smoking bans in different communities and the smoking ban, within a year you can see a 20% or a 15% decrease in cardiovascular mortality. So isn't there something like that for lung risk or a risk of lung disease or a risk of drop in lung function? Do we not know how rapidly the risk dissipates?

DR. CRAPO: I don't think there's any answer for the actual data, but there's data -- but there is an answer for quality of life, mucus production, cough, things like that, for smoking cessation. But in terms of mortality and change in emphysema rates, those aren't measured that I'm aware of, unless you have data.

DR. BARR: You can distinguish, sort of, acute effects and the effects of smoking bans stopping, and so in other words, in susceptible folks, like with asthma. Both stopping smoking and bans on environmental or passive smoke exposure will have, you know, big -- you know, reasonably large effects within days to months.

For COPD, however, the problem is it's -- and the portions that James talked about in terms of COPD. However, mainly for lung function, I mean, it's the chronic exposure, and it's a lifelong process. And so an exposure from age 15 to 35 is more

or less the same as 25 to 45, and so forth.

So if you get that, let's say, 20 pack-year exposure, as you age, you know, there's age-related decline in lung function and progression of emphysema. So if you die from MI at 55, you're never going to develop chronic COPD. The problem we have now is former smokers developing COPD. Not entirely, but that's the reason for the increase that I showed. So there is a bit of a reduction in risk. There's obviously a reduction in risk by stopping smoking and there is some reduction chronically, but there's no equivalent as there is for cardiovascular or lung cancer of that 5 or 15 year, going back to that of a never smoker.

DR. DRESLER: Dr. Tarran.

DR. TARRAN: Yeah, I was just going to say I think you mentioned that we have to not just look at COPD, because these new products of reduced harm may cause other types of lung disease. So say someone who's on the path towards developing COPD switches to a new product that's flavored with a buttery flavor and then they get bronchiolitis obliterans or something, so that's -- you know, just dealing from our genomic and proteomic data, now the e-cig users, I guess, technically are switchers. They're actually more different -- smokers are more

like controls than e-cig users are. There are more changes in the e-cig users group.

DR. JONES: Bobbette Jones, Reynolds American.

Since I have a panel of lung experts in front of me, with the discussion that's happening currently around nicotine product standards in which we would have a combustible burning cigarette that would just have lower nicotine values, even if smokers switched to that product, what kind of an impact would we see on lung function if they're still burning the tobacco and inhaling the smoke, even though it's just a lower nicotine product?

DR. TARRAN: I would say no change.

DR. CRAPO: If you're still breathing the combustion products, I think you still got most of the risk. I don't think that nicotine is the risk. The nicotine has its own set of issues, but the risks that we're primarily talking about are probably not nicotine.

DR. BARR: Yeah, I would agree. I mean, you can think about particulate matter in the context of air pollution also, which doesn't have so much nicotine in it. And, you know, at very low doses that we all breathe in, in the U.S. context these days, there is still demonstrable impact on lung

function, the FEV_1, and then if you go into higher exposed countries, it's much larger. So there's clearly -- you know, I don't think we really know in terms of cigarette smoke, but there's clear literature on particulates in multiple other contexts, none of which have nicotine in them for harm.

DR. DRESLER: So one last question. Tomorrow -- Dorothy, go ahead.

DR. HATSUKAMI: Yeah, I'm just going to go back to that issue, since I'm pretty much involved in that type of study. So the effect of reducing the levels of nicotine in cigarettes is to make them minimally addictive. And so what that would do is reduce the extent of smoking behavior, so it might reduce the number of cigarettes that are smoked, and it might facilitate abstinence. So in that context, then, do you think -- you know, for example, if you significantly reduce cigarette smoking, would that have an impact in terms of some of the lung function?

DR. CRAPO: I would say definitely, yes. If you're substituting nicotine, low nicotine for smoking, either decreasing or smoking cessation, then you've done something really good.

DR. BARR: Yes, with a caveat that we know -- I mean,

smoking one cigarette a day compared to, you know, 24 cigarettes a day, we know, is preferable. But if one is dosing based on nicotine and therefore smokes twice as many cigarettes with half the nicotine in each, I think most of us -- again, maybe not on the best scientific data -- we'll agree that that's going to increase the pulmonary harm rather than keep it the same.

DR. CRAPO: Yeah, you are going to backfire, and that would be a real problem.

DR. DRESLER: Okay. So I actually have two more questions. Dr. Tarran, for one of your studies you saw more changes in e-cigarette users than cigarette smokers. What do you think that result means in terms of disease risk?

DR. TARRAN: We'll tell you in 20 years.

DR. DRESLER: Oh.

DR. TARRAN: We don't know. I mean, data is trying to emerge. I've heard that e-cig users are more prone to infection than nonsmokers, so the immunosuppression profile could be indicative of more chance of respiration infection. And so compared to the average person, there would be more of a chance in picking up a cold or flu. Could that drive other lung disease? I don't seem to know right now. Do you know?

DR. BARR: There were data presented at the European Respiratory Society meeting, with vaping mice using the usual standard way that mice are smoked, generated as much emphysema -- sorry, this was e-cigarettes with nicotine -- generated as much emphysema as regular cigarettes. So I think we just don't know currently.

DR. DRESLER: Sir, do you want to go? Go ahead and ask another one.

DR. PRASAD: So in this session, we heard a lot about imaging techniques and how a meeting is helpful in linking biomarkers of effect to harm and then potential risk for disease. And Dr. Rennard also mentioned about some of the circulating biomarkers. Is there room for looking at circulating biomarkers in evaluating lung disease risk? Because these may be much easier to draw blood rather than sending to imaging, which may be more expensive. So probably in a postmarketing surveillance setting or even an epidemiological surveillance -- epidemiological studies, would any markers come up for any meritorious use?

DR. DRESLER: Do you want to address that? Steve, are you still there? No, I was waving at Steve.

DR. RENNARD: Yes, I'm here. Can you hear me?

DR. DRESLER: Yeah.

DR. RENNARD: Yes. Okay, thank you. Yeah, I think that, you know, ideally a biomarker that's going to be used has to have a number of properties, and a lot of them are more practical than anything else. So it has to be relatively inexpensive, it has to be highly reproducible, it has to be easy to perform, and it has to have a high degree of patient acceptability. I'm a bronchoscopist, and bronchoscopy doesn't meet most of those criteria, whereas simple blood tests usually do. In fact, peripheral blood tests are easier to do for most clinicians than spirometry.

Even though there's a needle stick, most clinicians are comfortable drawing blood and not so comfortable doing spirometry. And so I think that really to have blood tests that we thought were validated -- and we talked about a number of the issues in terms of validating a test -- I think it would be tremendously useful. And my own personal opinion is that a single biomarker isn't used in a -- and that we need to be able to assess the risks for multiple outcomes since one thing getting better at the expense of something else getting worse is probably not going to be good. Some biomarkers will be nonspecific, like fibrinogen, and it may integrate multiple

things, but they also need to be terribly sensitive. So I think that biomarkers will tell us a lot. I think that we will only learn more about them if we use them a lot.

I mean, it's kind of a Catch-22. If we want them to tell us everything, which they clearly cannot do now, and therefore we refuse to use them, we won't learn any more. So I think that we should accept them for what they are, that they give us some insight that can sort of help us with our thinking. And your decisions are going to be difficult, especially at first, but they will get easier with time, but only if we learn more.

And so that's kind of talking around the question, but I think the blood biomarkers, just because of the practical logistics, are extremely promising for use for regulatory purposes and probably -- and certainly much more useful than invasive measures that would sample the lungs directly. I think imaging is an entirely different category of measure and tells very different kinds of information. So I don't think comparing imaging with blood is really -- it's really comparing apples and oranges. They're highly complementary kinds of information.

DR. DRESLER: One last question. I'm more familiar with CT screening for lung cancer. Is the same CT scan able to do

the screening that you're doing? And those are currently at 6 months to annual repeats. Are they useful for COPD?

DR. CRAPO: Yeah, that's a very good question and one that we're actually changing -- we're proposing a change for the next few years in the COPD protocol to do a scan that's comparable to the screening one, to show that we can get the same data. We think we can, but we're actually doing those cross-comparisons, and we've done the first two or three hundred already, and it looks like we're able to get most of the data on the screening scan, which would really enable us now to reach hundreds of thousands of people at risk. And I think that's an exciting future for where we can take advantage of lung cancer screening to get both cardiovascular and lung disease data in addition to the cancer data at the same time.

DR. DRESLER: All right, good.

DR. CRAPO: Because you can read coronary calcium and --

DR. DRESLER: Yes, right.

DR. CRAPO: -- thoracic aortic calcium. So you can read -- and you can read emphysema, and you can read airway disease on it. So it's something that we have to do, I think.

DR. BARR: I agree. I mean, this has really turned everything on its head at some level in that we've been talking

for decades about whether a coronary calcium scan is helpful on top of Framingham, if spirometry is a helpful screening test, and now we have a lung -- now we have CT scans in at least -- albeit in a subset of the elderly population, where there's proven benefit for screening for lung cancer, and then we can also pitch in for emphysema, the other measures, and coronary calcium.

DR. DRESLER: It probably justifies the radiation risk, perhaps.

DR. BARR: Also, the doses are going way down.

DR. DRESLER: Right, exactly. The scans are getting better. So excellent.

Any other questions before -- I know we've gone a few minutes over. Thank you very much, panel, both for the presentations and for the excellent discussion.

Thank you, Steve, for being online.

(Applause.)

DR. DRESLER: Tomorrow morning, 8:30 we start. Thank you.

(Whereupon, at 4:45 p.m., the meeting was continued, to resume the next day, Tuesday, April 5, 2016, at 8:30 a.m.)

C E R T I F I C A T E

This is to certify that the attached proceedings in the matter of:

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