UNITED STATES FOOD AND DRUG ADMINISTRATION

FDA PUBLIC WORKSHOP: BIOMARKER-DRIVEN DRUG DEVELOPMENT FOR ALLERGIC DISEASES AND ASTHMA

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PARTICIPANTS:

DAVID KASLOW, MD

PETER MARKS, MD, PHD

JEFF SIEGEL, MD

STEFAN VIETHS, PHD

PETER STEIN, MD

RICHARD BEGER, PHD

LYNNE YAO, MD

ALKIS TOGIAS, MD

GURJIT KHURANA HERSHEY, MD, PHD

SYED H. ARSHAD MBBS, DM, FRCP

SALLY WENZEL, MD

ROBERT HAMILTON, PHD

MATTHEW ALTMAN, MD, MPHIL

MOHAMED H. SHAMJI, PHD

PAMELA GUERRERIO, MD, PHD

OLENA GOLEVA, PHD

ALEXANDRA SANTOS, MD, MSC

HUGH SAMPSON, MD

ERIK WAMBRE, PHD, MSC

WAYNE SHREFFLER, MD, PHD

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P R O C E E D I N G S

(8:10 a.m.)

DR. MARKS: Good morning everyone, in the room here, and online, I'm Peter Marks, director of Center for Biologics Evaluation and Research. I want to welcome you to today's public workshop on biomarker driven drug development for allergic diseases and asthma. Thank you for attending.

For those of you in the room and online, you should know that the meeting is being transcribed, thanks to our transcriber in the corner there and really want to welcome you. Thank you for taking the time to get through security, those in the room, for those outside of the room, you can enjoy that, you didn't have to go through security, but thanks very much, so.

Just to give you some opening, remarks here, and kick this off. I just want to, just put allergenic products in perspective at FDA. As people may be aware, FDA is divided into multiple different centers, and allergenic products are

actually handled by different centers, or parts of the allergenic world.

The Center for Drug Evaluation and Research handles small molecules and recombinant proteins, as well as Monoclonal antibodies, so those products would be, handled in their domain. The Center for Devices and Radiologic Health handles most in vitro diagnostics. We have a small number in our center, but they're mainly around blood transfusion, and then.

Our center, Center for Biologics Evaluation and Research has handled allergen extracts for diagnosis, or immunotherapy, and other in vivo diagnostics, such as patch tests for contact dermatitis. That's been in our domain because we tend to at our center. The nice way of saying it is that, we handle the complex biologics. Another way of saying it is, we handle the messy biologics, like the one you vacuum up out of carpets, but that's just the way it goes.

Our center actually has a long history that dates back before the actual Food and Drug

Administration was established. The Biologics Control Act of 1902, came into being because of two episodes of contaminated biologic products in the United States in 1901 that killed 22 children, so this was put into place in 1902, and. By 1903, the Hygienic Laboratory of the Public Health and Marine Hospital Service had the authority to issue, suspend, and revoke licenses to produce and sell biologic products.

Over time, this authority moved from different agencies, and this became the division of Biologic standards within NIH when it was the National Institute for Health, ultimately becoming part of, the National Institutes of Health, with research and review responsibilities. Up through 1972, when there was a little bit of a flu scandal, which you can read about on your own time, and. The administration transferred biologics over to the Food and Drug Administration, so then, the precursor to Center for Biologics Evaluation Research came into being here.

We maintained the researcher reviewer model that was in place at NIH. We now have state of the art laboratories with high quality core facilities. We had nice laboratories at NIH, they weren't, they were nice when they were new, when they were in the 1960s, but by the time we left them in 2014, they needed a little freshening, which they've now received, but. Our new laboratories, which are just to the south of us here, are really state of the art laboratories with high quality core facilities, imaging, we have a Vivarium that is reasonably large flow, Cytometry genomics, and Proteomic cores.

The Office of Vaccines Research and Review is the place where our allergenic products are, housed. Obviously, vaccines research and review is not a perfect name for it, because it's other products as well. Obviously, it handles all of the viral, bacterial, and parasitic vaccines, but also allergenic products, fecal Microbiota, transplantation, other live bio-therapeutic products, and Bacteriophage.

We have 31 principal investigators, who have independent, internally funded basic research programs, some have other funding from around the agency, and this research endeavor has actually contributed significantly to public health.

Between various publications and various technologies, including patents, have made a difference in global public health, one example being the conjugation technology that was licensed to WHO for the meningococcal vaccine, that has led to a less expensive meningococcal vaccine for the continent of Africa, so.

Back to allergic diseases. There has been an intense interest in biomarkers and allergic diseases, and you can see here, although there's been a little dip, the kind of steady rise here in the number of publications in this area, and. Today's workshop comes at a really, excellent time, I think, to have a further discussion of biomarkers in this area of allergic diseases and asthma. This is just as of February15th.

To give you an idea of who is here: 659 registrants as of February 15, 522 of you are virtual, 185 are in person. And 48 are double booked, they probably will be a little bit like me, I will spend part of the day in the room, and part of the day down South of here, in my office, taking care of some other things and listening in.

If you look here, we have about 61 percent industry participants, 12 percent academia, 8 percent including advocacy organizations and non-U.S. government agencies, and 19 percent government agencies, again based on the date when this was done.

We have a variety of countries represented, so very grateful to have you, you can see, I won't read through them all, but a list of European and Asian, including Australia and New Zealand, so thank you very much for joining.

I'll just conclude my remarks by saying, putting together one of these workshops is a huge undertaking, and I'm very, very grateful to all those who have really made this happen, there's a

lot that goes into it. You can see on this between Ron Rabin and Jay Slater, and others in the Office of Vaccine Research and Review, and those in the Cedar Office of New Drugs, Stacey Chen and others, as well as, Sherry and Lonnie in our Office of Communications, Outreach and Development. Thank you all to them for all their efforts making today happen.

I also want to just thank you all for attending today I really wish everyone a wonderful day of lectures and discussion. Thanks very much.

DR. SIEGEL: Good morning everyone, I'm delighted to be here. My name is Jeffrey Siegel. I'm office director for the Office of Drug Evaluation Sciences and the center for Drugs at FDA.

I'm going to be speaking to you about the way we approach incorporation of biomarkers into drug development programs. I'll begin by talking about the different types of biomarkers and how they can play different roles in drug development. Then talk about the process, for

accepting biomarkers in drug development programs, and then talk about some considerations for surrogate endpoints in particular.

Let's start with a definition of what a biomarker is. Biomarker is a defined characteristic that's measured as an indicator of normal biologic processes, pathogenic processes, or responses to an exposure or intervention, including therapeutic interventions. Molecular, histologic, radiographic, or physiologic characteristics are different types of biomarkers.

The FDA worked with colleagues at the NIH to develop a glossary of the different types of biomarkers, and this is called The Best Resource, it's available on this website. The Best Glossary defines a series of different types of biomarkers, and talks about the way they can be, incorporated in drug development programs.

We think a lot about surrogate endpoint biomarkers, which are particularly important, but it's important to consider that, there are many other potential uses of biomarkers in drug

development. At the top of the list, here are the biomarkers that are measures of disease presence and status. At the bottom are biomarkers that change with treatment, with treatment interventions.

The first one, I'd like to consider is a diagnostic biomarker.

This is a biomarker that has data behind it, indicating that it's capable of identifying patients with a particular diagnosis. Prognostic biomarkers are biomarkers that predict a particular outcome, a later outcome. These biomarkers have data indicating that the level of the biomarkers predicts which patients are most likely to have particular outcomes later on, and. It's particularly important for use in enrolling patients in clinical trials, to enrich clinical trials who are most likely to attain an endpoint. This allows smaller and shorter clinical trials.

Monitoring biomarkers are biomarkers that are associated with a particular aspect of the disease or condition and can be, used to

monitor, the status of that condition over time.

Then Pharmacodynamic, or response biomarkers, are a category of biomarker that changes with treatment. These can be, used in a variety of different ways. They can be, used to assess whether the drug hits its target, they can be used to gauge the appropriate dose, and in some cases where they predict clinical outcomes, they can be used as surrogate endpoints in clinical trials, and I'll talk more about that later.

When we think about biomarkers, we think about the best biomarker category that they belong to: Monitoring, or biodynamic and so on, and also, the way that, the biomarker is going to be used in clinical trials. We call that, the context of use, and we assess the type and quality of evidence that's required to support that biomarker for that particular use, based on how it's going to be used in clinical trials, so.

We think about analytic validation. This would be the sensitivity of the biomarker, the specificity, its reliability and its accuracy,

and then we think about clinical validation, which is evidence tying the biomarker to the particular clinical concept of interest, and.

For validation, we think about the benefit and risk of the biomarker, so. Benefit risk is obviously different from benefit risk for a drug. Here we're talking about the benefit for making clinical trials be more efficient, for example, and the risk is what are the consequences, if the biomarker doesn't accurately measure the concept of interest, so. For example, if you're using a biomarker as a surrogate endpoint, and it doesn't actually predict the relevant, later clinical outcome, then patients can be treated with the drug without having the benefit of the treatment, and that would represent a risk.

There are three different ways that biomarkers can be, incorporated in drug development programs.

The first, shown here, is the drug approval process would be for a pharmaceutical

company sponsor to submit the data to support use of the biomarker to their IND, their investigational drug application. Next is by scientific community consensus, which plays an important role.

And the third is through the biomarker qualification program, this is the program that's administered by my office. We have a program for clinical outcome assessments, another program for qualification of biomarkers, and a third, which is the I-Scan program for drug development tools that don't fit neatly as a clinical outcome assessment, or as a biomarker.

And these three ways that biomarkers can be incorporated in clinical development programs are not independent, they intersect in important ways.

For example, a biomarker can start out as a biomarker that's qualified for one particular use in the biomarker qualification program. Then pharmaceutical companies can incorporate that biomarker into their clinical trial and later, get

evidence that ties that to later clinical outcomes, so. It might be, used for example, as a reasonably likely surrogate endpoint, or as a surrogate endpoint biomarker.

The biomarker qualification process was, put in place by legislation, the 21st Century Cures Act. The process begins with submission of a letter of intent, and then, if that's accepted, by the FDA then, the requester will submit a qualification plan, stating the data that they're planning to gather and how they'll analyze it to validate the biomarker for the particular context of use. And then, they'll do their analysis and put together a full qualification package, and submit that to the FDA. If that's accepted, then we put on our website the biomarker, in the context of use, and. That biomarker can then be used in any drug development program for that same context of use, so this is a major advantage of, qualifying a biomarker through the qualification program.

Next, I'm going to turn to a discussion

of surrogate endpoint biomarkers, so.

Surrogate endpoint biomarkers are pharmacodynamic, or response biomarkers. It's important to consider that, to support approval, FDA expects substantial evidence of effectiveness that shows that a drug improves a clinically meaningful outcome, namely a way that a patient feels, functions, or survives.

In some cases, this isn't measured directly for approval, so. In one case with a validated surrogate endpoint, this would be a surrogate endpoint that's accepted by FDA, based on data that the effect on the biomarker predicts a specific clinical outcome. Validated endpoints have strong and diverse evidence supporting the relationship of the biomarker to the outcome, and these are, used to support traditional approval.

In certain situations, reasonably likely surrogate endpoints can be, accepted for accelerated approval. This would be an endpoint that's supported by strong mechanistic and or epidemiologic rationale, such as that, an effect

on the surrogate endpoint is expected to be correlated with a clinical benefit, but hasn't yet reached the level of standard for validation. These reasonably likely surrogate endpoints are, used for accelerated approval, for products intended to treat a serious or life threatening, disease or condition.

It's important to understand that there are limitations to the use of surrogate endpoints. They are not a direct measure of the way a patient functions, feels, or survives. Instead, they're intended to reflect and predict the clinical benefit, but that's not directly measured by the surrogate endpoint outcome.

With the surrogate endpoint, the benefit risk therefore has to be, based on assumptions and predictions of benefit. And there are situations where biomarkers may fail to predict clinical benefit. For a surrogate endpoint that's, reasonably likely to predict a clinical benefit, and is relied on to support accelerated approval. A post-marketing confirmatory trial is required to

confirm the clinical benefit.

Some of the limitations for surrogate endpoints are, shown here: It is helpful for use of a biomarker, if it's indeed on the causal pathway that's modulated by the drug, causal pathway to disease, that's modulated by the drug, but in some cases, the surrogate is actually not on this causal pathway, but is, correlated with outcomes. In this case, the drug can have an effect on the biomarker, but actually not have an effect on the clinical outcome, and. In other cases, drugs can cause adverse effects on the desired clinical outcome through a pathway that's not reflected by the biomarker, or the drug can have toxicities that affect the risk benefit of treatment, so. These are situations in which, the surrogate endpoint may not predict, clinical benefit.

There are, a wide variety of different types of pharmacodynamic biomarkers, some are on the causal pathway, some reflect, target engagement. They're biomarkers that reflect the

particular pathways leading to disease.

Some biomarkers reflect organ injury or organ function. These can, lead into clinical endpoints but may not, be directly related. An example of validated surrogate is Low Density, Lipoprotein cholesterol. This is, used as a surrogate for cardiovascular outcomes.

These are based on trial level, evidence of surrogacy, as shown on the curve on the left, where the differences in the achieved levels of decrease in LDL cholesterol are associated with the relative risk of major cardiovascular events. And you can see, these are displayed on the line indicating a close correlation between, the impact of the drug on the biomarker corresponding to the impact of the drug on the disease outcome, and similarly, in eight non-Statin trials shown on the right, a similar correspondence has been seen.

The situation is not always, seen with other potential surrogate endpoint biomarkers, so. Shown here is an example of High Density Lipoprotein as a surrogate. There was strong

epidemiologic data indicating that HDL cholesterol was highly associated with cardiovascular outcomes, as shown by the graph on the left here, where the level of HDL cholesterol was highly associated with the hazard ratio for cardiovascular events.

However, when drugs were, developed, that increased HDL cholesterol, it was found, that there was actually no correspondence between the increase in HDL cholesterol and cardiovascular events, so. This was a situation where a potential surrogate endpoint biomarker actually did not predict clinical outcomes.

A variety of sources of data can support use of biomarkers as surrogates.

Randomized trial treatment group level data is some of the strongest evidence that we have. Individual patient level data showing a correspondence between the biomarker and the clinical outcome is helpful.

Observational data, mechanistic data are very, very important in understanding how the

biomarker may be associated with clinical outcomes. Pharmacodynamic studies, human genetic data, and translational animal models can all be helpful, and shown on the right is a schematic that shows that, the more data is needed to support a validated surrogate, and quantitatively less data is generally seen with reasonably likely surrogate endpoint biomarkers.

I'm going to end with two examples of biomarkers that were shown to be reasonably likely surrogate endpoint biomarkers.

The first one I'll begin with is, total kidney volume as a reasonably likely surrogate endpoint biomarker for Autosomal dominant, Polycystic kidney disease. This work was based on a consortium that sought to associate total kidney volume with decreases in kidney function over time, and this consortium was put together by The Critical Path Institute, and included participants from academia, from industry, and from patient groups, to pull together all the data to support use of this biomarker in clinical trials.

What's shown here is the model that was put together by this consortium that was able to associate total kidney volume at baseline along with the covariance of estimated GFR at baseline and age to predict the likelihood of having a 30 percent decrease in estimated Glomerular Filtration Rate over time, as shown on the graph on the right.

These data allowed the biomarker to be initially qualified as a prognostic biomarker based on these modeling results. Subsequently, the biomarker was, supplied in individual drug development programs by pharmaceutical company sponsors and the data supported acceptance by the FDA review division of total kidney volume as a reasonably likely surrogate endpoint for accelerated approval.

The other example I'd like to share is proteinuria for IGN nephropathy as a reasonably likely surrogate endpoint.

There were, three types of data that were important, in this validation. The first

was, mechanistic data tying urine protein to kidney damage. The second was, epidemiologic studies showing a consistent association between the severity and duration, of Proteinuria and loss of kidney function. And the third was, interventional trials that showed an association between changes in Proteinuria and clinical outcomes.

The graph, shown here shows, three different conditions, kidney diseases, and the association between the baseline levels of proteinuria and loss in, and the slope of loss of kidney function over time. Starting on the right, IG Nephropathy, you can see almost a linear association between the level of proteinuria, as shown on the X-axis at the top, varying from zero to greater than five grams per liter, with the higher levels of proteinuria associated with a steeper slope of loss of kidney function over time.

An association between proteinuria and loss of kidney function was, also seen with focal

segmental glomerulosclerosis, in the middle, and membranous glomerulopathy on the left, but the association was less strong at lower levels of proteinuria.

In those two diseases, there was less of an association with loss of kidney volume, so some biomarkers may be specific for a particular disease and may not be applicable to other related diseases. Formally, trial level surrogacy was shown by associating the treatment effect of different drugs on proteinuria, versus the treatment effect on the slope of loss of kidney function over time, as shown on the Y-axis.

The four studies shown are where the vertical and horizontal lines intersect.

On the right are studies where there was relatively little effect of the drug on proteinuria, and similarly there was little effect on the slope of loss of kidney function. In contrast, with the five studies in the lower left quadrant, there was an association between reducing proteinuria, and a less steep slope to

the curve of loss of kidney function over time, providing evidence that the biomarker was indeed associated with later clinical outcomes.

Supporting a surrogate means accumulating variety from a variety of different sources, it's context dependent. Depends on what type of disease you're talking about.

It's important to think about what the risks of approval based on a surrogate endpoint might be. Different levels of evidence are, needed for a validated surrogate versus a reasonably likely surrogate, and multiple sources of evidence, are important, biologic plausibility, supported by a varying extent of clinical pharmacology and clinical trial evidence. And, finally, convergence of evidence is, really helpful if you have evidence from a variety of different sources that all point in the same direction. This is among the most persuasive ways of making a case for a surrogate endpoint biomarker.

And with that, I'll end and here are

some references for you for some of the things I've talked about, thank you for your attention.

DR. KASLOW: Thank you, Dr. Siegel, for the overview of FDA's biomarker, program.

I'm David Kaslow, and on behalf of the Office of Vaccines Research and Review, let me add my warm welcome to this FDA public workshop on biomarker driven drug development, and allergenic diseases, and asthma.

So this first session is, really meant to provide the regulatory foundation for the rest of the day. We've had one presentation from the FDA, there are five others, three more from FDA colleagues, one from the EMA, and one from U.S. NIH. Following these presentations, we will have a Q-A period and a panel discussion, so.

Next up is Prof. Stefan Vieths, who's been the acting president of the Paul Ehrlich Institute since the beginning of this year. From 2002 to 2017, Prof. Vieths was the director, and vice president of the Paul Ehrlich Institute. He's been a long designated expert in EMA and

European Pharmacopoeia Commission, as well as the Chairman of the Specialist Conference on International Paul Ehrlich Seminar, Allergen Products for Diagnosis and Therapy, Regulation and Science, since 2002, and he is well positioned to present EMA biomarker program. Prof. Vieths?

DR. VIETHS: Thank you very much, for the kind introduction, so. There will be some redundancies with the previous presentation, I think, but as always in Europe, things are a bit more complicated, so you will see that, it's not as straightforward as it is here.

I work for the Paul Ehrlich Institute. We are one of the two national competent authorities for human medicines in Germany, there is another one for veterinary drugs, and we are part of the European Network.

The European Medicines Agency is mainly coordinating the regulatory procedures, but not making the decisions. The decisions are always made by a committee, at the EMA, where the representatives in this committee are members of

the national medical agencies, so from this mechanism you can already recognize that, it will be more complicated than it is here.

In addition, when you go for a license of a drug, the EMA selects two member states as rapporteur and co- rapporteur, and these two member states do the assessment of a drug, and. In a similar way, the qualification of biomarkers is also a team effort, and is not, decided by a single medical agency. So how can I continue this one? Okay, so of course, I'm expressing my personal views here, and not the views of my agency, but of course my opinion has some impact at the agency, so.

I will give a short introduction to, the biomarkers field. This will be a little bit redundant of what you have heard before, and then I will go for the EMA qualification of novel methodologies, and then discuss a little bit, the regulatory aspects of biomarkers in allergen immunotherapy, so this you have already heard before. I will just address what is in this blue

box here.

I think biomarkers in clinical trials can help identify specific patient populations that are, more likely to benefit from a particular intervention. Biomarkers provide insight into the mechanism of action of, the drug or treatment, we will hear much more about this during the day.

Of course, biomarkers enable the assessment of a treatment response and efficacy, if they are, well selected. And if you define biomarkers performance, you have to consider scientific validity, so the rationale for biomarker selection, analytical validity of the biomarker, so there should be strong and reliable performance metrics of the biomarker, and accuracy, precision, and reproducibility of the test should be defined and known.

Clinical validity is important of course, this is the main aspect that the relevance of the test to the clinical condition is, understood and validated, and. At the end, what we want to know is the clinical utility of a

biomarker.

One thing that has not, been addressed in the previous presentation is companion diagnostics, so. When you use laboratory tests or other tests in a clinical trial, to define the population, for example, then you're using companion diagnostics, and these companion diagnostics also are biomarkers.

In the EU, there is a new regulation on in vitro diagnostics applied in 2022, or came into effect in 2022, and in this regulation, companion diagnostics are legally defined, so.

They are devices that are essential for, the safe and effective use of a corresponding medicinal product, to either identify patients who are most likely to benefit from the corresponding medicinal product, or identify before and during treatments, patients likely to be at increased risk for serious adverse reactions.

And these in vitro diagnostics are risk classified in the EU, and when they are used as companion diagnostics, they are at least Class C,

or higher. The highest is Class D, depending on the risk class, and they are required for CE marketing. They are requiring a conformity assessment by a notified body, including consultation of a competent medicines authority responsible for the corresponding medicine, and so.

This means that in the EU, when you run clinical trials where you are using in vitro diagnostics, for example to measure biomarkers, you have two separate regulatory procedures.

One is the authorization or licensing of the drug on the top of this slide, where the biomarker clinical trials are done and then, in a marketing authorization procedure, EMA and national competent authorities decide about the marketing authorization. Whereas for companion diagnostics, even if they are, used for the same kind of treatment, you have to perform clinical performance studies, and then a private organization, a notified body, decides about the conformity assessment of this biomarker, and then

in vitro diagnostics receives the CE certification, so.

These are separate procedures, but often of course done within the same development program, and with the same clinical trial, but you have to take, this into consideration, if you want to use a biomarker in your drug development program.

The perfect biomarker, of course would be consistent, fast and economical. It should provide us with quantitative differences that are sufficiently large to allow conclusions. It should be reliably and easily be quantified, like in biological fluids, and it should have a correlation with a relevant clinical outcome, that is verified.

This we have already seen before, there are a lot of different biomarkers used in clinical trials, from genetic biomarkers, to molecular biomarkers, protein levels, or gene expression patterns. There are cellular biomarkers, numbers, frequencies of specific cell populations, for

example. Imaging techniques are used and, also clinical biomarkers, could be used, like blood pressure, heart rate, or symptom severity for all of them.

Of course validation is, needed when you look at the development process of drugs. You can use biomarkers, in basic research, to for example, better understand molecular pathways. You can use it in discovery and design, to better understand mechanism of action, and select targets in nonclinical development, to define clinical safety in clinical development for stratification enrichment, patient selection, dose selection, to better understand clinical safety or efficacy.

And after, marketing authorization you may use them for monitoring therapeutic responses. On the lower part of the slide you can see some regulatory documents in this field, and I would now, like to address the EMA, the European Medicines Agency, Qualification of Novel Methodologies Program, so.

This is what you can see at the EMA

website, it's called Qualification of Novel Methodologies for Medicine Development. This is of course a quite broad scope, so it covers biomarkers, but it's not restricted to biomarkers, and if you look at the key aspects of this procedure, it's a voluntary procedure involving EMA committees.

SAWP is the Scientific Advice Working Party, and the CHMP is the Committee of Medicines for Human Use, also providing the opinions for authorization of medicines. This qualification guidance of EMA came into force in 2008, and the first clinical biomarker qualification by EMA was done in 2011, for Alzheimer's disease in general.

It is possible to involve non-EU regulatory agencies, like in a joint procedure with EMA and FDA, for example. There are two types, two steps, it's I think, quite similar to what you have here at FDA. The first is a qualification advice, where you come up with a concept or an idea, and discuss this with the CHMP, or the Scientific Advice Working Party.

When the data are still preliminary, you get an advice on future studies to be performed in a confidential document, which is a letter that the applicant receives, and if it's positive, you get a letter of support. If the biomarker is considered to be, promising, the second step is to go for a CHMP Qualification Opinion. This is defining the acceptability of the proposed biomarker for specific use. It includes a scientific assessment report and it can result in the amendment of relevant regulatory guidelines, so.

The essential considerations for successful qualification of novel methodologies is the definition of the context of use, selection of endpoint, a statistical analysis plan, demonstration of clinical utility, standard of truth or thoroughbred standard of truth, and appropriateness of the analytical platform, and some others may also be considered, so.

This is how the validation procedure looks like: You submit an application and the

procedure starts at day zero. The agency has 60 days, to prepare for it. At day 90, you should have a scientific advice working party recommendation, then when you go for the upper part, if you go for a qualification advice, this recommendation is adopted by the Committee for Human Medicines. And then, the consortium, if it's positive, gets a letter of support (in the lower part), this is the opinion procedure. You have, again a recommendation by the scientific advice working party. You get a discussion at the committee at the CHMP committee at EMA. The draft opinion is, published for public consultation, and once it is adopted, the biomarker is accepted as regulatory standard for the claimed use.

In addition, of course, what companies can do is to go for a separate scientific advice, without going for a qualification procedure. This could lead to, let's say, a recommendation to use one biomarker for one specific development program, and then it's not public, so.

What you have accepted as a biomarker on

the EMA website is only part of the picture of the biomarker being used in development of medicines in the EU. So now, there is a nice paper reviewing what has been, done in Europe, by Dutch authors I think, published in 2022, covering the years from 2008 to 2020.

There were 86 biomarker related procedures, seven procedures per year, at average, and a lot of follow up procedures. The main median duration was four months for qualification advice, and 11.5 months for qualification opinions, and there are now 13 qualified biomarkers.

The most common context of use was patient selection, stratification and or enrichment, efficacy, and safety. The most common disease area among all contexts of uses was Alzheimer's disease. There was, a low number of procedures relating to genetic biomarkers. So far, most procedures were going on with soluble and imaging biomarkers, and there is an increasing number of consortium initiated procedure. This

means that, for example, large EU research projects would go for such a procedure because they want to have the information available for everybody, and not just for one company, so.

The issues that, have been raised during these procedures are mainly, in three areas of: Either their validity, because there were deficiencies in the analytical method, validations, and methodological challenges, or in reproducibility, or standardization. Biomarker properties, in terms of clinical- relevant changes and usefulness, and chosen cutoff values, and general, study designs. For example, study population on period, responder versus non-responder definition, study setup, and so on. Whereas the other areas, like here, the context of youth, data analysis and evidence were with less frequency, leading to issues during the procedure, so.

Now, a few words about biomarkers and, allergen immunotherapy, I mean, we will hear a lot more about this in the afternoon, so there is of

course quite a good understanding, what happens during allergen immunotherapy.

A lot of parameters are going up during allergen immunotherapy, like antibody responses, cellular responses, and so on. And a lot of parameters are decreased during allergen immunotherapy, so this is, I think, good, and we understand much, much better what is going on now, compared to 20 years ago, so this is a good perspective for developing reliable biomarkers for allergen immunotherapy, well, we are now.

From the regulatory point of view, I would like to say is that there is not a lot, so no potential biomarker is currently validated for allergen immunotherapy in terms of regulatory acceptance, but they are commonly used.

For example, in those finding studies, provocation testing, immunological parameters, antibody responses, and so on, and even if a biomarker does not completely qualify as a full surrogate endpoint, it can still result in very valuable information. For example, decision

making on a company level, to continue pursuing or abandoning a specific product. And it can, also be very supportive in regulatory procedures, to argue that an immunologically relevant effect is observed, so.

It's quite regularly applied in Germany within marketing authorization procedures. For example, companies use IgE responses to include patients, they monitor responses to individual allergens during immunotherapy, and so on. What we have to keep in mind is that if you are in allergy immunotherapy, you want to treat, let's say, mite allergy or grass pollen allergy, you don't want to treat grass pollen allergy in patients with IgE, to P-1only. And if you ask the regulators for such a kind of stratification also, your indication will be a bit more restrictive.

And I think this is something that was also inhibiting a bit the use of, especially allergen biomarkers in the development of allergen immunotherapy products in the past. So at the end, I think next step that we need to follow is,

to identify and verify the most promising and consistent potential biomarkers for allergen immunotherapy.

Start validation programs for these biomarkers once promising candidates are available. Get in touch early with regulators, ask for scientific advice, and involve them in the validation programs. I think there is a massive understanding of relevant immunological and molecular events in allergen immunotherapies, so the ground-work is being laid to screen for promising biomarkers with higher precision than the ones that we have currently available in the literature, so.

With this, I think I'm finished. I would like to thank you for your attention, and acknowledge contributions by a few colleagues, Dr. Andreas Bonaz, Dr. Melanie Albrecht, Susan Kau, Jorge Engelbertz, and Sander from our Immunological Division, thank you very much for your attention.

DR. KASLOW: Thank you so much, Prof.

Vieths, indeed, a bit more complex in the EU, so. Next up is Peter Stein, who is currently the director of the Office of New Drugs. He joined FDA in 2016 as the deputy director, after a 20-year career in the pharmaceutical industry, focused on developing drugs for diabetes and related disorders. Dr. Stein will review the role of translational science in biomarker and drug development.

DR. STEIN: Great, thank you very much and good morning. Certainly, a pleasure to be here, and it looks like it'll be a terrific workshop. My task is really, just to sort of, give a high level overview of where, I might consider translational science fitting usual disclaimer.

Just as a sort of brief overview, it's worth thinking about some of the changes in the drugs that we're developing and the diseases we're targeting, which has impacted how we think about translational science.

I'll say a few words about, how

different disease population size impacts design and conduct of drug development, but also the implications of that for the use of translational medicine, and surrogate endpoints. Talk a bit about rare and small population, drug development and regulation, and. Just make the point that this really depends more on the use of translational medicine, than traditional development, which was 20 or 30 years ago, targeting large common chronic diseases, and then. A little bit more about the role of translational science.

I suspect you've seen this kind of graphic before, which really depicts the fact that we're seeing a transition. From drugs that 20 years ago targeted common chronic diseases I mentioned, and increasingly now are targeting rare diseases and small populations, subtypes of more common diseases, so that right now, about half of the new molecular entities are targeting drugs for orphan indications.

There are differences, and I think it's

worth thinking about that, because it has implications for the use of translational science. Obviously, stating somewhat the obvious in terms of the characteristics of development in the context of common chronic diseases. We have large populations, the ability to run two or more large, adequate well, controlled trials, a wide range of disorders from symptomatic to serious progressive diseases, but typically with a well, understood natural history. And more often than not, available FDA guidance, and certainly also, precedent endpoints that are generally well accepted and standardized, but when we move to rare disease drug development, we're talking about small populations, which can make recruitment difficult, need flexibility in study design, because of the variations in size and the knowledge about the diseases.

These are typically progressive, serious, life limiting or life threatening diseases, and with substantial unmet need. With also a lack of precedent for how to go about drug

development, and quite diverse, based upon both genotypic and phenotypic diversity, with a natural history that's not typically well characterized, and a lack of drug development tools and endpoints, biomarkers and the like.

And of course, with common occurrence in kids, which also modifies how, trials can be conducted. When we think about common disease drug development, we're really talking about, typically, is translational science having a very distinct role, I think. Just in the last talks, you've heard about the many different roles in earlier development, obviously, in terms of proof of concept, dose selection, exposure response, biomarkers that are developed to improve the efficiency of the larger trials. But the base of the application is really, two or more large, adequate, well controlled, randomized clinical trials for common diseases.

When we move to the context of a rare disease, drug development program, the role of translational science really is substantially

larger. Of course, we still have to have this on the base of a clinical trial, an adequate will control trial that can assess the effect of the drug in that disorder. But we also have to consider what translational science can deliver, so surrogate endpoints that might be part of a clinical trial, confirmatory evidence that might accompany the clinical trial, and.

I want to say, more about both, of these areas, where translational science is so important. But to step back, for a moment and just talk about in the US, the pathways and requirements for drug approval in a sort of simplistic way. If omitting other components that are necessary for drug approval, we can think about two particularly important components.

One is the demonstration of effectiveness. The standard that we apply is the substantial evidence of effectiveness standard, which is in statute, and is based upon adequate, well controlled, trials that are explicated in regulation. As those criteria for an adequate,

well controlled trial are exactly what you think of in any trial that is going to have scientific integrity.

The second step, though, is concluding that the drug's benefits outweigh its risk. So presence of substantial evidence of effectiveness and the benefit outweigh the risk, which is how we look at the safety that we see in characterizing the drug's profile. We have a guidance that was relatively recently released that talks about benefit risk in the context of drug regulation and drug approval. When we think about the substantial evidence of effect in this standard though, there are different ways that this can be, met.

As I said, for common chronic diseases, this is the top row, which is two or more adequate well controlled studies. But when we think about moving this into rare disease drug development, it can be used in other contexts as well, we can think of, one adequate and well controlled trial and confirmatory evidence, which is another way

that, we can meet substantial evidence of effectiveness. And I'm going to talk more about confirmatory evidence, because that's really something where translational science has a very large role.

Also want to mention the approval pathways, of course, we have two different approval pathways in the U.S., traditional approval, which is, based upon a clinical endpoint, an endpoint that reflects how patients feel, function or survive. We also have the accelerated approval pathway, and that's a pathway that's based upon a surrogate, or an intermediate clinical endpoint, a surrogate that's considered reasonably likely to predict the outcome, to predict benefit that the drug may offer, so.

Again, two different endpoints in the context of accelerated approval. A reasonably likely surrogate, which is, again, a surrogate measure that's not validated as predicting clinical benefit. That would be a traditional approval endpoint, but it's concluded to be

reasonably likely to predict clinical benefit, an acceptance of some uncertainty. It clearly still requires data to support that the surrogate predicts the clinical benefit of interest, but it does allow for more uncertainty.

An intermediate clinical endpoint, I'm not going to say more about, but a clinical endpoint that ultimately will predict durable, important clinical benefit. In the context of accelerated approval, we do require that there is a post- marketing trial that is preferably ongoing, or that's initiated that can verify and describe the anticipated clinical benefit, again, the clinical benefit on, how patients feel, function, or survive, so.

Now let's move to talking about the uses of translational science. And I want to say a few more words about confirmatory evidence, then turn to talking about surrogate endpoints.

So let's start with confirmatory evidence. Confirmatory evidence is a range of different things.

Unlike some, where we think about clinical endpoints or surrogates, confirmatory evidence is a wide range of different types of information. Indeed, it can even be, a body of information that, supports the finding from the clinical trial.

We recently released a guidance that talks about the different sources of confirmatory evidence, and it lists a number of different potential sources. Some of these are clinical referencing of a prior approval that's in a related condition, information from natural history, from other members of the pharmacological class, but very importantly, two components that are directly relevant to translational science, mechanistic or pharmacynamic evidence.

This can come from surrogates, this can come from biomarkers that show that the drug has engagement and is working in the pathway of the disease pathogenesis, but also evidence from an animal model. And of course, there are a wide range of uses of animal model in this context, so

here, we're talking about animal models that may be translational. Now, they don't have to be, and animal models generally don't perfectly replicate the human disease but, it has to have some basic similarity in its natural history, in its presentation, in its outcome, and what we understand about, its pathogenesis and the role of the drug pharmacology as it alters that pathway, so.

A translational animal model can also serve as confirmatory evidence. Now, I do want to just make the point that, the body of translational of confirmatory evidence can include different components. So there may be a translational animal model, there may be pharmacodynamic endpoints that correlate with what we see in the animal model, and then show that we see that in humans, but the extent, of confirmatory evidence, can vary.

When we think about that, it depends upon both, the setting, and the seriousness of the disease, the extent of unmet need, and that helps

us understand the extent of acceptable uncertainty, but also the risk. What is the safety profile of the drug, which also drives us to think about, how much uncertainty we can accept? And so, we also have to look at the robustness of the adequate, well controlled trial. How convincing was that trial resolve? And, then, also the robustness of the confirmatory evidence.

I could sort of illustrate that in this way, on the right, which is that, the stronger the adequate, well controlled trial, potentially the lesser the amount of confirmatory evidence, the weaker the trial, although it still has to provide evidence of the benefit that the drug is reported to have, the stronger the confirmatory evidence may need to be.

I want to talk now a little bit about surrogate endpoints.

And I know you've heard already some talks about qualification programs and the various roles of biomarkers, including as surrogate endpoints, and just to remind you, this is from

The Best Classification, the NIH FDA classification scheme.

We think about a clinical outcome, which describes or reflects, how a patient feels, functions or survives, as I've said already, a biomarker, I think you've heard the definition, so I won't repeat this. A surrogate is a biomarker that predicts, but does not directly measure clinical outcomes, and that's an important distinction.

Clinical outcome assessment measures the clinical outcome. Surrogate is a biomarker that predicts that we can expect to see that clinical outcome, but isn't a direct measure of the outcome. Again, I suspect you've seen this already, which is the wide range of biomarkers, which have a whole range of different roles from monitoring, assessing safety, helping us in the enrichment of populations for clinical trials, either as a prognostic biomarker, or a predictive biomarker. But I want to spend a little more time talking about surrogate endpoints, which are

response biomarkers, measures of response to treatment.

Now, when we think about the range of surrogate endpoints, we tend to lump them into this big bucket of surrogate endpoints, but I think you can start to think about dividing them into different types of surrogate endpoints.

There are surrogate endpoints that reflect the cause, for example, levels of a toxin, or measures of an infection, such as HIV RNA, or Hivrna. Those are, biomarkers that look at the etiology of the disease, where we clearly understand the etiology precipitating the outcome, the disease entity. There are many biomarkers that are, mediator biomarkers. These are biomarkers that are on the pathway, in the pathogenesis of the disease, for example, a disease which triggers an inflammatory cascade. Measuring a biomarker along that cascade could turn out to be a surrogate, if it's very closely tied to the outcome. There are certainly also biomarkers or surrogates that reflect the disease

outcome. These might be structural biomarkers, or functional biomarkers, for example, a biomarker that measures cardiac injury, CPK, or another type of enzyme, or a biomarker that reflects an inflammatory process, ongoing and structural damage, or. A biomarker that measures functions, such as EGFR, or for example, bone strength. Those might be functional biomarkers that could look at the impairment that's caused by the disease, that we're trying to develop a drug to target.

Now, when we think about a surrogate, because we're not directly measuring what matters to patients, how they feel, function, or survive, we're always going to want to prefer to have a clinical outcome, not endpoint, not a surrogate. A clinical endpoint outcome is always preferred because it's directly answering the question we have about the drug. How does it help patients? How do they feel better, function better, or survive longer?

Now, because surrogates are typically

continuous variables, they're more easily detectable. The effect of the drug on the surrogate is typically earlier, and this typically enables shorter, smaller trials. So certainly, a large advantage, particularly for settings where the disease course is prolonged or where the event rate is very low, and that can be in ultra rare disorders, or in common disorders. For example, drugs for Lipid disorders, for LDL cholesterol lowering, use that as a surrogate because, we recognize that the event rates in cardiovascular trials can be relatively low, and can take a long time to get enough events to be able to assess the effect of the drug.

Now, there can be a wide variation of, available evidence that can support a surrogate, and varying types of proposed surrogates as well.

I want to talk about something that I think we all worry about with the acceptance of surrogates, which is whether they'll work or not. Does a surrogate actually predict what it purports to predict? What can go wrong? And I'll give you

an example of something that went wrong, in terms of the concept that was put forward for the basis of a surrogate.

Well, conceptually, the framework for a surrogate looks like, what I have on the right side on top here, which is, you have the disease pathogenesis, the pathogenic sequence. Somewhere on that pathogenic sequence, the drug has its effect on its target, which modulates both, obviously, the pathogenic mechanism and the biomarker, which we assume is on that pathway, is on that causal pathway, and that modulates the clinical outcome. The biomarker change, therefore, is proportionate to the clinical outcome, the optimal circumstance, and we conclude that that biomarker does indeed predict the clinical outcome. The biomarker may be after the clinical outcome.

As I said, we can use biomarkers that look at structural or functional alterations, to look to see whether those are, modified. Does the biomarker reflect that there is increasing or

reduced damage? That can also be a useful way of determining, whether the drug has the effect it purports to have, but of course, biomarkers can go wrong, and this is in graphics.

If you've seen the classic article by Fleming, by Tom Fleming, that was published a number of years ago, that sort of explicated some of the things that could go wrong with a biomarker. Here, the drug modulates the biomarker, but that biomarker isn't on the causal pathway, it's on a different pathway, and the drug modulates, may modulate the clinical outcome pathways. But that relationship between the biomarker and the clinical outcome is not connected, so that it's not on the causal pathway, and the change in the biomarker may not reflect the clinical outcome.

The other possibility is, it is on the causal pathway. Here, on the bottom, we see a drug that modulates the causal pathway, the biomarker is changed, but if the drug has other effects, effects that perhaps reduce or attenuate

the clinical outcome, or lead to toxicity, then the biomarker is not working as we hope it would work.

In the situation where it leads to toxicity, we can't get a sense of the overall risk the drug leads to, because we may only have the biomarker reflecting the clinical outcome, and not the safety profile risks of the drug. In the other circumstance, where the drug has negative effects on attenuating the clinical outcome, the net effect of the drug is not, reflected by the biomarker. The point being, in all of these examples is, the biomarker and the assumption around the biomarker, may not hold, and so it may not reflect the outcome.

And that's something we clearly worry about when we're looking at a surrogate that's proposed in the development of a drug for disease. We worry about whether or not the surrogate behaves as it's expected to behave, so here's an example. In some ways, this was sort of the classic example of a biomarker gone awry.

Now, actually, this trial was, intended to look at whether the biomarker worked, whether PVC is premature, ventricular contractions actually did predict the outcome of improvement with antiarrhythmic therapy, so. This trial looked at patients after a myocardial infarction who, were having premature ventricular contractions.

There was an open label phase, during which several different anti standard, anti arrhythmic, drugs were, used and where suppression of the PVCs, substantial suppression of the PVCs was, demonstrated and then, those patients who had suppression of PVCs with antiarrhythmic drugs, were randomized to matching placebo, or the effective drug, and. The primary endpoint was survival and survival over time, and as you can see on the right, the results were, inverse of what was expected. The placebo survival was better than the drug survival.

Well, in thinking about this, we could look at what went wrong. Why did that happen? I

put up here, what I suspect was the intended model that, PVCs were a manifestation of the same pathogenic causal pathway as was ventricular tachycardia, or ventricular fibrillation. Therefore, if you reduce PVCs, you should reduce ventricular tachycardia, ventricular fibrillation, sudden death should go down, survival should be improved, but that's not what was observed, so.

What might have been going on, and this is just speculative to, just point out that, the models that we think about, in where a surrogate is placed, may not turn out to be validated. In the middle diagram, I'm indicating that it may be that the mechanism of PVCs and the mechanism of VTVF are distinct, and so suppressing a PVC may not lead to suppression of VT or VF.

Now, in that situation, one would have imagined that the drug would have had no effect on deteriorating or improving survival, and so you'd have to postulate, the drug had a negative effect on the outcome as well, (on the bottom). It may be that, it was quite correct, that PVCs are on

the causal pathway and represent the same mechanism, but that the drugs, have a direct toxic mechanism that increases VTVF even as the other pathway, through PVCs, reduces VTVF.

The point being that, as we think about developing surrogates, we have to think both, about the data that tells us where that's positioned along the causal pathway: Is it reflecting the etiology? Is it on the mediating pathway towards the outcome? Is it reflecting the damage, functional or structural, of that outcome? Is that the pathway it's sitting on, or is it on a different pathway, that either might mediate harm, or might not mediate benefit? In the former, where it actually doesn't look at the.net benefit of the drug, and in the latter, where it doesn't reflect the outcome of the disease, so.

What are some of the lessons that I think we have to think about with surrogates?

First, of all, that there's always some degree of uncertainty when we're using a surrogate. It may be relatively small with a

validated surrogate, surrogates that we know and have used for years and years, such as LDL cholesterol, or such as blood pressure for drugs, for hypertension. When we look at surrogates that we accept for accelerated approval, that is reasonably likely surrogates, we accept some greater degree of uncertainty. And I talked about How we think about how much uncertainty might be acceptable? How serious is the disease, what is the unmet need, what is the risk the drug provides, and what is the evidence we have to support that surrogate? The key is to generate high quality translational evidence to support the surrogate. Animal models, pharmacodynamic markers, genetic associations, really, in a sense, a convergence of evidence that supports that surrogate.

I do want to just take, sort of a sidestep for a moment, and say a few more words about this, because I think very often in development, what happens, particularly where translational science is going, will be important,

whether it's through informatory evidence, or supporting evidence of a surrogate.

It's not uncommon for a lot of work early in development, even before clinical studies have been initiated, for there to be work on translational science, development of an animal model, ideas about developing biomarkers into surrogates. But very often, as we move into the clinical sphere, as we move into particularly phase two or phase three development, the work on translational science comes to a grinding halt, or at least it goes down to a trickle, and so. By the time we have to think about that evidence, in concert with the clinical trial result, it's often not as well developed as it ought to be.

And that's something I think is a really important message, which is that, if we're moving towards a development program where it's likely to be based upon an adequate, well controlled trial, plus confirmatory evidence, and that confirmatory evidence is going to include translational science. The pathway of development that went

through development of the translational animal model evidence to support the surrogate has to continue in parallel with conducting the adequate, well controlled, trial.

And I would say, from my observations over the last years, that is often not the case, and so a little bit of, perhaps a message from the sponsor to say, that is a really important investment to make. If you're going to say translational science isn't just in early phases of development, proof of concept, or getting some sense of what exposure is appropriate, but you actually think that's going to be important in supporting the approval of the drug, that work needs investment, time commitment, and continuing effort.

As a last point, I'd say, as the last example, I hope illustrates, we really have to think about what assumptions we're making when we are posing that, a surrogate is going to reflect the effect of the drug on the clinical outcome that we're looking for.

And I would say, before you suggest a surrogate, think about all of the other models, so you have the model of what looks great, where the biomarker is right on the pathway, but think about what other models there might be, that might make the biomarker or the surrogate go wrong, and test those. What is the evidence that, that isn't the case? What is the evidence that supports your hypothesis of where that surrogate sits, and tests all the assumptions that can be, evaluated?

Well, with that very brief overview of translational science, and a small advertisement from the sponsor, thank you for your attention and enjoy the rest of your workshop.

DR. KASLOW: Thank you, Dr. Stein, for framing the critical role of translational science, and surrogates in the, regulatory review process, so we'll now turn to Dr. Richard Beger, who will join us virtually. Dr. Beger is currently, the branch chief of Omics modeling, imaging and chemistry branch, in the division of Systems Biology here at U.S. FDA. The Omics

Branch consists of Metabolomics, Proteomics, and Tissue Imaging teams that focus on discovering and evaluating translational biomarkers of toxicology and disease. Dr. Berger will review the role of Omics in biomarker applications and discovery, and it looks like. Is he on?

DR. BEGER: Yes, this is my slide, thank you. I'd like to thank everybody in the biomarker working group, especially -- for inviting me to give this talk here. I'm very sorry that I can't in person, but I love the talk so far, obviously, in favor of talks you're going to hear about from me, next slide.

Systems biology, Omics technologies, there's quite a few out there, there's genetics, genomics, transcriptomics, proteomics, metabolomics, and each one of these has many different ways of collecting that type of data, and they all can provide information for us and provide biomarkers for the next hit, so. NIH defined this as a biomedical research understanding the larger picture be at the level

of the organism, tissue, cell, by putting its pieces together, and. Dennis Noble said that, it's all about putting together rather than taking apart, integration rather than reduction, and that's going to be my whole point here, is biomarkers, they integrate. What's happening might be better than what we've been doing by reduction.

Next slide - So systems biology, OMICS Biomarkers, these can be used for discovery. All these things are happening in your cell, tissue and organ. You have genome transcriptome, your proteome, your catabolome, your lipidome, and these are all responding to your diet, drug, lifestyle, age and social interactions. It's best to provide, to try to limit those exposures, or to try to capture as much of that as possible before your study.

Obviously, the gut microbiome can also play a role on how you're responding. And hopefully, at the end of the holiday, you should get some kind of phenotype that can help us

predict, whether the side effects help somebody respond or not respond. That's it.

And I'd like to thank the previous speakers for talking about the models of biomarkers and the outcomes on that, thank you.

Next slide - So, the Omics biomarker workflow, this is a discovery process, but it can lead you to biomarkers that you need per validation. So each one of these major groups are the sample prep analysis, the bioinformatics data mining, and then you're applying the data, and each one of these has very strict. You should have very strict SOPs, when you're doing multiomics, you're going to have to start thinking about different sample collection tubes, where each one of those metabolomics has to be thought out beforehand.

Try to do this beforehand. Have the aliquots made, before putting them in the freezer, and all these other type of issues. So I'll give you very small examples of each type of these things going forward. And what you see at the end

of the day is, what we get is, some kind of, hopefully some identified pathways are changed. And also, you're going to start looking at univariate and multivariate biomarkers. I'll give you examples, all the way going through, and at the end of the day, really what you get is a hypothesis, or something that you need to validate when you go back through, what you're going to want to do is, actually analytically verify those biomarkers.

During this whole process, you're going to want to have policy control for each one of the genomics that you're doing and go through this process again. And I have to say, one other thing that's really, been bugging me lately is the reporting standards. When you actually report this out there, there's a big discrepancy of how people report metabolites, lipids, proteins in the literature, and. I'm saying that people, if they want to put these biomarkers forward, especially as patterns, we have to come to a concise area and have reporting standards.

Next slide, I will actually give a couple of examples down the road on using these processes for immune related studies, so for proteomic technologies, when I first got into CTR 25 years ago, or so, people were actually doing gel based, just these 2D gels. They were about the size of a table, and they were trying to find markers. And obviously, this is something that's not done too much anymore.

It slowly moved into what they would call, what I would call, mass spec approaches. These would be where you would try to break down, put them on another different gel. You would cut out the slices, you would use a protease to break them down into peptides, measure all the peptides, search the peptides to identify proteins, and when you have a couple of peptides, you would actually be able to say which proteins were changed.

Recently, a lot of things, have moved into what I would call the affinity based approaches, and what a lot of people right now, especially in this area, would be using the

multiplex immune assays. And these basically are looking at using antibodies to look at 100 or so proteins, these kits are widely used, and recently, in the last few years, what came on board is what these call, these large, I would call affinity-based approaches, but.

One of them is the SOMAscan assays, and these use slow off rate modified aptamers. These are sort of, chemical antigens that allow you to have really, reproducible results, and currently you can actually use a very small sample size, and collect up to 11,000 proteins. The Olink assays are very similar, except it uses antibody pairs. You can go on to things that can go on to the transcriptomic arrays, and right now this is up to about 5000 proteins so that you can get quite a bit of information in proteome technologies.

Next slide please. Previous back one of the issues with proteomics is, I can't really get a standard, so how do you validate that? And one of the ways that we've been able to do this is actually look at both using SOMAscan and Olin, and

do the elevation of the same samples, and so in this way, we're analytically validating the two technologies against each other and this is what we've done previously.

We were able to show some of the biomarkers that we found in the prediction of kidney recovery from dialysis were reproducible. Now it doesn't always work out perfectly as it did for like LS-6, but even there we had for FCF-23, we had correlation almost 0.5 and a very high P value because we had a lot of sample patients.

Next slide - So for metabolomics there's many, different weighted, Smith, many different flavors out there, targeted, untargeted, two seconds, about semi targeted. And what the standalone biomarker way of doing things, right now is the multipoint calibration curve with the ultonic internal standard isotope resolution and mass spectrometry, and this is what people use for the FDA biomarker guidance.

There's, other things out there now where you can be, targeted. Like you could have a

standard, additional, multi point, calibration curve that is normalized by similar, internal standards. What I say is, this could be like looking at all 20 amino acids but only having four or five internal standards that are, amino acids. Likewise, you could be looking at maybe 50 or 100 different bioacids and still, only be using five or ten bioacids. That would be examples of that, for three would be the same thing, but you're not using similar internal standards. Like if, we're amino acids, you'd be using steroids or something else, which not many people do, but it is out there.

And then what I would call now the targeted and untargeted approaches, where you have some stable isotopes that you're using for normalizing just for a few chemicals there, and you're still doing an untargeted collection of a lot of data, and you're using that for normalization, and then. You could have like a whole class there, where you have all the amino acids, but you're still collecting all the other

data that's untried.

And then, there's the third case, the last case, Number 6, which is a no-calibration curve, and there's actually many different flavors of this, where you're actually doing especially four and five, where you can do normalization and QC at the same time, and that kind of stuff. These actually are now, what I would call a different class, names, what are called semi-targeted metabolites, but. If people really want to start looking at multiplex biomarkers, we're going to have to actually standardize, and have guidance, I believe, for these other steps, two, three, and four, going forward.

Next slide - one way you can have really, good data in a metabolomics experiment, or similar type of experiment, is using really, good QC, and you can have QC standards for actually every step of the way, highlighted.

You can have it during your sample prep, your analytical sample analysis, data processing, and your data analysis at the end. There's out

there, there's reference libraries, standard mixtures that you can buy, that can help you.

These things that you can measure are actually one of the more important things, are like system suitability before you even start the experiment. What really helps down the road is that, for comparison's sake, they have these, what I would call reference and test materials, that like, NIST would put out, that every lab can use, and you can compare across labs. And what a lot of people use are actually like pool QCS, which are actually the closest samples that you might have to your study, but they don't allow you to go from one lab to another.

Next slide - one of the issues that you can come across is, as I said, you have to really follow your SOPs, and so.

We did a study a few years ago where we collected samples from 20 humans, and we did, and collected six tubes from each subject, and we did a different pre-lab sample prep for each one of those samples, and two of them for blood. One was

at zero degrees, one was for six hours at room temperature, one was a Hemolysis, one was a control, a normal way, and then one was plasma at four degrees for 24 hours, and plasma 24 hours at room temperature.

And here, we looked at metabolites, peptides and inflammation, cytokines and stuff like that. The HGA plot shows that having samples at room temperature can really affect your data analysis of what you see, so you have to be really, careful on your sops, and especially avoid room temperature.

Next, slide please. So now, I'm going to go on and talk about two studies that are sort of related to immunology.

One is Leishmania parasite, it is a blood borne pathogen that can be, transmitted by transmission or, bite of the inflected sampling. It is a rare disease (?), and currently, blood donor screening is not an option, because it does not meet the threshold for sufficient equivalence. Standard drug treatments to treat the infection

mod are limited and often ineffective. However, vaccination of U.S. travelers and military personnel is, stationed in areas that could be complementary measures to control the transmission of this life collection.

One of the things that's coming up, is they've used live attenuated listing of parasites that are under investigation for candidate biomarkers, and these ones are deletion of the centrum in the Leishmania parasite LMCN minus. Minus and this, leads to impaired cell division.

Next slide please. So the evaluation of this vaccine by immunization, by delay type, hypersensitivity responsively antigens and LCMS based pathologic studies. So these studies, what they gave is that, the vaccine that they're studying, the LMCM minus, or an IV treatment for eight weeks, and then they inoculated with the parasite LST, or excipient and then they did the imaging on the airs and measured these studies of the site by caliper.

They did this at 24 hours and 48 hours

and then they harvested the ears and did some close up commentary and craft reservoir, and we were able to get our hands on these samples for receiver, for two different studies. One is when we looked at the ears, one of them is when we looked, analyzed neutrophils -- I will talk about those in the next two slides.

So the preliminary mouse ear metabolomics data, we were only given so the Slide B over there, we were only given the native and the vaccinated. We don't have the third column here. Over here is the HME plot so, native and vaccinated, and is showing that there's lipid high increases in the vaccinated lipid species. And when we did the metabolomics analysis, we saw a lot of lipids that were changed. And here, what we really need to know here is, the KOH is the inactive mouse with the vaccine for eight weeks at the DSH site. And then, there's the KLB, which is the vaccinated with the buffer. And then there's the KOH, which is the vaccinated. But at the outside site on the ear, what we see, are the two

biggest changes in ceramides, C-14 Ceramide and the glycoceramide. And as I said before, these are discoveries processes, and our CBER collaborators are evaluating these further moving forward.

Next slide, please. When we looked at the neutrophils, we looked at the control, we looked at the vaccinated, and the resident, and the alien wild trite. And one of the pathways that we actually saw was, at a high level of reactive oxidative species, as metabolites, that are in the oxidative form, so retinoic acid that was, has for OXO, we had a couple of cholesterols that are oxidized form. These are, things that might cause the result of the future fill recruitment. And this is also something that's seen in other disease situations like COVID.

Next slide - so I know that everybody's probably heard a lot of stuff about COVID so, I'll be brief here. What our study is, we got day one samples from COVID positive patients. We put these into three different groups, the mild one

was one that didn't require hospitalization; a moderate group that was in the hospital, but without the ICU; and a severe patient population that was in the ICU, and these were about 30 per group.

And we tried to have age and gender maps. We've done Multiomics on these samples, we've done microRNAs, proteomics, metabolomics and lipidomics. Unfortunately, I got the responses of the groups just literally days before these slides were acquired, so some of the results I've given you are very preliminary here.

Next slide - So when we look at the significant changes in the microRNAs and proteins in the day one samples, we can look at the microRNAs, we can do either a P-value of 0.5, or a P-value with an old change of 2.0, or a false discovery rate of 0.5.

The MAQC said 20 years ago, when they did the study for transcriptomics, that really the best way to go forward is a P-value with a full change of 2.0, so that's what we're using for

microRNAs. When you apply that for the proteins, we see a major reduction going from the P-value because the proteins don't have as much of a fold change as the microRNAs, going forward. So we might have to adjust that going forward, when you're trying to find what might be significant pathways or biomarkers, going forward, and maybe an FBR actually would be more appropriate for them.

Next slide, please - so this is actually what you're seeing for the protein that was heard in the table previously, now we're showing it in the panel plot. Here we have on the X-axis, the ratio change in Log 2, then the P-values on the Y-axis, so you can see on the right, that the severe versus mild has most changes, and most of the changes on the protein side are actually increases.

Next slide, please - so what you can do with this data is, you can actually put it into IPA, or other types of things and look at pathways, and we've done that.

And when we do that for the severe versus mild just using what we would call the significant proteins, which we see is here, are the pathways over here. And if they're in orange, it means that they have the number of changes that were significant, but they're also in the significant direction they would expect for that pathway to be changed.

And we want to sing in. One of the biggest pathways that everybody's heard about is this Cytokine pathway, that everybody's heard about for the last 34 years. We also see the wound healing, and we have neutral fuel degeneration. When you look at severe versus moderate, we also still want to see as a cytokine pathway, and we see wound healing is also another major pathway.

You can also put these into what I would call networks and see what the correlation of a lot of these things going forward. At the center of all this is IO-6, forwards versus mild. We assume a similar versus moderate, similar patent

network, which one stands IO-6 in the middle of everything. But we're actually seeing a couple of negative correlations, shown here in blue.

Next slide - which is, more of a standard way of doing, analyzing, data sets, only data sets. This is what I would call these supervised methods by squares, discriminant analysis.

And then we have a semi supervised ACA plot on the right here, we can see for the metabolomic data, we can see the severes in the pink, the moderates in the middle in blue and in the mild, green. In this particular data set, we're not just looking at what I would call a nauseous thing. We're actually picking up quite a few of the drugs that people are taking during this stuff, and so that's actually playing a role in what we're seeing in the grouping there.

When you look at the ACA plot, we actually put it in order for mild, moderate and severe, and some of the tablets, that we are showing off, here have been previously put out, by

other people. Urinary pathway, the tryptophan pathway, what we've been showing here is also, we see a lot of oxidation of fatty acids, and we've seen a lot of proteolysis, amino acids and tripeptides.

Next slide, please. If you look at the tryptophan pathway, that's related to severe response, we see the tryptophan is actually down, but many of the actually other metabolites associated, especially in the indol side of it were actually increased. And we also see, downstream from tryptophan is the kynurenine, and it's another metabolite downstream of that were also increased. Next piece, here, these were actually also strongly correlated with creatinine, so previous people would actually report these as, kidney biomarkers, and functional kidney biomarkers.

Next slide - One of the things that you can do, and what we try to do is, start looking at cross correlation of the different elements, the data sets that we get. And so, just to see where

we might want to look, and other things, and so in this particular case, two ones I want to look at is, the left and the right, sorry, IL-6.

Everybody's heard about this being related to Covid. We see that on the top, the highest related things are actually some ceramides. Ceramides are probably actually instigating, sort of starting the IL-6 pathway, and this is a pro inflammatory marker of COVID, that many people have talked about.

Over on the left hand side, we have April lipoprotein 84, and this is a lipoprotein that's on the HDL, your good cholesterol, and actually, showed that we have many strong correlations with the lipids, which I would expect, if it affects our cholesterol, so it's a good thing. And it was, actually shown, it was anti correlated with the same ceramides that were actually highly correlated with interleukin. In this particular study, this patient, Sapphire, this was a bunker that was decreased, it didn't hit the FC, 0.50 percent reduction, was actually

at 0.57, but it had a very good FDR. It was something that we might go forward with because there was quite a few patients that were in the cardio and hypertension showing signs of color response.

Next slide - so the use of Multiomics analysis, kind of healthy service, what we have to figure out is, what are the rules? And so, we had our previous speakers give us some of that, a lot of that was really out of biomarkers. Multiomics can provide biological insights that we can follow up on. And really, the challenges are quality control, integration with multi element data. I acknowledge the provision of system biology to help with all the COVID response and evaluation receiver samples.

The Center of Toronto actually gave us the money to evaluate the COVID samples. CBER collaborators provided the samples, and our non-FDA collaborator Heather Smaller at the UTHSC that provided the color samples, thank you.

DR. KASLOW: Thank you, Dr. Beger, next

up is Lin Yao, who's the director of Division of Pediatric and Maternal Health in the Office of New Drugs in Cedar. As DPMH Director, Dr. Yao oversees quality initiatives, which promote and necessitate the study of drug and biological products in the pediatric population and improve collection of data to support the safe use of drugs and biologics in pregnant and lactating individuals. She also serves as the rapporteur for the IC he eleven a guideline, pediatric extrapolation and Dr. Yao will review the extrapolation of biomarkers between age groups.

DR. YAO: Thanks very much. I'm hoping to, in the next few minutes, describe the utility of biomarkers in a pediatric extrapolation approach. The subtext, or the subtitle of this talk should be, do not forget children.

There's my disclosure slide, and here's one of the, I think, most important slides that I can present to you today, may, and that is, as you're thinking about development products to treat or diagnose allergic diseases, that a lot of

that population is going to be adult, but some of those patients, almost in every situation, will be children.

And therefore, if your development program is going to be focused on adults, but, you know, children are going to end up being exposed to that product, then those product development programs should include pediatric information, and pediatric studies, because, of course, the very first line pediatric patients deserve access to products that have been appropriately evaluated.

As you've heard from other speakers, I think Dr. Stein, Dr. Marks, others have described some of the issues related to studies in rare populations. And fortunately for most situations, diseases that occur in adults and children, well, in children, it tends to occur less frequently because children tend to not have a lot of these conditions in as high or great incidence as adults. And so, that's a good thing for children, but it's not necessarily a great thing, if you're trying to develop that product for children.

In addition, we have special ethical considerations that require us to think carefully, before we would enroll a pediatric patient in a clinical trial, and in fact, you should have a justification for, why that child should be enrolled, rather than collection of that information, who can provide consent, for example. In an adult trial all of these factors make conduct of clinical trials in children a little bit more complicated.

And as part of FDA thinking about how could we develop and label drugs for children in an era where, we couldn't even do a clinical trial in children, came this idea of pediatric extrapolation, and this is what I'm going to talk about.

So if you have a disease in a reference population, and generally this is a reference adult population, and that disease occurs in children, if we can establish that the diseases are sufficient to a certain degree, and that that drug you're testing, is also likely going to

respond, or in a pediatric patient similarly. Then we may be able to leverage efficacy information, and indeed, safety information for that pediatric population, such that you don't necessarily need to have those adequate and well controlled trials, as Dr. Stein mentioned earlier, to support substantial evidence of efficacy, and to support a risk benefit analysis based on the safety data. So it's a really, important concept in pediatric drug development.

We've recently published a guideline, as Dr. Kaslow mentioned, an ICH guideline that's out in draft on the use of pediatric extrapolation, and this figure is from that guideline. I want to take a few minutes to go over it because, I think it's really important to understand conceptually how pediatric extrapolation works, so.

As I mentioned, you want to assess the similarity of disease, and the response to treatment between an adult population, and a pediatric population. And to the left, where you see red, when you have very little data, and very

little information to support that the diseases are similar, or in fact, you have a lot of data and you know that the diseases are different.

Then that plan that you're going to develop, those studies, that you're going to conduct in children, are very likely going to need to be, adequate and well controlled, trial or trials, to get that substantial evidence, but. As we move to the right, as we move to a collection of evidence, and hopefully it's high quality evidence that support that, the diseases between adults and children are similar, and we expect that, response of treatment will be similar between adults and children. Then you could imagine collecting enough information to support an approval in a pediatric population that may rely only on, identifying a dose that matches an exposure that was seen, that supported efficacy in an adult. That's what we call a PK matching approach.

This large area that's kind of going from orange to yellow to green, is an area where

we've had a lot of evolution in the last few years. So we have some similarities, we have some confidence that the diseases are similar, but we don't know that they're exactly similar. And that's for a lot of conditions in children where, we don't have a lot of information yet to support that, diseases between adults and children are really similar.

I'm going to spend a few minutes talking about how biomarkers can be used in that space, and in a pediatric extrapolation approach.

So you've heard about Dr. Siegel and Dr. Stein, talk about pharmacodynamic biomarkers. And I think that one important use of a pharmacodynamic biomarker in drug development is that it can indicate that, there's some biologic activity, but we're not necessarily drawing that conclusion that, it's actually a biomarker that will predict an efficacy, or disease outcome. In that case, pharmacodynamic markers can be used, to support that a disease is similar, that a response to treatment is similar between an adult and a

pediatric population, it can support dose selection in a pediatric plan. And it can support an actual extrapolation approach in that middle area that I just described, where maybe you can't just match an exposure, but you want to have a little bit more confidence that that drug is working.

Similarly, by using a pharmacodynamic marker in addition to matching PK, the bridging biomarker is where I really want to focus our attention on the next few slides.

And I'm not going to talk about surrogate endpoint biomarkers at all, because I think that's been discussed really quite thoroughly. A bridging biomarker, and it's defined here, is a response biomarker that is supported by really strong mechanistic evidence, is expected to be correlated with an endpoint that will assess a fields function, survives outcome, but you don't necessarily have enough clinical data to show that, that's a validated surrogate.

So this type of bridging biomarker can

be used, and has been used in a pediatric extrapolation approach, when we've concluded that there are sufficient similarities between an adult and pediatric population to allow for it to be used.

And how do we establish the evidence for that bridging biomarker in a pediatric extrapolation approach? This slide is a little bit busy, but I want to focus your attention on the middle box, that level of evidence for a bridging biomarker, so: 1) It requires that you have established some evidence to support the similarity of disease between an adult and a pediatric population. 2) That in adults you've established efficacy based on a clinical endpoint. 3) That in both adult and pediatric settings, that that biomarker captures an effect through a causal pathway.

We've heard a lot about the causal pathway by previous speakers, and similarly, as we've heard before that, that biomarker in both adults and children, that that treatment effect

goes through the biomarker and is not really, there aren't other effects that are captured by treatment that are not reflected in changes in the biomarker. And then finally, through really rigorous analyses, that the net effect of the exposure, the experimental treatment on the clinical outcome can be explained by changes in that biomarker, so.

A lot of, requirements to use the bridging, biomarker, and by the way, I might say that, as you heard, that in translational sciences and the use of such an approach relies on, that you're collecting data in adults during adult development that can help support children.

And I'll give you that case example, this is a drug called Sacubitril/Valsartan. Subcubitril is a neprilysin inhibitor, and Valsartan is an angiotensin receptor antagonist. And you can see that, this product, the trade name is Entresto, but it's a combination product and was approved in 2015 for the treatment of heart failure, with reduced ejection fraction.

Now, at the time of the adult approval in 2015, we had very little information to support that heart failure in adults was similar to heart failure in children. Most heart failure in adults is ischemic heart disease. Most heart failure in children is really due to congenital heart disease, and so we thought, okay, etiologies are different, the presentation is different, the prognosis and clinical course are different, lots of reasons to say, these conditions are not the same. And therefore, pediatric extrapolation, as an approach to support and approval in the pediatric population was not accepted in 2015.

What was required was a double blind, randomized, active, controlled study in pediatric patients with heart failure, based on important clinical endpoints. And you can see the original endpoint was, time to event for death, heart failure, hospitalization, transplant, and other important clinical outcomes, so this was going to be, a trial that we knew was going to be hard to conduct, a time to event trial, in children. It's

going to take a long time, it's probably going to take a lot of patients, and there aren't many events that are going to happen. So we knew going out that, this was going to be difficult to conduct, but we didn't feel like that, we had a way scientifically to bridge efficacy in adults to efficacy in children.

In the meantime, in 2017, FDA, along with the Centers of Excellence for Regulatory Research and Innovation, CERSI, regulatory science and innovation, CERSI, convened and hosted a workshop that was specifically to ask the question: Are there populations of children with heart failure that are similar to adult patients with heart failure?

And through conversations at that meeting, which we had a paper published about this as well, it was determined that there is a subset of heart failure patients in adults that actually are very similar to pediatric patients, and those are adult patients with non ischemic dilated cardiomyopathy. They tend to be, younger they

tend to have diseases that are not related to myocardial infarction, or atherosclerotic disease.

And so for that reason, we thought, okay, maybe pediatric extrapolation could be considered, if we had a subset of patients with dilated cardiomyopathy not due to ischemic diseases in adults, and compared them to pediatric patients. The problem with the study in Sacubitril/Valsartan was that, most of the patients had ischemic cardiac disease. But there was a subset of patients with dilated cardiomyopathy, they tended to be younger, who could be evaluated.

But you see, because the adult trial wasn't powered to look at efficacy in that subgroup, we didn't have a lot of confidence, that you could directly extrapolate and just say, okay, let's look at the PK in those patients, and we'll go ahead and just get PK in pediatric patients. We thought there had to be something more, to bridge the efficacy, and so we looked at NT-ProBNP Prob.

Now, this is a very busy slide, and it's really intended for you to take a look at, at your leisure, after the meeting. But NT-ProBNP is short for N-terminal pro brain, naturic peptide, and the reason that NT-ProBNP was very interesting as a candidate biomarker to bridge efficacy, it was because we know mechanistically and biologically that, this hormone is secreted by cardiac myocytes in response to stretch, or dilation of the ventricle, that occurs in both children and adults. And if we look at those five criteria that I mentioned before about, how to collect the information to support Probnp as a candidate bridging biomarker, each one of those steps was evaluated and established.

Now, I'm not going to go into details, just for the sake of time, but I really want to point out here that, this doesn't happen overnight. There was a lot of information that had to be collected and integrated, both from the clinical trial data in the, adult, paradigm HF trial, as well as, data that were collected in the

pediatric heart failure populations, that supported the use of this biomarker. And in fact, the sponsor collected NT-ProBNP, as a biomarker, as part of their phase three trial. So there's a way to directly correlate changes in NT-ProBNP, with changes due to the drug and clinical outcomes in adults.

So that's a really critical piece of information that, that's the kind of information that you have to consider collecting, as part of the adult drug development, in order to support potentially an extrapolation approach in children. So what did, we decided, after review of the NT ProBNP data, remember I told you that the endpoint for this trial was an outcome that was death, hospitalization, heart transplant, and we changed it to, changes in ProBNP at 12 weeks.

Okay, so a very, very dramatic change in what we were going to accept as establishment of efficacy in children. So a primary, endpoint that was going to be a time to event to a primary endpoint, that we were going to measure at 12

weeks, and a change in a biomarker. You can see why we agreed to that change, and again, because it was, correlated to changes in outcome in the adults, and that, that correlation was not just a casual one. In fact, when FDA did, and the sponsor did an analysis of the data from that trial, that over 80 percent of the treatment effect could be explained by changes in that biomarker.

Here are the primary efficacy results from the pediatric trial after the endpoint was changed. You can see Entresto, which is the trade name, that the NT-ProBNP from the ratio, from baseline to 12 weeks, was cut in half, compared to the comparator Analypril, which was cut about two thirds. And you can see that, the comparison from Entresto to Analypril, there was about a 15 percent improvement, relative to Analypril, in the treated group, so.

What were the conclusions by FDA, that the evidence in adults and children, that FDA concluded that, NT-ProBNP could be used as a

pharmacodynamic bridging biomarker, to bridge the efficacy from adults, where we had clinical efficacy, clinical, hard clinical endpoints, to children. Where we were not asking for the clinical endpoint anymore, that we used that biomarker to bridge, and that ultimately, when we compared the changes in NT-ProBNP from pediatric patients, to adult patients with dilated cardiomyopathy, those changes were very similar.

There are a couple uncertainties that I need to let you know about. That is that, the active comparer that we used in the pediatric trial, Hanalopril is not, approved for use in children. It is standard of care, however, and that reflects sort of, the lag that we have, and the difficulties we have in developing and approving drugs for pediatric heart failure.

And I also want to point out, as you might have noticed in the last slide, that the difference between Analypril and the treatment Sacubitril/Valsartan, was not statistically significant. It was in the adult trial.

Why was that? We don't know why, because we don't know what, and we didn't know going in, whether Analypril was going to lead to the same changes in NT-ProBNP, as in adults and actually. We weren't as worried about that because, the important comparison was: How did NT Probnp change, between adults and children, not with treatment, with Entresto, as opposed to with treatment with Analypril, but that does remain something that we do need to actively investigate.

How do children differ in terms of treatment, with any drug, in heart failure? How do those changes, how they are similar or different to adults?

So some final thoughts: We've made a lot of progress in development of treatments for children, based on innovative strategies under the umbrella of the use of pediatric extrapolation and biomarkers, as I've hoped to demonstrate to you, play a very important role in the use of pediatric extrapolation. But there's a very important, but here, and that goes to, what are the assumptions

we're using, when we take that leap and say, we can extrapolate.

The assumptions have to be that, we have a certain degree of similarity between the adult and pediatric forms of the disease, and that, that similarity has to be assessed very carefully, because if we're wrong, and the diseases are different, then we are absolutely going to approve a drug in children that doesn't work. So the work that has to be done upfront, to support a pediatric extrapolation approach, has to be collection of the data, rigorous analysis that supports similarities between the adult and pediatric population.

And then finally, I just want to say that, on behalf of all of my colleagues, we share an important job of increasing the availability of safe, effective and affordable treatments for pediatric patients, including those who suffer allergic diseases. Thank you for your time.

DR. KASLOW: Dr. Yao, thank you for your, compelling call to action to ensure, access

to drugs in pediatrics, through use of biomarkers, to extrapolate the benefits from adults to pediatric populations.

So, last but not least, Dr. Alkis Togias, an allergy immunologist, and chief of the Allergy, Asthma and Airway Biology Branch at the Division of Allergy, Immunology and Transplant, NIAID Support of Biomarker Research in Allergic Disease.

DR. TOGIAS: Thank you. Thank you very much to the FDA for this very kind invitation. We are thrilled to see researchers, industry, of course us, and the FDA, getting together to discuss this very important topic. I have the same disclaimer as a government employee, I just put it in a shorter sentence, but it's exactly the same issues.

I want to talk about what we, or I, consider as biomarkers of interest in allergy. And once I talk a little bit about that, I'll give you a few examples of what we're trying to do, in terms of attracting research proposals for

biomarkers. And then I'm going to go through a few examples of where, I personally think, we have some real gaps, or problems in some of what we use either as biomarkers or as endpoints in some of the trials.

But I think it is quite logical that we're looking for good diagnostic biomarkers in allergy, because in allergy, there is this big issue of, when does sensitization to an allergen reflect true disease or not? And that is, a very common problem we deal with. And obviously, these biomarkers would be applied in clinical trials, in epidemiology, but also in clinical practice.

We also care about prognostic biomarkers in allergy, because as you know, there is a high chance for a kid with, either recurrent wheezing, or food allergy, to outgrow these problems. And it will be great if we know from the beginning, whether that is going to happen or not.

Of course, we care a lot about therapy in the context of therapy in allergy. There is your classic predictive biomarker, which will tell

you from the beginning, if you put this particular patient on this treatment, what are the chances that this individual is going to do better on this treatment or not? And this always differs, again, in the world of allergy, based on two concepts, the concept of desensitization, meaning that you are essentially reducing the chance of a reaction, versus the concept of long term tolerance, where you have modified the disease, which is something that theoretically can be done in allergy.

And then, we have the question, particularly in allergy and immunotherapy, of can we monitor therapy while it's happening, to predict whether it's going to be effective after a year, or after two years? Because, in immunotherapy we may have to treat for several years, and it would be great if, we know right away once we start it.

So here is what we would take, a monitoring biomarker, that can become, a response biomarker, in the middle of the therapy, and use that to predict success. Again, the issue of

tolerance comes here as well, because it would be wonderful, even in the middle of therapy, to know that, we have a response biomarker that predicts tolerance. So these are some efforts that we're doing in most of our RFAs these days, that are called NOFOs, or Notice of Funding Opportunity. We have entered elements that have to do with biomarkers.

This is our most recent COFAR RFA, and you can see there that, we're asking specifically for studies to accurately assess the incidence and prevalence for epidemiology. We need something more than, just somebody telling us they have food allergy, and studies to improve the diagnosis of food allergy, aiming actually at replacing oral food challenges. It's in our aims, this is another initiative that is on the streets right now, that have to do with vaccine and antibiotic allergy.

And again, you can see we are emphasizing the need for research for biomarkers to identify people at risk for reaction, or to

confirm reactions to specific antibiotics or vaccines. And this is our omnibus solicitation for SBIR/STTR, and some of you may be very interested in that, in that omnibus solicitation. When it comes to allergy research, we're emphasizing that what we really want is, to fund research for biomarkers as diagnostic markers, or disease severity, and predictive biomarkers for treatment, so.

Now why are we really interested? Well, we're interested in the biomarkers a lot because we want to facilitate, of course, like everybody in this room, the development of new therapeutics. But for us, from the perspective of a research institution, we want to make sure we can use those biomarkers to study subgroups, phenotypes, endotypes, that will allow us to understand the disease way better than we do today. And so, we want to use them in clinical trials, we want to use them in observational studies.

But there is also something else about biomarker research, and that is that, it can allow

us to dig deeper into the mechanisms of disease. So you, on one hand, identify a biomarker with all the uses that we talked about already, but then, at the same time, it unveils all kinds of aspects of disease that we didn't know, and so we have double interest in those biomarkers because of this reason.

Now, I'm going to take you through a couple of examples, not to show what we're doing in general, but only to raise some concerns and some discussion, about things we are not sure about. We need more research, classically, in the airway allergy situation, what we use, as you know, to enter patients in a trial, but also in clinical practice, is the combination of symptoms and irrelevant allergic sensitization. That is the gold standard.

However, what do we know from epidemiology? We know that percent of the US population is actually, sensitized to at least one aeroallergen. And we also know in the case for allergic rhinitis, that the symptoms of allergic

rhinitis are pretty much the same, with slight differences from those of non-allergic disease. And we also know very well that, allergic and non allergic disease probably are superimposed, there is something we call mixed rhinitis, so we have a conundrum here.

Do we know for sure that if we choose patients who have symptoms during the ragweed season and the ragweed allergic, that these are patients with allergic rhinitis to ragweed? We certainly don't, I think, and this is an example of one more, observational study we did, with the Inner City Asthma Consortium. We took children with asthma and followed them for an entire year, in terms of symptoms of rhinitis. By the way, 96 percent of these children had rhinitis.

And you can see then, clustering their symptoms in terms of their seasonal variability, that there are two top clusters. One of them shows a typical spring and fall peak of symptoms of rhinitis, and then the other one, which we really didn't know that it existed, seems to be

showing a late fall and winter peak of symptoms. And these are the top two clusters in terms of severity.

So we hypothesize that, perhaps they differ, really, in terms of their allergic sensitization, that one of these groups, for example, may be much more allergic to trees and grasses compared to the other, and here's what we find.

These two groups absolutely have no difference in terms of sensitization to any tree, to any grass, or to mold. So all of a sudden, we have this reality, which is those clusters, but we cannot necessarily say that, what differs between them is, sensitization to a particular allergen. So that does not matter that, within one of these groups, there are children who do have an allergic problem due to those allergens. But what it does say is that, once you select on the basis on symptoms and sensitization, you may have very well a good number of participants in your study, for whom wear allergy is not relevant.

So we do need more diagnostic and predictive biomarkers in the case of allergic rhinitis. And of course, one of the discussions that we all know has been going around for more than a decade, probably a couple of decades, is the story of, whether we should also be using an allergen challenge. And I'm looking at Stefan because he knows that this has been a strong, big discussion in Europe.

Here's another problem. Allergen immunotherapy and IgG4, I think that most clinicians will actually say that, IgG4 increase in the course of allergen immunotherapy is an indication that something is happening, and that many people still believe that. There is, a relationship that is causal in that, IgG4 increase does help in the induction of desensitization or tolerance in large immunotherapy.

This is, a negative study, that was conducted by the Inner City Asthma Consortium with cockroach subcutaneous immunotherapy, it hasn't been published yet. Ed Zoradi is the principal

investigator. What you see is a beautiful effect of SCIT on increasing IgG4, with no effect on placebo, of course, but absolutely no clinical effect.

Now, the outcome here, the endpoint here, is a nasal challenge, as opposed to a field type of study. But even here, you can see a big disconnect between IgG4 and symptoms.

And you can say, well, why didn't we know about this before? Well, this is a negative study. Most of what we see are, positive studies of allergen immunotherapy, and of course, in positive studies, if you're giving the allergen, and that is the reason why IgG4 gets increased, you're going to see some form of a relationship. Here we have a negative study, and we still see IgG4 going up.

Now, in food allergy, one of the issues that we feel needs to be addressed is, the issue of oral food challenge. It is a major advantage that we can do oral food challenges, and that we can actually take medications to approval on the

basis, of oral, food challenges. And we use them for diagnosis, we use them for efficacy of therapeutic interventions.

But we need to consider that oral food challenges have a list of issues, safety considerations, a personal problem to those parents, and those little kids that are exposed to these foods that they know they may react to, and what it does to their psyche.

Time consuming, expensive, partially objective, and still without adequate standardization. Although there is progress in terms of standardizing, but sometimes even the way that, the data are, presented following food challenges, are problematic.

This is a comparison of three major studies, two in the New England Journal, one in Lancet showing efficacy of either, epicutaneous immunotherapy, The Epitope Study, or oral immunotherapy, The Palisade Study, and impact that was done by the immune tolerance network. All I want you to look at is the response to placebo,

because on one study is 33 percent, in the other studies is less than 10 percent.

This is not because of the methodology. It is probably because there are criteria at which a response is, called a response, which are different between these studies. And I'm not raising this to criticize Epitope, I'm raising this to say that, for any reader of these studies, unless they really go deep and think about what is the difference, they will think that there is a problem either in impact or in Epitope, that the response to placebo is what it is. So those are flags for us, that we need more, we need to do more.

So the value of a biomarker to replace oral food allergens, or to use in food allergy also has other issues. We need to deal with the type of allergen, so every single different food allergen may actually behave differently.

And we need to take that into account, and the age of patient. Dr. Yao raised this issue and we are very, very sensitive to the fact that

it's a very different thing to diagnose, or to treat food allergy in infancy, than it is at five years of age. Presence of risk factors already present, those may change the behavior of a biomarker, the stage of diagnosis, is it done before, any exposure to that particular food has happened, versus after years of eating this particular food? And of course, the nature of the therapy.

A biomarker may behave actually differently when we're looking at allergen immunotherapy versus, let's say, a microbiome manipulation.

Just an example of, behavior of biomarkers in the diagnosis of food allergy, this is Corinne Keats work. And in this analysis, she took infants younger than 12 months of age, and they had not been exposed, they've had risk factors for peanut allergy, but they had not been exposed to peanut. And she identified, of course, a number of them that, even at infancy were already allergic to peanut. And what she's

showing is, in her case, that RIH-2 seems to have the strongest accuracy and predictive value for that diagnosis in infancy, in children who had not been exposed before.

However, Corrine herself has published another paper a few years ago. When she's looking at five to seven year old children, things are not looking so good for RIH-2. And that's again, the important point, that we need to take age into consideration as we're looking for biomarkers, at least in children.

And the other point I wanted to make is that we may want to be looking more carefully at models of biomarkers, as opposed to a single biomarker for diagnosis. And this is an exercise by the Leap Group that looks now, in children who had undergone oral food challenges, what else would be predictive of their response to oral food challenge?

In their case, they combined, as you see here, peanut skin testing, RIH-2 antibodies, peanut specific, IgE, IgE to RIH-1, and IgE to

RIH-3, and they come up with an AUC in the rock curve, that looks pretty good, despite the fact that sensitivity may be, in my opinion, still an issue, but. It is a concept that, I haven't seen many people pursuing. In my opinion this needs to be, pursued more, it may not be, in other words, a single biomarker. That will give us the answer.

So, planning for the future, at least from my perspective, what needs to be done?

In the field of allergy, we need two types of studies. We certainly need observational studies, we need large, prospective, longitudinal observational studies, and we are already doing one. Most of you must have heard of our new, birth cohort and the Sunbeam birth cohort, where we will standardize methodologies, standardize clinical evaluations, standardize collection and handling of samples, as one of the previous speakers emphasized.

And we would be looking for diagnostic and prognostic biomarkers for susceptibility and risk. And we also have to, then take clinical

trials into account. And here we need, as again, it was emphasized, multiple clinical trials, where the same kind of approaches are going to be followed, looking for predictive monitoring, response, diagnostic biomarkers.

How can we do all that? Obviously, NIH will do as much as it can. We have a limit of what we can fund, and we will fund whatever we can fund to do this, but it really requires a collaboration between us, and investigators, and the industry. And it needs the input of the FDA, which we are getting, and we want to get more of that, so that this effort is an effort that is with the future in mind.

And then there is a need for a platform where these analysis can be done, that is an open, public platform. And what we're really thinking is that in the future, we need to have something like an allergy data commons, where it's not going to be simply a repository of data like we have with import, which, as you know, you can put all your data in there, but it needs to be downloaded.

But it will bring out a platform where people can actually analyze these data towards mixing, bringing multiple trials together, and allowing for these biomarkers to be produced and validated. So, thank you for your attention.

DR. KASLOW: Thank you, Dr. Togias, actually, come back this way. We're going to have everyone come up, the presenters, to come up to the table here, and I'll start by thanking all of the presenters and inviting them to have a seat up here, and hopefully, we still have Dr. Beger online.

What we'll do is, we've got maybe about 15 minutes for questions and answers. For those in the room who have questions, please come up to a mic in the middle of the room. Please have a seat, and just state your name and affiliation, and we will also be looking for - thanks, Ron.

DR. DRAZEN: Jeff Drazen, from Boston - What I'm taking away from this is that biomarkers are probes into disease causality, where diagnostics are generally syndromic rather than

molecular. We don't understand diseases at a molecular level in many cases, heart failure, asthma, hypertension are many examples, and that we, in a way, use biomarkers to give us a more quantitative and precise definition of a disease. And then, therefore, by using biomarkers, we may be able to divide what we have, large disease categories into smaller, more therapeutically approachable diseases.

So to some extent, biomarkers are teaching us about biology of disease while we do clinical trials, or while we do observational studies. So how does FDA feel about the identification of biomarkers in quantification of them, so that it can be a standard biomarker?

I think Alkis is showing that the various tests for IgE versus skin tests, which actually turned out to be the best. So that when I use a biomarker, I know I'm using the same biomarker that someone else is using, because otherwise we have heterogeneity added to a problem of trying to do disease discovery.

DR. KASLOW: Thank you for the question, I think I'll first turn to Dr. Siegel, I'm sorry, Dr. Siegel.

DR. SIEGEL: So I think you're raising an important question, which is if you have different assays for a particular biomarker, how do you know that you're measuring the same thing?

And this is a difficult question. I think that consortia of academic groups with government and sometimes involvement of industry can be helpful to create standards, for what's being measured in the biomarker qualification program. We qualify biomarkers, and we don't qualify a particular assay.

So if you think about high sensitivity CRP, we don't qualify one particular assay for high sensitivity CRP, we use measurement of one assay for the qualification, and then that biomarker is what's qualified. Someone who wants to use a different assay to measure the same thing, needs to show that what they're measuring is the same thing that's being measured by the one

that was used for the qualification.

So when we qualify, we put the information that was used to qualify the biomarker, on our public website, and the specifications that would be part of that qualification would be publicly available. And others, who want to develop a new assay, would want to mirror those specifications to make sure they're measuring the same thing. I know your question was quite broad and that I was just answering one part. Did I at least go part of the way to answering your question?

DR. DRAZIN: Most of it, but just the National Bureau of Standards provides standards for things. Is there an equivalent for standards for biomarkers?

DR. SIEGEL: It would not be in my group, we work collaboratively with NCTR and other groups, and there are standards for, certain things, but I can't say there's a single place where there are standards set for biomarkers.

DR. KASLOW: Actually, before that, any

comments from Europe on that question?

DR. VIETHS: Maybe to the question of, having standards for it? When you do these companion diagnostics exercise you have to do, to show you're not forced to use one commercial test in Europe, you can use whatever you want, and then if somebody wants to address the same marker, you have to qualify, that your test has the same performance as the other test. And so, this is a kind of standardization of the approach, which I think is quite important in the biomarkers field.

DR. KASLOW: Maybe turning to you, Dr. Beger, in terms of reference standards, and your thoughts that may be related to that quality control.

DR. BEGER: Yeah, I mean, you can purchase for a lot of lipids, a lot of metabolic(?) labels, but proteins are a little bit more difficult. And obviously, you can generate other ones for the other omens, but there are a lot of ones that it's very difficult to get standards for. That was a very valid question.

Will you do that please?

DR. SIEGEL: I think there may be one other way to answer the question that may be helpful. So what we qualify in the biomarker qualification program are, use of biomarkers in drug development programs.

There's a separate way to get approval of biomarkers as in vitro diagnostics through the Center for Devices. So they would qualify a particular in vitro diagnostic test, for a particular use, and then, other tests that want to be used, would have to be cleared based on having similar specifications to the initial one that was cleared. So in that case, when it's a device that's been cleared by the Center for Devices, there's a clear pathway to showing equivalence between one test and another one. Hope that's helpful.

DR. KRISHNAN: Gary Krishnan Eli Lilly so, from a sponsor standpoint, more and more, we realize that the information around how a drug responds, or doesn't respond in a disease is

really embedded in the target tissue. And rarely do we see that signal show up in more accessible samples like serum or urine, et cetera. And the question to the panel is: What is the burden of evidence that one needs to generate?

Because a lot of these are ideated in the clinic as we progress through phase two, and by the time we refine what to measure, and why we should measure, the train has left the station of phase three. So can the panel opine on retrospective evaluation? What is the burden of evidence, how do we overcome this latency? That's perhaps inherent in how we discover and develop.

DR. KASLOW: Dr. Siegel?

DR. SIEGEL: So I think it all depends on the type of biomarker. Obviously, surrogate endpoint biomarkers require much higher level of evidence than a prognostic biomarker, or a diagnostic biomarker.

I think it is important to recognize that when you assess a data set for the performance of a biomarker, you need to verify

that, in an independent data set, to have confidence that the hypothesis is borne out. So if you can work into your clinical development program, that process of hypothesis generation and then hypothesis testing in a separate data set, that can be very helpful for those purposes.

DR. KRISHNAN: I think you're right. And the challenge for us is pre-specifying, having enough time to analyze the data in phase two, in a real world situation, and then pre-specifying it in phase three.

DR. KASLOW: Great, thank you, for your question, it sounds actually that question had, I think, two parts. One was a temporal part, but I thought I heard another part of that question being a compartment problem, which is, we have limited compartments that we can sample, like the blood, but oftentimes the action is, someplace else, and so, any thoughts on that?

DR. TOGIAS: Yes, please. I think this is a very important question, and we haven't figured it out in allergy, but again, in allergy,

we do have tissues that are accessible quite easily beyond blood and urine, for respiratory allergy, especially for allergic rhinitis, the nose is very accessible.

And through the Inner City Asthma Consortium, and there will be a speaker discussing this, we have established, essentially almost an invariable use of either nasal swabs or nasal lavage to continuously measure biomarkers there in transcriptomics or other omics, that will help us go up to the tissue, and. I think Dr. Altman will discuss how that doesn't necessarily agree with what we see in the blood or in the skin.

Another methodology, simple methodology, has also been devised in, atopic dermatitis with the skin tape strips, a very interesting methodology where you can certainly assess a lot of functionality and structural aspects of the skin, with a very, non-invasive way.

So those are techniques that I think should start being used, even if they're not at this point validated as true biomarkers, but

should start being used in clinical trials just to see what we can get out of them.

DR. KASLOW: Okay, so there was just one question from Dr. Rabin, just one second, Ron. I think one of the things that I want to emphasize, I think Dr. Stein and others have sort of implied or explicitly described, is to know translational medicine.

We've sort of grown up with the idea that it's benched to bedside and it moves in one direction, right. That you have a hypothesis and you look at some of these in vitro, most of those are biomarkers. You look at mechanisms and then you say, okay, well, I think I have enough, I'm going to move into the clinic, and it's going to be clinical from then on.

But I think what we're saying with biomarker utility in drug development is that it doesn't always move in that direction. If we can collect as much information as we can, on those biomarkers you use, to sort of jump to the bedside and keep evaluating those biomarkers, and others

all along in clinical development. I think to that one questioner's point that, you may create that sort of evidence that can support at the time you're doing that clinical trial, if you've followed those biomarkers all the way along, it can give you a lot of information.

So I think the idea is that, translational medicine isn't necessarily all just one way anymore, that it's kind of got to move back and forth, to ultimately make the most use of the data that you're collecting in a development program.

DR. KASLOW: Dr. Siegel?

DR. SIEGEL: And then we'll go to Dr. Rabin, and I'd like to make a plug for substudies. We all like to find the most convenient source of samples so we can study lots of people, which is usually blood. But recently I've heard several examples where biomarkers in the CSF aren't reflected in the blood. And the AMP study of rheumatoid arthritis found a lot of very insightful findings by synovial biopsy of the

joint, and none of that was reflected in the blood. So if you can incorporate a small substudy with more intensive sample collection and a biomarker study as part of the larger study, that can be a very helpful way to get some important scientific insights.

DR. KASLOW: Okay, we have now two questions, virtually, one for Dr. Yao.

Is there an FDA age definition for children? At what age is a person considered an adult in the context of clinical studies? And is there an age definition for transition from newborns to children?

DR. YAO: Yes, so I'll direct you to a guidance that's been published out for a while now. It's called: The ICH-11 R-1, which is an ICH guideline that describes basic rules and concepts related to pediatric therapeutics development. There, we provide very explicit sort of age cut offs for what's considered a neonate, what's considered an infant, a child, adolescent, et cetera.

Now, I will say that, regulatory definitions differ, even within FDA, so if you look at CDRH, the Center for Devices, their cutoff for child to adult is 21, for CBER and CDER, we consider the cutoff to be 17.

And my point in bringing that up is that, regardless of what regulatory definition you see or you reference, the point is, scientifically, we want you to base your development programs on the populations that, make know age is actually a biomarker, too, and I would argue, a very poor biomarker in most cases. So we really want you to think scientifically, about what age groups you really need to be studying, or apply, as opposed to just. Well, FDA says a child is two, so we'll, just study down to two.

DR. RABIN: Okay, thank you, and the last question before the break, then.

Has the FDA become aware of biomarkers that are not intended to be used in drug development, but rather commercially, such as genetic screening services? If so, would these

products be subject to the same regulatory requirements? Anybody, want to take that?

DR. SIEGEL: Yes. So the question has to do with biomarkers that are not intended for use in drug development programs.

DR. RABIN: Yes.

DR. SIEGEL: I guess what the questioner is saying is, if something pops up on 23-and-me what would the FDA do with that information? I think that's kind of where the question is coming from, not sure.

DR. RABIN: We'll deal with it when it happens, I guess.

DR. SIEGEL: Yeah, you can ask me. This one's not going, why don't you go ahead? I guess I don't completely understand what the question is, so: What would the FDA do if a biomarker pops up on 23-and-me?

DR. KASLOW: I mean, I guess it really depends on the context of use. How are you going to use that information, and what medical intervention or action are you going to take based

on that information? So I think it's the usual. It depends on what the context of use is.

DR. VOLLMERS: Well, thank you for saying that, because that's exactly where I'm going, so. Thomas Vollmers, allergist in the Food Allergy Biomarker Alliance.

What I want you to speak more on is, really context of use, in the context of food allergy in a surrogate biomarker, and which clinical trial designs are going to provide the most evidence, to get to a surrogate endpoint? And keep in mind, context of use, does that make sense?

DR. SIEGEL: So the question is: What clinical trial design would be most helpful to get the evidence to validate a biomarker as a surrogate, endpoint biomarker in food allergy?

In food allergy, I would give a general answer for any therapeutic area, and you could apply this to food allergy. It would have to be a therapeutic study, where you see an impact of the therapeutic intervention on the biomarker, and

you're also, collecting evidence on later clinical outcomes.

And if you see a very strong correlation between the therapeutic intervention and the clinical outcome, that's evidence to support that the biomarker can be used as a surrogate. If you then see that, in a variety of different therapeutic trials, with different classes of drugs, where the change in the biomarker corresponds to a certain change in the clinical outcome, and that relationship is preserved across drug classes, that would be very strong evidence to support.

DR. VOLLMERS: Thank you.

DR. KASLOW: Just highlight that point that came up multiple times, I think with Dr. Yao, Dr. Stein, and others.

What are the underlying assumptions you're making in those studies, as it relates to mechanism, populations, et cetera, et cetera? So, really understanding, what are you assuming as you're designing those clinical trials? One last

question is that, yes, one last question.

DR. VON MUTIUS: Erika Von Mutius from Munich in Germany, I'm a pediatrician, and. If you would consider running a study where you want to identify the biomarkers, and you want to have multiple evidence, like you have mechanistic studies, normally analogy, this would be mouse studies, and you would have clinical studies, or epidemiological observations.

What I've always been puzzled about is the mouse models. There are so many ways of, having mouse models. Is there any standard that the FDA or the IMA would like to see? Is it about haste mite? Is it about cockroach? Is it about Alternaria? Is it an acute model? Is it a chronic model?

I mean, there's so many of these, is there any sort of standardization in that way? That because, we know that these models do not necessarily reflect what we see in epidemiology, or what we see in clinics, I'd like to sort of get your feeling.

DR. KASLOW: Do you want to take that one first?

DR. VIETHS: This is a new battlefield. I think, in general, the models are useful for a certain purpose, I would say. And so, it's very difficult to draw general conclusions from a very specifically designed, animal model, to extrapolate to humans. So we would look at it in a very specific way, we would think they are, mostly hypothesis generating, especially in the field of biomarkers, and then, you have to validate your hypotheses.

DR. KASLOW: Dr. Siegel?

DR. SIEGEL: So the question of, how animal models support use of biomarkers in humans is one that comes up very commonly, across many therapeutic areas.

Again, going back to the symposium, from the accelerating medicines program, a couple of weeks ago, from the foundation of the NIH, they spoke about studies of lupus and rheumatoid arthritis in humans, and. Found that the

mechanisms that were driving disease, were quite different than, what had been seen in animal models.

So those would be situations where the animal models actually did not offer useful insights for the human. But there are other situations where, animal models mirror the human disease very closely.

So I think that, you would want to put together a dossier of evidence, indicating that the manifestations of the disease in the animal are similar to in the human. The interventions that impact the human impact the animal in a similar way, and other biomarkers that behave a certain way in the human, behave a similar way in animals. That's a tall order, and often you won't see that.

Another way to look at animal models is they may be a way of assessing one particular aspect of the pathophysiology, but may not be relevant to the whole disease in humans. Hope that's helpful.

DR. KASLOW: Dr. Togias, you want to say anything on animal models, from NIID perspective?

DR. TOGIAS: No, nothing to add, I mean. I really see a two- way approach to animal models. On one hand, they do give us hints about mechanisms and ideas about biomarkers. And many times when you do the research in humans, you will get a lot of ideas that in order to get to the bottom of them, you're going to have to bring them back to an animal model, and then look at what exactly the observation in humans means.

DR. KASLOW: Great. Okay, I think we should probably leave it at that. Thank you all, and thanks again for your presentations.

DR. HERSHEY: Our first speaker is Dr. Arshad from the Isle of White Study, Southampton UK. Good to see you, again, wonderful study looking at the natural history and risk factors for the development of allergic disease. So we're really happy to have him kick off this session. Thank you.

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DR. ARSHAD: Thank you, for the kind introduction, and the FDA organizing committee, for inviting me, to participate in this symposium, and I'm so sorry that I couldn't travel.

I got a moderately severe claim. I felt better not share that, so my task today is, to discuss the current clinical endpoints for efficacy and I'll try to summarize those.

Next slide, yes, I looked at the biomarker endpoints and another two resources from the FDA, and these are the categories of the biomarkers that are listed, and try to put the asthma related outcomes onto that.

Next slide, next hit, next hit. If these are the kind of clinical endpoints of biomarkers that, we use in different categories within the book, I notice that there are sorts of examples of different diseases, and biomarkers that are enumerated to show, how these biomarkers can be used in clinical trial, and. I searched for asthma and LSD, and didn't find even one example, where asthma and LRD was listed.

And, I suppose, that shows that we do not have the best markers that can qualify, and that's why we are here, to discuss more research is needed, and as we previously called so.

Next slide is just another example of what happens, when we don't have good biomarkers that reflect the disease activity. This was just copy and pasted from, protocol from a pharma company that contacted me last month for a biological treatment to be tested in asthma, and primary marker is analyzed as magnetization rate, and we'll come back to that, but.

There are 18 secondary endpoints listed, trying to cover all the aspects of asthma, partly reflecting asthma and the heterogeneous condition, but also indicating that, we don't have one or two markers, that can capture all the disease activity, or the endpoint.

Next slide. So there are markers for the diagnosis that would be used. If we are trying to prevent asthma, the aim is to treat, or manage asthma, with a new drug. We will be

wanting, to have markers, which reflect asthma control.

Next. The asthma control is generally gauged in two domains, one as a current asthma control, which would be symptoms or reduction in medication, and the next, which is equally important, is for any drug, which can reduce the future risk of adverse outcome, exacerbation, or future loss of lung function.

Next slide. Broadly, we can divide biomarkers that we use in asthma in, those which are objective and are reproducible, but. They may not be so relevant to the patient, and they may include lung function, for instance, which is widely used, easily done, but it is relatively insensitive. Especially for instance, pillow that has been used previously, it's not been found to be sensitive to various interventions. Airway responsiveness is most sensitive, but generally requires, more procedural sort of time and resources, and tend to have a weak correlation with many other asthma features.

Allergy, bone shape provocation test is very specific, because it's an experimental model, and mimics features of allergic asthma. But again, previously, about 20 years ago, this was used frequently at the early stages of drug development in asthma, but it was found that, those drugs which were found to have an effect in the allergen provocation model, did not then succeed, when they were biased with the exposure, so this model is much less commonly used now.

Next slide, so there are other patient relevant outcomes, which are increasingly more popular and used because they reflect what patient is experiencing. They are in three categories of symptoms, or the Q medication use, which is easy to gather information, but they are subjective and.

One of the problems is that, for symptoms, the definitions vary widely, and rescue medication use changes depending on individuals. The other is the corticosteroid reduction, which is often used. It's a desirable outcome for any

new drug that can be found useful for, asthma associated with various asthma effects. And the third, which is more commonly used now is exacerbation, partly because it is very closely related to the patient's well being, and long term health, but also it has implications on healthcare resources. One of the issues with exacerbation, as an outcome is that, used in different trials, in various definitions, including the number of exacerbation, number of patients with at least one exacerbation time to first exacerbation, or annualized penetration rate, and. The other issue is that, it might not happen in a shorter duration trial, because it may be not very frequent.

Next slide, the asthma quality of life is, assessed in many trials, partly because it is very relevant to the patient. This graph showing a combination inhaler, improving patient's quality of life, it's often used as a secondary outcome.

Next slide, there are various (in the next slide) a number of other instruments are available which provide very similar information.

Next slide, so as none of the individual outcomes that we have discussed are ideal, because they have advantages, but also some drawbacks, in recent trials, composite outcomes have become more popular. The rationale is that, these composite outcomes have more than one, different aspect of asthma features being assessed.

Secondly, most of these give a numerical score, so. Asthma controlled tests, and since it includes the symptoms, and medication, asthma controlled questionnaires, when you add, the lung function into that assessment, cells and, the last two compacts, and test, include not only the symptoms, medication, lung function, but also include a number of exacerbation. And that has the advantage that, if the exercise did not happen to the degree where, it can be assessed in the two arms of a drug trial, there are other outcomes that can be combined to give invasion of indefinite.

Next slide. This is a recent publication in ERJ last year from one of my

colleagues in Southampton, where they put together five domains of asthma: Clinical life, clinical outcomes, exacerbation use, cortical steroid, and asthma control, into a measurable instrument, to assess response to biological therapy, and this is developed separately for adults.

Next slide, a similar outcome has been, suggested for children.

Next slide, so I think this has been, previously shown that a good biomarker, or a perfect biomarker, if you like, should be easily measurable. It should have some partial link to the mechanism, it should be reliable, and reproducible, provide information about the disease prognosis and clinical outcomes, and be, cost effective.

Next slide, so this is borrowed from the Yaki Physician Paper, where they enlisted endotypes of asthma, and we understand and link it to the treatment, or type of treatment to be tested, as a relevant biomarker, and that has been used in the trials for these medications. For

instance, eosinophil in either in the blood or sputum

for asthma therapy, or IgE for olimizumab.

Next slide, so one of the issues with asthma as we understand it, is that it's not easy to characterize asthma into all the endotypes that we discussed, or shown in previous slides. And most commonly, a more practical way to endotype asthma is, to divide it into classified, into T2 and non-T2 asthma.

For T2 asthma phenotype, nitric oxide is often used as efficacy marker, and this recent paper showed that, it can be also used as a prognostic marker in T2 asthma.

Next slide, the recent paper again, also suggested that periostin may be a marker for T2 asthma. When they put together the data for periostin, compared to the FeNo blood use results in IgE, they show that the specific sensitivity is better than other markers, so this can be a candidate to be used, although it is harder to

measure.

Next slide, so as I said, for T2 asthma ESNFL is commonly used. It predicts response to Anti IL-5 therapy and one of the problems is that, as methyl can be raised in other conditions, and it's not specific for inflammation, and same is the case for phenol actually, that it is influenced by various other factors, but the advantage being that it is safe, and fast, and easy to measure.

IgE is also easy to measure, and has been, used but the cutoffs used are arbitrary, and have not, been validated. There are, other, potential markers, that have been proposed in the literature.

Exhale breath contains a very large number of molecules, which have been, put together in various publications. One called, Electronic nose, or in a different pattern, but by and large, this hasn't been found popular in the trials, because of the inconsistent results period we talked about for non-T2 markers, we do not have

good validated markers.

Neutrophils from the biology of the non-T2 asthma and some of the other cytosines, have been possessed, but they have not been validated. So that's where personal research is, so just also to point out that, when collecting the clinical endpoint, one needs to remember that. What is the type of treatment that is being, tested?

So for instance, the bronchodilator trial, lung function is very reasonable to be clinically the endpoint, but for a controller treatment, such as anti-IL5 therapy, exacerbation will be most appropriate. One for safety assessment, when the plasma cortisol would be good if the drug contains therapy.

Next slide. Another thing to remember is the time it takes, for the clinical efficacy outcome to be apparent. So for instance, symptoms and lung function might change with a drug trial within few weeks, and clinical trial duration, which is for two to three months, cannot have an

area responsiveness, which takes months to improve.

Next slide. So this is just as an example of what happens with different affected endpoints using the same intervention.

And this is a trial from about 20 years ago, by Woodcock in North England, where they used a mite impermeable bed covers in the primary care, and

mild to moderate asthmatic in a randomized control trial. The efficacy endpoint in this trial was, peak flow variation and changes in asthma medication and.

Next slide. No significant difference, you can see, was seen, but a more recent trial using the same intervention, that is the, mite-impermeable bed covers which was against for years duration, than the mite cover trial, but on this occasion the efficacy endpoint was, exercise and emergency department visit.

There seems to be a significant difference. Now these two trials had been done

differently, so it's not necessarily that, only the outcome difference made a difference, from non- significant to a significant trial.

Particularly the second trial was, done in children, but. This could be an example of where one needs to be very careful about selecting the outcome, which suits the intervention, so.

Now just touching on the efficacy endpoint for primary prevention, because when you're trying to prevent asthma, the endpoint is, asthma diagnosis in the two groups, so pediatric asthma diagnosis is challenging anyway, especially in early childhood. The question is

Bronchodilator is generally, suggested in various guidelines, with the addition of FeNO in the UK Guideline, but not in China. But by and large, in epidemiological studies, include a very large number of different definitions, that have been used because of the lack of the consensus.

This is the recent paper, which suggested that 53 different definitions were, used and that makes the problem. Asthma selecting

rather, got a clear outcome in asthma prevention of asthma so much more challenging.

Next slide, again trial was to show what happened. So this was a GAP trial, very well known in the asthma allergy world, where those who have grass pollen allergic bronchitis were given grass pollen immunotherapy in order to prevent the development of asthma, which is generally, these children have a very high risk.

The primary endpoint was, to prevent asthma, diagnose a symptom and a change in FEV more than 12 percent, and the trial fails to achieve that, which is disappointing. Given that the cost of analysis, when they did, to see what happens with all the individual components of asthma symptoms, medication use, and combined symptoms and medication use, as well as lung function were, all better in the intervention. There was certainly a sort of clinical indicator that, the allergoid therapy reduced, or did something in these children, where they had less symptoms, and did not require medication for

asthma for the clinical endpoint.

Another issue with the primary prevention trial is the duration, so often primary prevention is kind of agreed. It is generally agreed that, that needs to be implemented, or at least, it started very early in life, maybe in the infancy, or toddler age group, but. Asthma diagnosis cannot be, done until later in the childhood, which makes the trial very long, so we need some surrogate marker in between, which can be, assessed to indicate which children will develop asthma later in childhood.

And one of the exercises we did recently was to combine the large UK cohorts of about 7,000 children, and looked at various combinations of risk factors to see, what can predict asthma leak, in not only childhood but also up to the young adult life, and. After the serious modeling, we came up with the element of utilization, plus frequent B's, which you call Atopic frequency at the age of three to four.

Work, which will very strongly, indicate

those children who will develop asthma in the later childhood. Vaccine is possibly, used as surrogate marker and we show that, nearly 25 percent of these children who have ERW will develop asthma with area, become so.

Just to summarize, asthma control includes two components, level of clinical control by features such as, symptom control, and personally, and the risk of exacerbation, or future loss of control. And any biomarker or combination of biomarkers, should try to assess, or include both of these domains, and the neurons, and various numerous composites exposed, that have been used, especially compact in a set try, to cover both these aspects.

We need to also, remember that, asthma is a variable condition, so it is preferable to assess any marker, or biomarker, or efficacy endpoint, not just at the beginning and end, but also at multiple time points, during this trial duration. We don't have, a good objective efficacy marker, they're not that precise, and

there is a huge need for research in this area, which is, when selecting the efficacy endpoint to consider endotype of asthma, the type of therapy, and the duration of the trial. That can make a difference in the success and failure, and pro primary prevention needs, certainly need therapy months, thank you.

Next slide, the next slide, that's the end of my talk. Thank you.

DR. HERSHEY: Thank you. Similar to the previous session, the question and answer panel will happen at the end of this session, so I'm going to go ahead and introduce our next speaker, Dr. Sally Wenzel.

Dr. Wenzel chairs the Department of Environmental and Occupational Health at the University of Pittsburgh, and the title of her presentation is: Biomarkers and asthma endotypes.

DR. WENZEL: Thank you, Nero, and thank you to the organizers for the opportunity to present today. I really enjoyed the morning so far and I think it's been very provocative.

I was wondering in advance, all right, so I was given the topic of biomarkers and endotypes, and I think we're not quite to endotypes yet, so I'll give you some of my explanations.

In 2024, I think we're moving from molecular phenotypes to endotypes. Asthma has made a lot of progress over the last 15 to 20 years, looking at clinical characteristics, looking at molecular characteristics, and really, I think, starting to come up with molecular phenotypes, as opposed to just clinical phenotypes. But over the next several years, I think we need to continue to incorporate responses to specific targeted therapies, the biologics that are available to us, and really define the pathways that define endotypes.

So at least from my perspective, and a few other people, defining an endotype means actually discovering and treating the causative pathway of a disease that, when you modify that pathway, you substantially improve and maybe even

cure that specific disease, or endotype, so we're not yet to endotypes.

We think of asthma as an umbrella. It's got a lot of different heterogeneous clinical symptoms, exacerbations, lung function changes, and I think, at least in 2024, we can certainly define it by Type-2, the presence of Type-2 inflammation, or the absence, or at least low amounts of Type-

Inflammation, and. Type-2 inflammation of course, alludes to the activation of cytokines IL 45 and 13, which we now can generally identify using reasonable biomarkers, eosinophils and exhaled nitric oxide. When we think about that, low or no Type-2 inflammation, we really don't have any biomarkers for that, it's really the absence of the Type-2 biomarkers.

Now, I said these are general biomarkers, and I really do mean these are general biomarkers. This is an example of why, I think they're quite general.

This is a study that we did now, 10

years ago, looking at epithelial brushings, and looking at gene expression in the epithelial brushing, and relating it to exhaled nitric oxide. And so, we took actually the top 500 genes that were related to exhaled nitric oxide, and then clustered them, and.

In this, checkerboard that you see up here, every column, is a patient, every row, is a gene. I think what you can see is that, the patterns are really quite different. In those, of course, we had two clusters that appeared that had very low levels of exhaled nitric oxide, you can see those green highlighted areas, and you can see that the gene expression patterns in them are really quite different.

But, in addition, we had three different groups that all expressed very high levels of exhaled nitric oxide. The last one was really, probably what we would consider the most typical for asthma. As we were taught when we were in medical school, these were young people, highly allergic, 50 percent African-American, et cetera,

but then, there were also two other clusters that had equally high exhaled nitric oxide levels, where the gene expression pattern was, totally different.

I think, this really emphasizes that despite a similar biomarker, what's driving it, and that maybe, even the responses to it, can be dramatically different.

When I think about our current asthma biomarkers, I am clustering them into Type-2 molecular biomarkers, and of those, I've listed, sputum eosinophils, blood eosinophils fraction, exhaled nitric oxide, and a combination of exhaled nitric oxide and blood eosinophils. Then I'm listing IL-6 as another biomarker, but I'm not really categorizing it as T-2 or non T-2, I'm classifying it as a separate biomarker, in and of itself, so. I think sputum eosinophilia was perhaps the first, true asthma biomarker.

Certainly we've had atop skin sensitivity, but I think those are better biomarkers for allergy, as opposed to asthma, so I

think sputum eosinophilia was really the first, true asthma biomarker, we know that it's predictive of the efficacy of inhaled corticosteroids. There have been several studies that have been, published on that, as well as the efficacy of eosinophil targeted biologic therapy. We know that, eosinophils are, decreased when we treat patients with corticosteroids and Anti-Il-5 targeted therapies, with the decrease generally predicting the response. However, in the US, maybe not in some other countries, we're very limited in our ability to use sputum eosinophils because it's expensive to do, it's poorly standardized, and certainly not every patient can actually provide an adequate sputum sample, but. Here you can see, again is the example of the efficacy of an Anti-Il-5 therapy, which really only showed up when you targeted patients who had sputum eosinophilia.

All of the earlier trials that had been, done had been negative, but when you selected patients that had sputum eosinophilia in the first

pass, you were able to demonstrate efficacy.

Well, that was kind of a breakthrough study, needless to say, and so that was very exciting, that sputum eosinophilia could predict response. But we all knew that, that was going to be way too expensive, complicated, et cetera, so. The interest increased again in looking at blood eosinophils, blood eosinophils as a biomarker for predicting responses, to biologic therapy.

This was really, despite decades, I think, of research that had been unproductive in determining whether blood eosinophils, or its products, could be a successful biomarker. Overall, the previous studies had showed that, the relationships to sputum eosinophils, or lung eosinophils, was actually quite poor. And there'd been several studies published in that regard.

But lo' and behold, when bloody acidophils were used as a surrogate for sputum eosinophilia, in some of the early Anti-Il-5 studies, in fact, you were able to predict people that had about a 50 percent reduction in asthma

exacerbations, when they were treated with Anti-Il-5 therapy, using 300 eosinophils per microliter as the cut point. Again, I think you can see that, that efficacy curve is actually pretty, similar to what I showed you for sputum eosinophils.

Now, that's using kind of a random, random but certainly a specifically defined targeted cut point of 300. How do we come up with that 300? I think this is still, sort of, a moving target, this is an early study that was done with benralizumab, looking at improvement in FEV-1, on the basis of starting a, starting level of blood eosinophils going from 200, to 300, to 400. I think it's quite clear that as you increase the number of eosinophils in your blood, there seems to be a better response, so.

Where is that actual cut point? It probably varies from patient to patient, the context of the inhaled corticosteroid dose that they're taking, and maybe even whether they just had a viral infection not that long ago, or were

exposed to the neighbor's cat. So there's a lot of issues, I think, on what is the best cut point, that still remain.

And then, of course, you have to bring up the concept of, do you need to sample several times? How predictive is a single blood eosinophil count as a measurement of elevated blood eosinophils?

And this was, I think, a very important study published a few years ago, where patients in a placebo arm of an anti IL5 receptor antibody study were actually followed over the course of their study with multiple eosinophil counts obtained.

Basically, of patients entering this trial on placebo, 35 percent of those individuals were with low eosinophils on entry, so less than 150 eosinophils per microliter actually reached the 300 microliter threshold at some point in the trial, so really quite a bit of movement, but only 22 percent of those with greater than 300, dropped below the 150, so. If you get someone who's over

300, I think it's a pretty reasonable indication that they probably generally run high eosinophils, but if they're low, I think that, it does suggest that you should probably measure it several times, and, of course, if you use 150, you have even more movement back and forth.

Now, I already alluded to exhaled nitric oxide, and how exhaled nitric oxide can be elevated in a variety of different biologic situations, and I think the same is true for eosinophils. Just the presence of elevated bloody eosinophils, does not necessarily, give you the best indication of how well someone will respond to Anti-Il-5 therapy.

This was a study by Jean Blaker and colleagues, the reference got cut off here, that was published a few years ago looking at the predictors of, response to Anti-IL5 receptor antibody therapy.

You can see that, there were several reasonable predictors, certainly nasal polyps, perhaps not surprising, since nasal polyps are

traditionally associated with high levels of eosinophils in the blood, and oral corticosteroids, more severe disease, but then, there was another one, which was the age at onset of disease. So the later, that you got your disease, the more likely you were to respond to an Anti- IL5 receptor antibody, as compared to early onset disease.

The differences were really pretty, dramatic with about a twice as high a number of patients who got their disease later in life responding. I think this then has implications, and I'm glad we had the discussion earlier, about pediatrics, because I don't think you can apply the same standards from adults to children. When the study that was, done looking at mepolizumab in children showed efficacy, yes, indeed, the drug was efficacious in children with high levels of blood eosinophils, but.

The efficacy was much less than what we had seen in adults, really, about half of what we had seen in adults. And certainly, if you looked

at time to exacerbation, there was actually no difference in the time to exacerbation, so clearly differences, despite the fact that the starting bloody acidophils are, almost exactly, the same.

A little bit of a summary about blood eosinophils. Clearly inexpensive, well standardized, every laboratory in the country, and most of the world can do it. It predicts response to all current biologics, but many with elevated levels, will poorly respond. So again, it's very sensitive, but not as specific, levels are impacted by corticosteroids, and are variable. Cut points are loosely applicable, and you may need several measures to determine whether someone truly has elevated eosinophils.

They are not currently response biomarkers. For biologic therapies, eosinophils almost always, go to zero with all the Anti-IL-5 therapies that are out there without relationship to clinical response. And of course, with dupilumab, you almost always get either no change or, sometimes even an increase, and you'll still

get a clinical response, so really, not a very good response biomarker.

Let's move to exhaled nitric oxide. Unlike bloody acidophils, where you're really measuring a systemic compartment, this is a measurement of local lung airway inflammation. It's a gas measured in exhaled breath, it's generated by an enzyme called, Inducible Nitric Oxide, that is expressed in high amounts in the airway epithelial cells. It's induced by IL-4 and IL-13 in airway epithelial cells, but also induced by Type-1 cytokines. It's up- regulated in a range of patients, and generally responsive to inhaled corticosteroids, and for that reason, it's been used really as an indicator of poor adherence, but.

It can also predict patients who are going to be oral corticosteroid dependent, so it can go from very mild allergic rhinitis patients, all the way to very severe, oral corticosteroid dependent patients. So although it's helpful, it has a lot of, issues but it's probably as good or

better, than bloody eosinophils, as a predictive biomarker.

And these are data from the anti-TSLP study looking at the improvement in exacerbations with increasing levels of bloody eosinophils. And you can see that there's a very nice improvement in exacerbations, as bloody acidophils increase, but similar to that, you saw a similar increase in reduction in exacerbations as levels of exhaled nitric oxide increased, so seemingly pretty, good indicator for responses in exacerbations.

Now, the thing that differentiates exhaled nitric oxide from bloody acidophils, in my opinion, is that it's a pretty good response biomarker, so.

FeNO declines with Anti-IL4 receptor antibodies, and anti-TSLP antibodies doesn't decline with Anti-Il-5 antibodies, and this is from an early study that we did, looking at the effect of dupilumab on exhaled nitric oxide.

You can see that within four weeks there was a pretty, nice reduction in exhaled nitric

oxide. And interestingly, that degree of reduction in exhaled nitric oxide, actually correlated quite nicely with an R-value of 0.4 with the improvement in FEV-1, so it really did have a response that was tracked with the clinical response, but. Interestingly, it doesn't predict the reduction in exacerbations, that is actually separate.

I give you a summary of exhaled nitric oxide: Exhaled nitric oxide requires an FDA approved device to measure. The actual cost is low, but often, it's not approved by insurance. Absolute levels cover a broad range, no little correlation with severity or control. Cut points remain fluid, and contextual predicts response to Anti-IgE, IL4R, and anti-TSLP, it's a response biomarker for FEV-1 with Anti-IL4R and possibly anti-TSLP, but. The lack of decrease in FeNO to biologics, or corticosteroids, may indicate that there's complexities underlying that inflammation, which suggest that, the loss of that decrease is due to increasing complexity of the disease

itself, so.

Can an index better predict response? Bloody acidophils and exhaled nitric oxide alone, are of modest predictive value, so can we combine them? Can we combine a systemic biomarker and an airway biomarker, to lead to better prediction of responses?

And elevations in both, could in fact indicate, the greatest Type-2 inflammation. So I actually thought about this and went back to the severe Asthma research Program SARP database, an NIH sponsored study, and pulled out the patients in our dataset, and this is about 500 patients, and divided them by their bloodiest infills, greater than or equal to 300, their exhaled nitric oxide. Oxide greater than or equal to 25, in the 18 year olds and above, so this is only adults, and I think what you can see is, as you go across from left to right, the people on the left, less than 25, less than 300, have very little elevation of Type-2 biomarkers. On the end, you have those individuals who are elevated exhaled nitric oxide,

elevated blood eosinophils.

There's actually, five times greater sputum eosinophils in those who have both biomarkers elevated, as opposed to one or the other. There's a fourfold increase in the Type-2 gene mean, again, consistent with a lot of Type-2 inflammation. They have a 50 percent greater exacerbation risk, they have a lower FEV 1, percent predicted, and they have a higher bronchodilator response.

You do not see this pattern in children. I went and looked at the SARP database by children that relationship does not, exist in the children, so it's very specific to adults and asthma.

How does that apply to responses to biologic therapy? Certainly, we actually have some data that have suggested that that combination of an index might be better than either biomarker alone.

This is with dupilumab data, and again, the four boxes are generally here, with low exhaled nitric oxide in the orange box, the

highest exhaled nitric oxide and blood eosinophils in the green box. You can see looking at rates of exacerbation, as a start, the rates of exacerbation are considerably less in patients with less, elevations in these biomarkers. If you have one elevation or the other, the exacerbation rate increases a little bit, but when you have the combination, you have the greatest degree of exacerbations.

Then, when you intervene with the biologic, in this case, dupilumab, there is the greatest reduction in exacerbations, and it's independent of dose, both doses worked equally well. Are there any non Type-2 biomarkers? Well, as I began, the lack of Type-2 biomarkers currently defines Type-2 low, and Type-2 low may actually be a corticosteroid effect, that they have suppressed most of the Type-2 inflammation.

Sputum neutrophils, are highly variable, poorly predictive, CRP is, only occasionally, elevated, so what about IL-6? We know now, from several years ago, that high plasma Aisle-6,

associates with components of asthma severity, components of the metabolic syndrome.

This is Michael Peters' work it was, published, several years ago. High IL-6 bears no relationship to Type-2 biomarkers, and it can be present in patients with high Type-

Biomarkers, or in patients who have no Type-2 biomarkers. It certainly associates with more severe disease exacerbations. Lower lung function does associate with higher BMI metabolic abnormalities and, it also, interestingly associates with, higher all cause mortality.

In our SARP data set, it generally associates with poor outcomes across diseases. I actually think, it's a biomarker of badness, as it were, no matter what your disease.

It's also, interestingly, independently influenced by pollution, and we're going to present some of that work at ATS. This is just plasma IL-6 in relationship to exacerbations, again, in the SARP network. I think you can see that in the panel to your left, that as IL-6

levels increase, there is a very robust predictability of exacerbations measured longitudinally, which is actually better than the ability of bloody acidophils alone, to do that, so.

In conclusion, Type-2 biomarkers, bloody acidophils, and FeNO, have greatly improved our ability to phenotype and treat, asthma and severe asthma. The prediction of responses to biologics is imperfect, and they likely perform less well in children.

Blood eosinophils and IL-6 are all predictive biomarkers, but only FeNO and sputum eosinophils are somewhat response biomarkers. Development of FeNO blood eosinophil, maybe, even with and without IL-6 in adults, could greatly increase our predictive, our ability to predict the response to biologics, and.

Type-2 low asthma, remains with limited biomarkers, but in fact, I actually think that IL-6 may be the best predictive biomarker for the most severe outcomes, but we have no idea whether

it's a response biomarker.

Thank you very much --

DR. HERSHEY: We'll move on to our next speaker, Dr. Robert Hamilton. He's a professor of medicine and director of the Dermatology, Allergy, and Clinical Immunology Reference Laboratory at the Johns Hopkins University.

The title of his talk is: Component resolved testing and IgEE quality as prognostic and predictive biomarkers.

DR. HAMILTON: Thank you very much. Well, I've changed the title a little bit to focus on IgE antibody as an ideal diagnostic monitoring response, predictive and prognostic biomarker in respiratory allergic disease.

I'm going to extend it, not only to allergic asthma, but actually to other forms of respiratory allergic disease. My theme today is to try and, convince you that, it's time to transition from a single-plex technology that we've been using since 2010, when the NIH and the FDA held their biomarker meeting on asthma years

ago, to a new technology that's come out of Europe. It's cleared in Europe, and is poised to be maybe cleared by the FDA here in the United States.

First, I would like to begin by over viewing IgE antibody, identified as the core bought biomarker for atopy assessment in asthma studies that we identified in a 2010, NIH FDA biomarker workshop on allergic disease, very much like what we're holding today.

Second, I'd like to describe how the IgE antibody fits into multiple biomarker categories.

And third, I'd like to examine the pros and cons of the technology that was used back in 2010, that was available, was most cost effective, and how today we have a new technology, that we need to consider its pros and cons as well.

The allergy explorer, the microarray that came out of Vienna, the following of, the ALEX 2 chip IgE and, how it can actually help us in assessing atopic status in respiratory diseases such as allergic asthma. And also, it has impact

on food allergy, that we'll hear about later from Dr. Sampson and a variety of others.

I don't have to convince you that IgE has a central role to play in allergic reactions, and so therefore, IgE antibody can serve as a very good confirmatory marker for atopic status. That's what it was identified at this 2010 biomarker meeting, the presence of IgE antibody is a risk factor for allergic disease, and we all know that it needs to be linked with a clinical history to make a diagnosis, so it's not definitive, but it's very important.

As a risk factor, IgE helps us confirm changes in atopic status following exposure to environmental allergens and therapeutic interventions. It helps us confirm immune responses following exposure to environmental allergen sources, so it can be a response marker IgE antibody to select allergenic molecules components.

And part of my talk is to convince you that, we now have to begin looking more at the

component specific IgE responses, and so it can help us in identifying certain risk related risks associated with serious allergic reactions. And we've heard about Corey Keith's work with Area-2 that, maybe didn't span out in later studies in adults. We'll hear more about that.

Finally, IgE as a prognostic biomarker can identify the likelihood of allergic responses, only when it's linked with the clinical history, with a convincing, objective association of symptoms with temporal exposure to the allergen itself. So IgE antibody discriminates asthma subtypes very well, and possibly, as possibly the most important discriminator to help us define whether in fact it's allergic triggers, or it's non allergic triggers that you have to focus on when you're managing your allergy patient.

If you look at IgE in terms of the best guidance document listing of a biomarker, IgE has all the properties of a very well defined biomarker. It has UniProt code, it's found in serum, biologically, it's very, very linked. It's

linked well with the binding TIGEFC receptors on mass cells, and basophils and involved in the release of, mediators following exposure to allergenic molecules, and associated with, chronic inflammation in bronchi as it relates to allergic asthma.

Now, back in 2010, the best technology we had, which was the least expensive analysis, was the Phadiatop system.

This multiallergen screen on the Immunocap system, and as a single-plex assay system, it allowed us to get a discrimination between the presence and absence of, allergic disease to 10 major aero allergens, that we thought drove most of the allergic disease in the United States.

The problem was it was a plus or minus, yes or no. And every asthma study that was funded by the NIH after 2010 was asked to measure Phadiatop, as an indicator to separate atopic from nonatopic asthma, as a biomarker. In my lab, we've participated in a lot of NIH sponsored

analyses, studies such as, the echo crew, and a variety of other multicenter studies, all of which had Phadiatop as a target.

Now, oftentimes they wanted, after the Phadiatop positive response, to discriminate between the actual specificities of the allergens that are driving the allergic response, and that required measuring IgE antibody to individual allergens. The problem was that was very costly and really, not in the purview of most of the NH sponsored asthma and allergy, respiratory allergy related studies that we actually were involved with.

So back us to 2010 and this very important NIH and FDA sponsored biomarker study workshop. Alkis Togias was coordinating it at that point. We had many of you actually in that meeting, and of all the biomarkers that were identified, and. I don't want to diminish in any way, iron oxide or eosinophilic measurements, the core biomarker that was identified for recommendations for every asthma study that was

supported by the NIH, was the Phadiatop, the multi allergen screen, that allowed discrimination of atopic versus nonatopic disease, the presence of IgE antibody.

The problem was, at that time, and it was the best technology for the cost, it had ten aerology measurements, and so, it was marked as a core biomarker, and all the others were either supplemental or emerging. The publication that Dr. Scheffler, and Dr. Wenzel chaired and published two years after that meeting, sort of set the stage for NIH sponsored respiratory related disease studies to almost, require a discrimination of atopic versus non-atopic, status.

So the Phadiatop, let me just, for those of you that are not familiar with this technology, quickly indicate that, the Phadiatop has two components to it. It has a calibration component, where we have an anti IgE that binds to an IgE reference serum, it's the third IgE reference preparation, and it's detected by an anti-IgE 2-

site immunoenzymetric assay, on the other side of the assay system, so runs simultaneously. We have a solid phase allergen.

Now, in the multiallergen screen, we actually have 10 aeroallergens linked to that solid phase so. We're measuring IgE antibody to 10, one-to-ten aeroallergen measurements, and we add the patient serum, if we can add the patient serum, and then it binds to maybe one of the aeroallergens on that solid phase, and we detect it, with the same anti IgE detection antibody.

Now, the reason why this works, and it's reasonably quantitative, is because when you dilute out the calibration curve with the specific IgE measurement, they dilute out in parallel. So you can actually get close to quantitative measurements of IgE antibody. And in fact, the IgE antibody assay that we have today, is possibly the most quantitative antibody that's used in all diagnostic immunology today, because of this technology that has been fostered both by Thermal, Fisher and also by Siemens.

Now, the strengths of the Phadiatop, which were identified in 2010, were that it's a single screening assay, typically between 30 and $50 per measurement, and it could discriminate, or determine IgE antibody to the 10 aeroallergens that were believed to be, the major aero allergens driving most allergic respiratory allergic disease in the United States.

The Phadiatop in Europe had birch, instead of replaced birch with oak. This single test was required for most aeroallergen related NIH sponsored studies, and it required only 40 micro liters of serum, with a hundred micro liter dead volume. So the quantity of serum was very reasonable, the cost was very, very minimal, for getting this very key piece of information, but. What were its limitations?

Well, it was a qualitative measurement, positive or negative. She didn't know if it was a specific IgE response to dust mite, or to oak, or to any of the other tenor allergens that were identified. And now FDA really didn't want us to

indicate to you what the specificities were, but because we're moving way beyond the Phadiatop, to a multiallergen screen with almost 300 allergen specificities, this becomes irrelevant.

So we were measuring dust mite, cat dog, Alternaria, two tree pollen allergens, tree oak and elm, two grass pollens, the meadow fescue, Canadian blue, and the Bermuda, and then two weeds, ragweed, and then saltwort.

Now, the problem was, as we grew after 2010 we realized, well, we're missing a mouse, we're missing cockroach, both very important indoor air allergens driving a lot of asthma. It had no definitive specificity data. It was positive or negative. That didn't tell us very much, but it did help discriminate, at least in the early days, between atopic and non-atopic asthma.

And it didn't give us any information about what we know today to be, these molecular allergen cross reactive families, profilins and thermomycin. And so for all these reasons, along

came the group in Vienna that developed the ISAC, that was sold to Thermo-Fisher Scientific, and they developed a new technology called the ALEX Explorer.

The Allergy Explorer has a tremendous number of strengths and a couple of limitations.

It's a multiallergen specific IgE antibody, chip based array that has on it measures of IgE antibody to 117 individual allergenic components, sorry extracts, and 178 individual allergenic components. Very powerful technology, very, very amazing, requires 100 microliters of serum with a dead volume of 40, it's almost equivalent to the Phadiatop, but.

Its strengths are that it gives you quantitative measurements for all of these allergenic specificities and close to a quantitative, almost close to a quantitative measurement with greater than 0.3 units per liter, which is very close to that 0.35, which we all claim is clinically relevant for Immunocap. It can go down to 0.1, but at 0.1 to 0.35 there's

some question about the clinical utility of those measurements.

It gives you definitive specificity data. It gives you information about cross reactive, allergenic family IgE antibody reactivity, very important in both respiratory and in food allergy and, it's becoming even more important in venom allergy.

Now, limitation is, and I can see Bob Wood, the pediatric allergist who runs the clinic at Hopkins saying, this is absolutely, unacceptable. It's a fixed panel, so you're measuring IgE antibody to almost 300 allergen specificities, which means you're going to detect venom in a person who has asthma. Is venom specific IgE relevant to asthma? Well, probably not, but is it useful to know about it, well, maybe it is.

The other thing is, it detects asymptomatic sensitization. You can be positive for IgE antibody, but not have any clinically relevant objective symptoms, so the fixed panel is

really, one of the constraints, and.

This technology involves a chip. On the chip, there are almost 300 allergenic specificities marked in little dots, and it has, a number of the dots around it are actually controls, it has built in control, and it, believe it, or not, even has a total serum IgE that's been indicated.

Now, if you compare the IgE and the ALEX-2 version, it doesn't agree perfectly well with the Immunocap and the Immulite based on the College of American Pathology Proficiency Program that we have in the United States, so. The Phadiatop, as we know, has these ten aeroallergens, and I've marked in red the specificities that match really the Phadiatop. As I mentioned, what's missing is, at least for asthma studies, is Inner City Asthma Studies is mouse and cockroach.

And I want to also point out that, it has molecular molecules that allow you to discriminate and identify IgE antibody to all the

major 10 aero, and food, and venom, cross reactive allergen families. These are the profilins, the PR-10 family, the nonspecific lipid transfer proteins. And I'll go on and on, and you can actually look and look at it in the molecular allergology user guide, that was published by EAACI back, actually this past year, and the references at the bottom.

Now, to investigate the utility of the ALEX-2 in comparison to the classic Phadiatop, we went to University of Chicago, and they were studying two genetically identical populations. One was Amish and one was Hutterites, and the Hutterites had a threefold higher frequency of asthma than in fact, the Amish, and the question was why?

We knew that the Amish used non-mechanical farming horse plows and they rode around in their horse buggies to church, whereas the Hutterites actually used mechanical farming machinery, and they were very, very state of the art. We evaluated the atopic status of these

asthmatic children using, both the Phadiatop and the ALEX-2, to confirm the specificities with the ALEX-2, because I was a little bit skeptical of some of these.

I confirmed, a lot of these specificities, with the Immunocap individual measurementS. If you take and look at two groups, one group was the Phadiatop negative population, a total IgE, less than 100 international units per ML, and a negative Phadiatop negative for 10 aero-allergen specificities. The second group we looked at, were the positive Phadiatop greater than 100 international units of total IgE, and a positive Phadiatop.

Now, if you take just an example of the individuals that were negative for a Phadiatop, and had a total IgE less than 100, so they'd be really classically defined as nonatopic. And look at six of the individuals, three of them had IgE antibody, to allergens that either were poorly represented on the Phadiatop, such as the individual number two that had IgE antibody to

drop 23, poorly represented on the Phadiatop. Because of the limited binding capacity of the actual matrix, where you can't bind all the allergenic molecules from dust mite, when you have ragweed and all the others on there.

We also detected one of the children had an IgE antibody response to honeybee venom. Now these are farm children, they're out playing with their honeybee hives all the time, so that's relevant, but it's not relevant to asthma. It was relevant to the well being of the child. If we look at the second population time, second population, we have a discrimination zero allergens, such as all of the grasses in the trees, but also detected IgE antibody to aspergillus.

Now, alternaria was on the Phadiatop, not aspergillus, but isn't aspergillus as important as alternaria for sensitivity to respiratory disease? And also we detected, IgE antibody to rabbit, these kids all happen to have rabbits as pets, so those were detected in

addition to the aero allergens.

Finally, the IgE antibody measurements with the ALEX-2 allowed detection of the specificities to the allergenic components. And the example I'll give you is an individual child who actually had IgE antibody not only to some of the major aero allergens detected in the Phadiatop, but also to the profilins, where they had some reactive, some oral allergy symptoms related to melon, because of the cross reactive molecules between the profilin melon and the bridge pollen responses.

Now, the two challenges I see with merging, or moving from a Phadiatop to a multiallergen ALEX-2 are twofold.

First, there's just an overwhelming amount of information that's provided here.

You get 300 allergen measurements, many of which are extract and component comparable. So you would detect similar, but the company also has allowed us to get information very rapidly, just a minute. Allowed us to get information rapidly by

using software called Raptor, which calculates the data rapidly, and then Raven, which actually allows interpretations using sophisticated AI related information.

The second challenge is really, the asymptomatic sensitization.

I know some allergists are going to really be, unhappy with detecting honeybee venom in a person who, they would only search out for IgE antibody to the aero, allergens or, to a food allergen. Like Bob wood, that just really would say, this is ridiculous, I shouldn't be detecting IgE antibody, I can't explain, because there are no symptoms in this.

This is just a simple summary to show you where in fact we stand. The Alex-2 is pending clearance with the FDA, it can't be used right now, probably only for NIH sponsored studies.

It measures many, many more allergen specificities, the amount of serum needed is comparable to the Phadiatop, the cost is about threefold higher, the sensitivity is slightly

less, but it gives you quantitative data. It allows you to deal with this issue of carbohydrate, cross reactive, determinants that, it deals with directly by an inhibitor.

One of its limitations, well established, is the high levels of IgE and IgE-4 antibody after immunotherapy can inhibit the actual binding of the IgE to the limited amount of binding capacity on the chip.

One last thing, I have to go, and I would like to suggest to you the following: We are starting a brand new research study called the US atopic status study, which will follow in the vein of the N. Haynes, which will be done in two years time. We are recruiting new fellows from 15 centers in the United States to collect serum on 100 individuals. Send it to us, and take a standardized history, and they will receive an Alex-2 profile, plus an Immunocap profile, and AI supported interpretations of those data.

The educational opportunity -- you're going to push me off, one second, I'm almost done.

I view this as a great educational opportunity for new fellows coming into allergy, where they'll learn about IRB submissions, about the power of molecular allergy, and also get their name on an authorized paper that's peer reviewed.

And with that, I'd like to say that -- it's maybe now time to begin considering the new technology on the block for discriminating atopic status, the Alex-2. But we have to remember the golden rule of diagnostics, and that is that asymptomatic sensitization tells us that, even with any method that we measure, we detect IgE antibody, we have to always link it to the clinical history, objective temporal relationships between the symptoms and the exposure, to actually make the diagnosis of allergic disease with that.

Thank you very much for being patient, appreciate it.

DR. HERSHEY: All right, thanks everybody. We're going to break for lunch now, and as Ron said, there's going to be food available, I think, at the same place where coffee

was available this morning. I'm not sure how long it's going to take to get all through there, so I'm a little reluctant to shrink our lunchtime. So let's plan to meet back at 12:15, okay.

(Recess)

DR. HERSHEY: All right, everybody, we're going to go ahead and get started. It's 1:15. This is continuing Session 2, Biomarkers in Respiratory Allergic Disease. So please take your seats and I'll introduce our next speaker. Our next speaker is Dr. Matthew Altman. He's an Associate Professor in the division of Allergy and Infectious Diseases Department of Medicine at the University of Washington, and he's the head of the allergy section. The title for his presentation today is Host Prognostic Biomarkers for Childhood Asthma. Thanks, Matt.

DR. ALTMAN: Well, thanks, Nero (phonetic), and thanks everybody. Hope everyone had a good lunch. And I'll start slow as people make their way back in. But it's a

pleasure to be here. So, Nero mentioned the topic, but I'm going to talk about prognostic biomarkers for childhood asthma, and I'm going to talk largely about some of our work, which is through the Inner City Asthma Consortium, now CAUSE Consortium, as sort of an example I think, more than any currently established biomarkers, but an example of what we're doing and what can be done.

So, we actually got this background. I'm going to just have like two slides on background and then kind of get into the current science. I think Sally Wenzel set this up very nicely. This is just to kind of give an example that there are certain biomarkers that we use in asthma. This is actually adults. They've been nicely summarized in this New England Journal article from about a year ago, and they are, in effect, blood eosinophils, FeNO and the presence

or absence of allergic sensitization. And certainly, those are useful, but as I'll show in a moment, they're far from perfect.

And again, just a little background. I have another talk where I go through this in detail. So how did we get to these biomarkers and why do they matter? Well, I think the example of blood eosinophils and anti-IL-5 therapies is a perfect example, and this is just to illustrate that we had several studies of, in this case, mepolizumab. They're the first four listed there outside the green box that attempted to use mepolizumab to treat asthma, asthma allcomers without a biomarker, and they failed. It was only when blood eosinophils or other metrics of eosinophilia were incorporated that we saw positive outcomes and led to the ultimate approval of the drug and really the explosion of biologics and asthma that we've had over the last decade or so.

And this is just to illustrate it. This was the Lancet paper showing the efficacy of

mepolizumab, specifically in those adults with sputum eosinophils, elevated sputum eosinophils, FeNO or peripheral blood eosinophils. Now, the trouble still with this, and this is what I'm going to illustrate in the next point, is that there's an efficacy there. You can see a reduction in exacerbations by about 50 percent, but it's far from complete. So, if blood eosinophils were truly a marker of response to this drug, you'd expect something closer to 100 percent reduction in exacerbations, which, of course, we don't have.

So, what is lacking with our current therapies, or r maybe I should say with our current biomarkers? It's to say that while they're effective in the studied populations like mepolizumab, in that example, all of them -- I mean, I showed you one of many RCTS looking at biologics in asthma, it's about 50 percent at best. Which means one of a couple of things. Either 50 percent of people don't respond despite having the biomarker of elevated blood

eosinophils, they all have a 50 percent response rate, or somewhere in the middle of that. And I think the reality is exactly that. It's somewhere in the middle. You have some people who have a perfect response, some people who have no response, and some people who have a partial response. And in part from not having a lot of molecular data in the studies, we don't know terribly well who those responders and non-responders are.

Just sort of, to some of the other points we heard earlier today. One, we don't actually have comparison. So, we have the same biomarkers in effect for all these drugs, but we don't know which works for whom and what biomarkers may be better for one drug or the other. And we don't really have molecular data of the response or non-response to best understand these drugs.

So, what we've been doing in the Inner City Asthma Consortium is to ask, can we use these rationally designed drugs, these rationally

designed biologics, but this could really apply to any therapy, to better understand the mechanisms of treatment response and failure. Which, if we understand the mechanisms, that should give us more precise biomarkers and then ultimately, more precise treatment selection. And to identify, ideally, novel treatments for those who don't respond to the current drugs.

So, I'm going to spend most of my time talking about this study that we carried out in the Inner City Asthma Consortium. It's called MUPPITS 2, and it's really just to illustrate -- and at the end, I'll give you several other studies we've done, sort of towards the same approach, but just to give an illustrative example. So, here we were looking at mechanisms underlying asthma's exacerbations, prevented or persistent with, in this case, mepolizumab therapy. These were 6 to 17 year old urban children with relatively severe asthma, at least two exacerbations per year, and they met the criteria of having elevated blood eosinophils.

And I don't write it here, but they all had allergic sensitization as well, with varying levels of IGE. And it was just a simple RCT of mepolizumab versus placebo, plus guidelines based management for a year. And with most asthma studies, the primary outcome was rate of exacerbations. I guess what set it apart, is what's listed there at the bottom, is we integrated a lot of ancillary mechanistic studies, and I'm going to focus really on airway transcriptomic profiling, though I'll draw a slight contrast to what we see in the blood because that was brought up earlier. We've also looked at sputum, blood flow, cytometry of the eosinophil and other things. But again, I'll focus on the transcriptomics.

So, the clinical question was pretty simple, does the drug work or not? And actually, Dr. Wenzel showed this outcome earlier, but in effect, it did. So, we did see a significant reduction of exacerbations, but not even at that 50 percent rate that I alluded to for most of

these studies. Rather, it was.73 rate ratio, indicating probably this drug is somewhat less effective in children, and maybe we can find out why. And I guess that's actually important, because if we want to get it, responders or non responders, we need this sort of clinical outcome.

So, then my question for the study was, as I alluded to, what are the actual mechanisms/what predicts responder status? So, I'll just sort of outline this in general. I won't go into a ton of detail. But as we heard from Alkis earlier, you can actually get a lot of information from the nose. So, in this case, we use sinus lavage, which is a heterogeneous mixed cell sample. We can get a lot of information from that. What I list here is virology, cell differentials, and host MRNA sequencing. Same with blood, and we can look at airway and peripheral response over time in these children.

This is the most complicated figure I'll show, so I'll walk through it somewhat slowly and you can, of course, read about it in detail in the

publication. So, from that nasal lavage sample, we measure 15,000 genes, the expression of them. In prior work, we had already done a dimensionality reduction to understand what are the fundamental pathways that you can identify in this. 12 of them are listed here. These are, of the 50 plus pathways we had previously identified, the ones we found to be most associated with the primary outcome. So again, rate of exacerbations. This is a sparse partially squares regression analysis. So, we're selecting out the features that show the greatest relationship in either placebo or mepolizumab. And the bars indicate, as you can see in the bottom, a propensity to either a higher or lower exacerbation rate. And red is the Placebo group and blue is the mepolizumab group. But basically, what you can see -- and this is all at baseline, so this is before they received the drug at the time of randomization.

So, what you can see is that there's a block of eosinophils associated modules related to type 2 inflammation, eicosanoid metabolism,

cytoplasmic proteins that indicate if you go on placebo, you're going to do poorly, you're going to have a high rate of exacerbations, and if you go onto mepolizumab, you're going to do well. That was relatively expected, albeit there's sort of more detail here than simply blood eosinophilia.

In contrast, we then found that whole block of epithelial pathways that showed somewhat the opposite. So higher expression of those tended to indicate you're going to do worse if you receive mepolizumab, probably than you would have even done with placebo alone. And then you see some that are sort of mixed and invariant to the drug, like the eosinophils activation mucous secretion pathway, where you're kind of going to do poorly regardless of treatment if you have high elevation of that. And then at the bottom, there are some protective ones, but it gives us an overall profile of, sort of, who does well and who doesn't do well on the drug.

And just to then take it a step further,

we had baseline sample collection. We also had sample collection at time points throughout, and here we're showing end of study. But you can see, as you would expect, those eosinophil pathways are decreased by mepolizumab therapy. That's the first column with the down arrows. And again, quite curiously, we saw that mepolizumab actually increased the level of some of these epithelial pathways at the group level and placebo had no effect on any of these. So that was nice. It gave us sort of an indicator of responsiveness.

What we've gone on to do since then is really more targeted towards this development of a biomarker. So can we use a combination of these or ultimately then a combination perhaps of a few genes to really predict response to therapy? So, looks like a little bit of a PowerPoint issue here. But the method we use, this is sort of all machine learning approaches, we used here model based recursive partitioning, which is just a flexible approach where you can kind of use interaction models, you can use a negative

binomial distribution for the rate of exacerbation. So, it's just useful for this data type and you can now partition your population to identify what are the cut points for responder and non responder.

So, when we do that, if we take these 12 pathways, that I showed in the PLS model, and just plug them in, we can kind of pick, again, one or two or even three of these modules and the cut point at which you see a clear response or not. So, in this case, the first one is an epithelial associated extracellular matrix module that cuts a group that's going to do poorly, then one of the eosinophils modules that's going to cut a group that does well. If you plug that back into a more familiar view, in effect, what you can see is we now, in the middle graph here, identify those who are high by that cut point in the eosinophils pathway, low in the epithelial pathway. And suddenly we see a better, albeit still not perfect, response to mepolizumab.

In contrast, on the right hand we see a

group, again, that has the high epithelial, that's showing a trend anyway towards a poor response, and then a fairly large subset of the population that does relatively well in terms of exacerbation rates and well, whether they're on placebo or not. So, this allows us to, in effect, partition based off of a couple of biomarkers who in the population really needed the drug, who probably should not have been in the study for one reason or another. But we can do better than that. And again, we can ultimately do this in a way that might be clinically applicable into the future. So those modules are composed of hundreds of genes. There's a lot of bioinformatics necessary to do that. Ultimately, if we could have a 1 or 2 or 3 or 4 gene panel, that would probably be much more clinically relevant.

So, in this case, again, we can go towards sort of, you know, machine learning to probe this large data set. I just show this to kind of motivate the method we're using. This is LASSO Regression with cross-validation and test

and training to make sure that we're fitting the data well without overfitting it. And from that, selecting out a parsimonious set of genes which are going to best partition our population. So, you can use various metrics here but we'll select down to a small number of genes, something like 20, that explain most of the variance of the cohort. And then from there go back and use this model based recursive partitioning to understand how our population should be subdivided.

So, when we do that, we can now move to a 3 gene panel, which I have listed here. And these are by no means the be all, end all of biomarkers, but it's a first approach or a first attempt at what works well for this study population. And what you can see is there's an eosinophil associated gene SWAP-70, a neutrophil associated gene, and an epithelial associated gene, where again, we can partition the population now into four groups, and in this case get an even better understanding of response and non-response.

So, on the left hand side of that, you

can see those who have really a dramatic response to the drug and those who would be theoretically the ideal candidates in this cohort to be treated. In the third panel, again, this idea that perhaps there are those who actually do poorly on the drug, and we see that now as more statistically robust, those who have high ACE-2 expression in the epithelium, and then two other groups that are sort of more modest or a null effect. This sort of approach has now given us, at least again within this cohort, this, of course, all needs validation and many other steps down the road, but a way of best selecting out responders and non-responders.

Before I leave this study, I mean, that was really the take home point. I actually don't know how much time I have left, but I just wanted to say one more thing, so I'll plug this in. In addition to sort of profiling at baseline, I think what's been very important in this study and other studies we've done is to then actually understand what's going on during illness. So again, in

childhood asthma we're usually looking at exacerbations rather than just profiling during wellness, we can profile during illness, and compare expression patterns in an exacerbation happening on drug or off of drug. And what we see, again, this is a lot of information, but here is I'm highlighting those pathways in red that are particularly elevated during on the left mepolizumab exacerbations versus placebo exacerbations versus non-exacerbation events. On the right, two columns, and you can see they look very different. And in fact, many of the same pathways that I indicated as predictors for a poor response are also highly elevated during the mepolizumab exacerbations. Really just sort of adding additional evidence from new samples at a new time point that these are probably pathogenic to a certain extent in those kids who are doing poorly on the drug, where again we see that eosinophils pathway is playing a major role in the placebo exacerbations.

So that's all, really, just to say a lot

of information can be gained. I guess that's my next slide, probably. So, what did we learn from this? From adding molecular profiling to a mepolizumab RCT in children? Well, we learned that the drug works. It didn't work as well as we would expect. But taking it a step further, by using nasal profiling of the transcriptome, we could pretty accurately identify both the beneficial and deleterious responses to the drug. And using some fancy machine learning approaches, we can distill a lot of data down into probably a handful of genes where, and this point came up earlier, a combination rather than one or another, give us a pretty accurate predictor of response.

I didn't really show this -- well, this was my last point, that then, in addition, if you profile during illnesses, you can understand other pathways, and in particular, not to get too deep into the biology, but a lot of non T2 stuff came up in this analysis as being relevant to residual exacerbations. And there's probably, I didn't show it, but some reciprocal relationships among

these pathways.

Just to -- so, that's one example I've given you. I threw this slide in over the lunch break just to say it's not only in this Inner City asthma study, this is a recently published immunotherapy study, this is not asthma, this is not kids. But just to show the same idea of nasal profiling of the transcriptome, we were able to understand, in effect, where tezepelumab plus subcutaneous immunotherapy was or was not effective and identified novel mechanisms, which you see at the bottom of this graphical abstract of a mass cell gene expression signature in nasal brushings. And again, not to belabor the point, but we've done this across a lot of different studies and any of these would be good examples to think about. Both the method we use, how we generate our data, how we do the Omics analysis towards this idea of mechanisms/biomarkers of clinical response and asthma outcomes, and pediatric asthma.

So, with that, just acknowledge this

work is all funded by NIAD through Inner City Asthma CAUSE and or the Immune tolerance Network. Dan Jackson, Jim Gern, and Bill Busse are the ones who have really led that and just privileged to be involved in my group in Seattle at BRI and UW who do this analysis.

DR. HERSHEY: Thanks. Okay, our next speaker in this session is Professor Mohammed Shamji. He is from Imperial College in London and he's a leader in respiratory allergies and how disease modifying treatments affect immune responses. The title of his talk, which is the last in this session, is Cellular biomarkers for response to AIT for respiratory allergies.

DR. SHAMJI: Thanks very much. This doesn't seem to be my presentation. Seems like Eric has jumped the hoop. Eric, shall I present your talk here? So, while they're fixing the tech issue, I'd just like to thank the organizers for this meeting, particularly, and especially Ron, for inviting me here to talk about biomarkers for monitoring immunotherapy and particularly focusing

on cellular biomarkers. I thoroughly enjoyed the meeting, the regulatory meeting, that are organized in Europe by Stefan Veith. And it's just nice to be invited here to have a feel of the U.S. perspective.

So, these are my disclosures. My research group is based at Imperial College in London, and this is my group. And the data I'll show you is generated by these team members. If you happen to be in London, pop by and say hello. And I've been tasked to talk to you about cellular biomarkers for monitoring response to immunotherapy for respiratory allergies. And now I'll be focusing particularly on aero allergens for this particular talk. But I might give you a bit of a snapshot for food allergy as well.

So, allergen immunotherapy as we know it, is a highly effective treatment for IgG mediated diseases, particularly it is associated with reduction in symptoms, need of rescue medications, and improvement of quality of life. But most importantly, I think as Alkis mentioned

it nicely, it induces immunological and clinical tolerance. And what this means is that you have to give it for a period of three years to be able to induce tolerance. And if you don't give it for three years, then you are on the road to tolerance. But how do we find out? What about biomarkers?

Well, in order to understand the underpinning mechanism or the identify biomarkers, we need to understand the underpinning mechanism of allergic disease. And our current understanding, really, is that when we think about an allergic individual sensitized and allergic to aeroallergen, firstly, what you see is the disruption of the epithelium integrity. Where you see the allergen are dissipating through. Then you have the immature dendritic cells that are capturing the allergens and then they migrate towards the draining lymph node. They activate into 9T cells into 2T to 2A cells, anti follicular helper cells, and then they help V cells to differentiate and proliferate into IgG producing B

cells. And the IgG sensitizes MAR (phonetic) cells. And following allergen exposure, subsequent allergen exposure, we have degranulation of MAR cells and basal cells in a local target organ.

But when you give immunotherapy over a course of three years -- so when you are, in a way, you are inducing immunological tolerance. What you see, really, is that you have restoration of the epithelial barrier integrity. So you have less of the alarmins, the cytokines that drive type 2 responses, such as R25, TSLP, and IL-33, coming through the epithelium or activating innately for cells. You have induction of regulatory LC-2s. You also have induction of regulatory DCs as well as regulatory T cells, the FoxP3 T cells, the ALTEN inducible T cells, the L-35 producing T cells. And what you have, for example, L-35 has the capacity to prime B cells to become regulatory B cells. And these B regs produce IgG-4, but also have the capacity to suppress T effector cell functions on a cell to

cell contact dependent manner. But also, these V regs have the capacity to produce IgG-4. And if we evaluate the IgG-4 that is induced following immunotherapy, all it tells us is exposure in a sense, but not necessarily the function. But IgG-4, particularly that has high affinity, high avidity, and specificity, is able to inhibit the earlier phase response, such as activation of MAR cell and basal cell, but also the T cell responses.

So, you need to give immunotherapy for three years. And this is an elegant study by Stephen Durham that elegantly showed in 1999 that you give immunotherapy for three years. What you have there is, you have 36 patients at the end of three years. On the right hand side, you have, the top row shows the pollen count for the third year and then the subsequent years. And then you have the second row, symptom scores, rescue medication scores, and visual analog scales. So, clearly, at three years, those who received immunotherapy had lower symptom scores, rescue

medication scores and the VAS scores were lower compared to placebo.

But after, in the follow up group particularly -- so these were randomized to either be on maintenance treatment or discontinued treatment, and they recruited just normal allergic individuals. And clearly, those who were more maintained treatment, but also discontinued treatment, had lower symptom scores, rescue medication scores and VAS scores. And similarly, what we also see is that whether you give SCIT and SLIT, if you give it for three years, you induce tolerance. So, this is another randomized blind placebo control trial. Steve was very much involved in this. This was a European study, a large study, three years of treatment in patients with allergic rhinoconjunctivitis and three year follow up. And clearly you can see that. So three year treatment and two year follow up, and you see a reduction, sustained reduction, of symptoms at follow up, one year and two years.

So really, the key is that we know that

if you give treatment for three years, you induce immunological tolerance. But we need to really think about what's really happening in the context of biomarkers. And I'd like to go over three simple vignettes, in a sense. So, the first thing I'd like to talk to you about how grasp on SCIT and SLIT is both associated with the generation of distinct subset of regulatory innately for cells. How SCIT and SLIT induces, suppresses Th2A cells, as well as inhibits activation of T follicle helper cells and induces T reg cells.

And finally, what I would like to do, rather than just focusing on biomarkers of tolerance or biomarkers of efficacy, I'd like to highlight how we can use biomarkers to actually think of a novel approach of immunotherapy, in particularly thinking about depictment polymer as grass pollen extracts for immunotherapy. So, innately lymph cells are immune cells that belong to the lineage negative cells, negative lymphoid cells, and lack T cell receptor, and play an important role in immune hemostasis, infections

against microorganisms, but also play an important role in chronic type 2 inflammation that can be grouped into three subsets. So, ILC-1s, ILC-2s, and ILC-3s. And ILC-2s particularly responds to TSLP, lL-33 and lL-25, and secrete a lot of cytokines, particularly type 2 cytokines like L-4 and L-5, particularly 5 and 13.

And what we had done several years ago, we monitored the frequency of ILC-2s in grass pollan allergic individuals in and out of the pollen season and those who received immunotherapy. And we were able to demonstrate that in the immunotherapy treated patients there were a blunting of the increases in ILC-2s during the pollen season, while in the allergic individuals there was a clear high frequency of ILC- 2s. And the question then was what was regulating at the innate immune compartment? What were the regulatory mechanisms? Is there induction of ILC-2s that have regulatory capacity? So we were very much interested in looking at L ILC-2s that produce ILC-10 and whether they have

the capacity to regulate innate immune responses at the local target organ. But as a surrogate, we looked in periphery.

So we were able to generate ILC-10 producing ILCs in the lab. And the way you do that, you purify ILCs from peripheral blood, more nuclear cells. You stimulate with RA27 and 33 and you stimulate them with a retinoic acid. And what you see is that you then look at the population of lineage negative cells that are RCD-127 positive and look at a double positive that CRTH2 positive and KLRG1 positive.

But because we do 18 color or 36 color, flow cytometry it's very difficult to really be objective about the way we do these analysis. And one way we did is we use unbiased machine learning algorithm analysis for visualization, where we reduce the dimensionality into 2-D of all the parameters. So, each dot represent a cell and the cells that are close to one another are more likely to be expressing the same marker.

So you have, firstly, you have, in the

absence of retinoic acid, we're looking at CRTH2, KLRG1, and IL-10. And in the presence, what you see is the red dot flacking in the area of CRTH2, but predominantly KLRG, and there is a small island where you can see the IL-10s. And if you look at the bottom, what you see here is that you only see induction of ALTEN producing ILCs in the cells that have been stimulated to retinoic acid and are typically expressing KLRG1. And you can measure protein expression of IL-10 over the course of 8 days in a time dependent manner. You can also take the cells and throw them in the air and they fall like a tree. And then you can look at individual node and the PI (phonetic) represent the expression of the markers. And we were able to identify, so this called flosum (phonetic), we're able to identify two meta classes, particularly meta classes 6 and 10, that were flagged in the presence of retinoic acid as a mark of whether the cells had the capacity to produce IL-10. And you can see increase in abundance in the meta classes 6 and 10, but also expression of

IL-10.

So, the most important thing is so what we're seeing, we're able to generate IL-10 producing ILCs in vitro, but so what? So, we recruited non-atopic controls. Cross-pollen allergic individuals and patients received immunotherapy during the pollen season and were able to look at evaluate the frequency of these IL-10 producing ILCs. These were clearly dysregulated in a grass pollan allergic individual, so lower proportion compared to non-atopic controls and somewhat restored following subcutaneous immunotherapy, to our surprise. We've done a lot of studies and looked at several correlations, but we were able to see a modest correlation of symptom scores versus the proportion of IL-10 producing ILCs, an inverse correlation which was reproduced in a context of symptom scores, but also VAS scores as well.

We went on to validate this into a randomized double blind placebo controlled trial with sublingual immunotherapy. I had a fellow who

came from Switzerland, we worked very closely together with Steve, and performed a randomized trial on sublingual immunotherapy. So this was initially a 12 month study, placebo and active. Patient received treatment for a course of 12 months and then they were followed up for up to 24 months in the actively treated group. So at baseline, if you look at the TNSS for placebo and SLIT, they match at baseline. And at 12 months there was a reduction in the TNSS area under the curve measured by -- so this is TNSS measurement of TNSS after nasal allergen challenge at 12 months, or at baseline, and we looked at the proportion of IL-10 producing ILC2s. So, these remained very much unchanged in the placebo group between 9 and 12 months, but somewhat they were elevated in the actively treated group. So, we also looked at the association between the TNSS scores as well as the frequency of these IL-10 producing ILC, but the level of IL-10 that we're secreting. So, we're able to demonstrate a linear inverse correlation between IL-10 producing ILC2s

and the TNSS scores, as well as the levels of IL-10.

So what we're able to really do here, to really have a look at first glance, what happens right beneath the epithelium and where are the underpinning mechanism that we're seeing following immunotherapy and whether we could have a very nice biomarkers. Where ILC2s they, initially, they come from ILC precursors, an express CD117, and they can differentiate into an NKp46 or KLRG1, immature ILC2s, which has the capacity to become ILC2 when exposed to TSLP, and IL-

Or IL-10-producing ILCs when it's exposed to IL-7, IL-33, and retinoic acid. And we're able to demonstrate in this particular study that there was a clear dysregulation of these IL-10 producing ILCs in allergic individuals, which was somewhat restored following grass pollen immunotherapy, SCIT and SLIT. We did a lot of functional data analysis where we were able to look at the capacity to restore epithelium integrity, how they can suppress Th2 effector

cells, and also highlight their potential use as potential biomarkers. And we're currently validating this in the large studies.

Secondly, what I would like to highlight, when we think about cellular responses, well, Th2 responses are prominent in allergic disease. And so what happens following SCIT and SLIT? Well, in an elegant study, again, this was a study that was funded by ITN and NIH, and Steve was the primary investigator, it was a randomized blind placebo control trial of SCIT and SLIT single center study 100 and we had 36 patients in the active arm SCIT, 34 in the placebo, and 36 in the SLIT group. But this was a way of comparing SCIT versus SLIT in the same group, a single center study. So, it was a 1 year study and 1 year of treatment. And at baseline, we performed intranasal allergen challenge, collected TNSS scores, during the pollen season we collected symptom scores, rescue medication scores, quality of life scores. And we did this at baseline, year 1 year 2, and we stopped treatment and followed

them up for year 3.

And if what you can see here in the TNSS, if we look at the TNSS score, this is data that (inaudible) actually generated. And it was very nice to see that what you see here, at year 1 and year 2, SCIT and SLIT are associated with reduction in TNSS. But at year 3 we lose that effect. And we also had a placebo effect. But when we looked at the total nasal symptom scores, a change in increase for both SCIT and SLIT at year 1, year 2, and this effect was lost and the placebo remained pretty much the same. But the key point here is that it's very difficult to come across the studies where you have response and no response. So, it was really good to be able to have a study that, where we can actually really look at the certain biomarkers in collaboration with Bill Clark and Eric. What we did is we looked at the frequency of Th2A cells. And if I just get you to focus on the top right panel, what you see is that SCIT and SLIT had reduction in Th2 cells at year 1, year 2, and we lost that effect

at year 3. So again, mirroring similar to what we saw with the total nasal symptom scores.

We tend to focus about T cells, particularly Th2 cells, IL-4, IL-5 producing cells. But we tend to forget about the cells that are really important in driving IgE responses. And T follicular helper cells are critical in helping B cells to differentiate into an antibody producing cells. T follicular helper cells are T cells that express CD4, CXCR5 and PD-1. And in order for them to differentiate into a Tfh cell, they need to interact with dendritic cells and they require particular signals such as lL-12, IL-27, IL-6. And these signals provide a downstream activation of stat 3 and stat 4, which allows them to express IL-21, CXCR 5, and PD-1. And IL-21 is critical for helping B cells to differentiate and switch into an antibody producing cell. So it's very crucial. And when you have a combination of IL-4 and IL-21, you have a huge boost of IgE. And this is an experiment that illustrates this, where you can culture

peripheral but more nuclear cells, stimulate the cells with IL-4, lL-21, and CD40 ligand. But when you have a combination of IL-4 and IL-21, you have a massive boost of IgE that is produced in vitro.

So, we performed a cross-sectional study, a cross- sectional study where we recruited non-atopic controls, untreated grass pollen allergies, SCIT and SLIT treated patients. This was a pilot study, a small core study, and these are the patient demographics on how we selected our patients. And we looked at the rhinitis total symptoms scores during the pollen season. What you can see here is the treated group, SCIT and SLIT, have lower RTS compared to the allergics. The non-atopic controls are behaving themselves. Then you have the IL-21 producing cells are elevated in the grass bone allergic compared to non-atopic control, but somewhat restored in SCIT and SLIT treated patients. And we also looked at the cells that regulate Tfh cells. These are Foxp3 TFR cells, and there are somewhat the reverse where they were lower in the grass pollen

allergic individuals compared to non-atopic controls and somewhat induced following in the treated group, SCIT and SLIT.

So, what about the evidence of IL-21 in the nasal fluid? So, this patient had underwent intranasal allergen challenge with collected nasal fluid. And we looked at IL-4, IL-6, IL- 21, IL-6, particularly because IL-6 drives IL-21. But we're also very keen to look at the combination of IL-4 and IL-21. And these were increased in a time dependent manner in the allergic group and somehow had inhibited in the SCIT and SLIT treated group and not much in the non-atopic individuals. We went on to do a lot of analysis, including ataxic, looking at the epigenetic effects of SCIT and SLIT at the chromosome level in the Tfh cells and T follicular regulatory cells. And what you see here on the left, firstly, in Tfh cells there's more accessibility in the chromatin region, in particularly allowing expression of IL-4, IL-21, IL6, and less in the Tfh so that they have restriction in terms of functionality. And on the

right, this is reverse where they are much more functional and the Tfh have much more, less, chromatin accessibility and unable to produce more IL-4, IL-21.

So, this is what we know in terms of the underpinning mechanisms of when we are moving towards induction of tolerance, we influence the innate immune compartment by induction of IL-10 producing regulatory cells. We are dampening Th2 cells, Th2A cells, and modulating T follicular helper cells by inducing T follicular regulatory cells.

But how can we use biomarkers in terms of thinking of using and identifying a candidate that will be able to induce tolerance when we use it for immunotherapy? I won't go much into this detail, but I think it's important to highlight that when we think about biomarkers, they're not just for diagnostic or prognostic, but we can use them for drug development. And I think it's a very nice way of being able to understand by performing basic research and really identify the

relevant key molecules, and how we can think about understanding the mode of action, whether there can be a therapeutic target. Can we use them in pre-clinical studies? Can we use them for stratification of patients? Can we use them later on for qualification in terms of relevant biomarkers for validation and use for clinical trial, but also real world evidence studies or in a clinical practice?

So, here's an example, thinking about optimizing allergoids as a novel approach for immunotherapy. And this is really a way of using a larger molecule that is unable to activate T cells or Th2 cells particularly. And what one can do is purified -- you use an extract which undergoes a mild acid treatment. It can become depegmented, can be polymerized, chemically polymerized. One can look at the IgE binding and look at, particularly the epitope, where you have IgG binding. And we had one candidate, that as you can see, in the native flip the top row, you have the various different epitope, IgE epitopes.

This is reduced when you depigment it and when you undergoes polymerization you have less IgE epitopes, but the IgE epitopes remain the same. You can use basophil activation to look at reactivity or activation of basophils and select the candidate that is a little bit more hypoallergenic.

And what we did is we performed single-cell analysis, RNA-Seq, in three patients and we're able to demonstrate by the power of using single cell transcriptomics. We were able to demonstrate that the polymerized Depigoid molecule was able to reduce regulate IL-4, IL-10 pathway, suppress antigen presentation, induce regulatory T cells that express CD-52 and express their functionality towards Siglec-10. And then we validated this in in-vitro study with 16 grass pollen allergics and 12 non-atopic control to be able to demonstrate that they were the right candidates and they were unable to stimulate Th2 cells, Tfh cells, but they were prominent inducing B regulatory cells and T-regs but also were

hypoallergenic.

And I think what we were able to really do here is demonstrate that you can use biomarkers to also select the relevant candidate that one can use for immunotherapy. I think we're also doing similar in a context of using virus like particles expressing peanut and moving into the peanut allergy field. And we have some very elegant data showing reduction of basophil activation, IgE mediated, Th2 activation, Tfh2 cell responses Tfh follicular regulatory induction, IL-10 producing B cell induction, and Tfh1 responses.

I think with that, I would like to just acknowledge the team that have worked towards the data I've generated you. But I just want to just leave you with a thought. I think we are at the stage where we have access to all this wonderful -omic technology, but also we are thin the era where we can use a lot of informatics to make sense of what we're doing. And I think what we need to do is to think about our patients, how heterogeneous they are, put them through the

relevant pipelines where we can do a lot of measurements, and do the relevant data sign that's validated to be able to identify responders, non-responders, and low responses. And I think this is really the way forward.

And just on the last slide, what I would just like to highlight here is that what we need, really, is to think about biomarkers of desensitization, efficacy, and tolerance. And we need to really think about whether it's for SCIT or whether it's for SLIT, or whether it's for modified allergen. We should be really putting them into one pot.

And finally, I'd like to invite you all to Valencia, where we have the European Academy meeting, and hope to see you there. Thank you.

DR. HERSHEY: Matt, Sally. Dr. Hamilton, if you have any questions, just please move to the microphone. One of the microphones.

DR. KRISHNAN: Gary Krishnan, from Eli Lilly. This is a question for Dr. Altman. Really enjoyed your talk. Have you pressure tested the

reliability of your thresholds in your models using prospective studies? Especially when it comes to gene expression, which in individual patients varies quite a bit.

DR. ALTMAN: Yeah, no, it's a very good question. We have certainly not pressure tested anything to the point of saying this is a clinically reliable test that we would implement in a study. As to that sort of variability over time, I mean, we've used these exact approaches and these genes and these modules now across multiple studies and have seen really a very consistent pattern. We don't have another mepolizumab RCT, you know, to sort of fully validate it. We are currently validating the utility of some of those genes in observational cohorts to look at just exacerbation rates in general. So, you know it's a work in progress. But as with anything, you would want truly external validation to generalize the result.

DR. HERSHEY: Any other questions?

DR. RABIN: There was one virtual

question for Dr. Hamilton. A questioner wanted to know whether or not it was worthwhile in the context of some reports that there's IgE against several viral antigens, like RV and such, and whether or not it would be worthwhile to add that to a multiplex cartridge such as the one you spoke about?

DR. HAMILTON: I don't think that's ever been raised before, actually. And we've talked about alpha-gal and a whole, and a whole host of other allergens, but never viral antigens or allergens. Good question.

DR. HERSHEY: Wayne? DR. Shreffler: Hi, so, question for Dr.

Shamji. Mo, do you have a sense of the relative contribution of IL-10 from this ILC population versus adaptive T cells or other sources?

DR. SHAMJI: Yes.

DR. SHREFFLER: In the context of, you know, allergen IT?

DR. SHAMJI: It certainly, we've done

comparative analysis where we've stimulated RCs, purified RCs, with RA and lL-7 and lL-33, and also stimulated B reg with CpG and CD40 ligand. Sorry, my asthma is kicking in a little bit. My voice is coming down --

DR. SHREFFLER: We'll get you a lozenge.

DR. SHAMJI: -- while talking about the relevant topic. Sally, maybe you can help? But the point being is that pound for pound, they produce a lot of IL-10, and they have the capacity to sort of support the epithelial integrity in terms of, you know, induction of IL-1 and so on. So certainly, they do have not only the capacity to suppress Th2 cells, they produce a lot of the IL-10 concentration is really high, but also helping the epithelial cell integrity regeneration.

DR. HERSHEY: Lady in the back?

QUESTIONER: This is a question for Dr. Hamilton. Dr. Hamilton, this new ALEX technology that you showed was very interesting. Do you think that we will ever be at the point where

those of us who order serum specific IgE testing for foods will actually get a result that says something other than just greater than 100? And do you think otherwise that this is something that we should be talking about thinking about?

DR. HAMILTON: So, your question is whether laboratories will ever report greater than 100 kilo international units of allergen specific IgE? Do you find levels above 100 to be clinically useful?

QUESTIONER: Well, I don't know. I've never had them, so I haven't had the opportunity. But I like that I get a total serum IgE, and I do wonder sometimes if my level were 102 versus 10,000 if I wouldn't do something different with that.

DR. HAMILTON: Well, it's a very good question. We actually, in the clinical lab that I run, do titer out all serum above 100 to the level by doing a dilution analysis. So we actually do that. But in reviewing the College of American Pathology Proficiency survey data, very few

laboratories do that. In fact, I'm still surprised that there are many labs still reporting in class units which we've tried to eliminate. So, it's a very good question. Thank you very much.

DR. HERSHEY: Dr. Drazen?

DR. DRAZEN: Jeff Drazen from Boston. For the biomarkers to be useful they need to be reasonably repeatable. And I think reasonable idea that IgE is reasonably repeatable within an individual over relatively short periods of time. But of the other biomarkers we've talked about, Sally mentioned in, and you mentioned, about the variability. How do we deal with this variability within a given patient? Because we're going to label a patient. Do we label them as low all the time, high all the time, or somebody who jumps around in that middle group? It's not going to be terribly clinically useful. So how do we categorize the repeatability? How do we use it to our advantage or understand it to our disadvantage?

DR. WENZEL: Jeff, as always, that's a great question. You know, I think we use it to our advantage. The noise is often where the signal is. You just have to look for it. And we published a paper in SARP, we actually published now a couple of papers, looking at variability of FeNO and variability of sputum eosinophils and sputum neutrophils, and, you know, those that are persistently low, persistently high, and the ones that wiggle around. And interestingly, probably the most intriguing ones are the ones that are changing, and they seem to have some of the worst symptoms, the more exacerbations, et cetera. And, you know, I think it's because it is contextual, right? That if you had a virus a month ago or if you were treated, you decided to become more adherent to your corticosteroids over the last two days because you knew you were seeing the doctor. You know, all those sorts of things can influence the variability. And I think those patterns of behavior may be part of it. And then your environmental exposures are obviously going to

vary, too. But the noise is actually probably where some of the most important exacerbation and severe patients lie. The noisy ones.

DR. HERSHEY: Matt, did you want to say something?

DR. ALTMAN: Well, I would second that. On the one hand, it makes it tough to actually come up with drugs and clinically treat because, you know, asthma is variable over the course of the year, it's variable over time. We've seen in our studies that your transcriptome state in the nose, at a given point, sort of predicts the time to your next exacerbation. So, they're at risk periods. And for example, we see in kids in the fall, those who have a low interferon basal tone are the ones who exacerbate that fall. So, you know, that makes it tricky because you treat them with a certain drug at that point in time and then a different one at a different point, that all gets muddy.

But there's real data, there's real information, within that variability if you know

how to look at it, you know. So, I think it's important that we understand that. We're doing a study now where we're looking at T2-high and T2-low, and surveying throughout the course of the year, to better understand that variability and how it relates to types of exacerbations.

DR. WENZEL: And I'll just add one other thing. So, I showed the data about pheno and blood eosinophils. That was one measurement. But it seemed with that one measurement to predict things 3 years and 4 years down the line. So, a single measurement was actually pretty good. But I think we, as a collective, should come up with some standards that to actually know whether somebody is high eosinophils or not, you need to measure it three times. I think a single measurement is probably not enough. You need to measure exhaled nitric oxide three different times and three different occasions. But if you pass the ceiling, whatever that ceiling ends up being, you have that background. I actually pretty strongly believe that. That that background of

type 2-ness, whatever we want to call it, is there and doesn't go away. I've never seen it go away with.

DR. SHAMJI: Biomarkers to assess immunological tolerance, for example, following immunotherapy. What we always do is you start with optimization validation, analytical validation in a pilot study and then you take it into a clinical study, and only when that's reproducible and informative. Then the key then is to take it forward for qualification. And the key element here is, you know -- the graph that I showed in terms of mechanism, all the work we've done in terms of T cells, B cells, RC-2s, and so on, in terms of reproducibility, they are reproducible and they can be done by independent operators. And if you think about the differences between in small pilot study, starting with atopics and non-atopics, before we actually evaluate in treated group, the concept is to always think of a pilot study where you're optimizing and validating these biomarkers. Then,

you can then take them into a clinical study to validate them, prior to valid qualification.

DR. HERSHEY: Thank you. Next.

DR. ORTEGA: Hi, and Hector Ortega, San Diego. And I was interested, Sally, in the comment about IL-6 and recognizing there is a major gap on the non-type 2 biomarkers. And do we know if the elevated IL-5 is, I mean IL-6, is correlated with the low response on the drugs that are now used biologics for all commerce, like tezepelumab or even dupilumab? And that's one comment. But also, probably this workshop should be an incentive to look for additional biomarkers in the non-type 2.

DR. WENZEL: Yeah, no, I don't know whether it's a predictor of response to type 2 biologics. Like I said, it's fascinating. It's there in everyone. It is completely independent of type 2 biomarkers. But if you look at the people that have type 2 high inflammation, and you start them on whatever your biologic of choice, is there a difference in response? We can look at

that in SARP. We have not done that yet, but we can look at that in a pretty rigorous way. So, thank you for that suggestion.

DR. HERSHEY: I have one question while we're waiting for this lady to come up. I have one question for our panel. We heard from several of you biomarkers at different tissues, right. And I think it's becoming increasingly evident that allergic diseases are systemic. They manifest in the blood, right, with the immune responses, and they manifest locally. And I just wonder what you think about that in terms of the future biomarkers. I mean, are we going to need -- Matt showed how we had to use two or three different genes, and Sally presented some examples. And I wonder how we're going to really think about this collectively and bring it.

DR. ALTMAN: I mean, I can tell you, I appreciate that comment. That, you know, systemic, and we can measure things in the blood. I mean, my bias from a lot of data sets now is that, and we kind of heard this earlier, looking

in the airway, and we're lucky, and that we can conveniently sample the airway. I mean, upper airway at least. The signals are much more robust there in all of our data sets. There are things that you see in the blood. Some of them are genetic, you know, some of them are not. Interferon is a classic example where you can see sort of basal interferon tone well in the blood. But I think a lot of these tissues eosinophils, tissue epithelial things show up better in a nasal sample. So, I'm certainly a proponent of that. But we're always going to look at both and see how they associate to one another, or at least that's what we're doing in our studies.

DR. HERSHEY: I think with skin types we might have even more that we can add in.

DR. WENZEL: And I would just add, I think there could be differences in children and adults with this, too. That, I think I'm hypothesizing here, that childhood disease could be more target organ focused. But as you go on with longer duration of the disease, more

environmental exposures, et cetera, it could become more systemic. And I will say, that the IL-6 in the blood has zero connection with literally any gene expression in the lungs, in sputum, or in epithelial brushings. So, it's a completely separate compartment, which we don't understand at all, to be quite honest with you.

DR. HERSHEY: Interesting. Did you have anything you want to add?

DR. SHAMJI: Just to echo what Martin and Sally mentioned, really an integrated approach of looking at the local target organ with all of the novel technologies we have. Looking at, for example, proteomics with oiling, looking at special transcriptomic with the signal cell approach, I think it's only going to tell us more about the underpinning mechanisms and the immunological response. We can then take that to translate into relevant biomarkers.

DR. HERSHEY: Thank you. Last question.

DR. TRIPATHI: Hi, Anubha Tripathi, FDA CBER. Thank you all for your talks. This

question is really directed at any of you, but, Dr. Hamilton, you first touched upon it, and then Dr. Shamji touched upon it. I think as your last slide, you talked about the Raven AI software, and I was just wondering if you can -- or with the multiplex. Did I get that right? I was just wondering what your experience is with that? And I guess, along the lines of what we're discussing today, how you see that working into perhaps the biomarker discussion.

DR. HAMILTON: So where does the multiplex fit into the future of diagnostic?

DR. TRIPATHI: Well, you actually, on your last slide, you talked about using Raven AI software.

DR. HAMILTON: Oh, right. Yes. So, one of the problems with having data from 300 allergen specificities is that it's extremely overwhelming. So, we're fortunate that computer AI has simplified it by not only allowing us to analyze those data quickly, but also provide general interpretations based on the allergenic profiles.

And what's very nice is that you get information about cross-reactivity and suspected or expected symptoms, that in fact may relate to cross reactive allergenic of families that we wouldn't really know.

In the United States, allergists are not really well versed in the details of molecular allergology like they are in Europe. Because actually most of this actually grew up in Europe. Most of the molecular allergens. That's why the major companies that are producing these multiplex components based assays are actually in Europe. So, we have an educational gap here in the United States, I view, which we have to fill, and we can fill that by AI programs that allow us to get some general interpretations of data, both for extract and component reactivity.

So, I see the transition of serologic measurements of IgE antibody in the United States, and the world, transitioning from the singleplex assay to a multiplex technology, because multiplex technology is the only cost effective way for us

to actually get measurements to components. Otherwise, it just can't be done. So, I don't know if I addressed your question or not.

DR. TRIPATHI: Well, my question was, what is your experience with, how does the -- so, that's what the Raven AI software does, is put together cross reactive and then symptoms, and then the physician checks? Is that what you -- what's the readout that you get?

DR. HAMILTON: Yeah, the readout is actually an interpretation, providing profiles of symptomatology based on the allergenic profiles that might be expected to be seen. For example, in the case of profilin, the one case I presented, the child may very well have an oral allergy symptom to melon, even though their primary sensitivity is to birch pollen due to Bet v 1 one cross-reactivity -- or Bet v 2 cross-reactivity, sorry. So that type of interpretation is very helpful as a starter, but ultimately comes down to the allergist ability to actually interpret the data. And that requires knowledge about the

clinical utility of the allergenic components. And that's not as trivial as we would like to think, unfortunately.

DR. TRIPATHI: Right. And I was just wondering, Dr. Shamji, you mentioned it as well, have you used machine deep learning in your?

DR. SHAMJI: Yeah, thanks for asking. Actually, we are. We are using a lot of machine learning algorithms and working with bioinformatician to really data mine studies from clinical trials, real world evidence studies, and so on. So, it's really, it's early stage because the thing is, the output, the key is how do we integrate the data nicely to make good sense of it. And actually, even if we do and we have some interesting data clusters, we do need to validate them into real clusters because otherwise we are only talking about virtual clusters. So, I think we have to go through the process and then we have to be very thorough in terms of really looking at the data. But thank you.

DR. TRIPATHI: Thank you.

DR. HERSHEY: All right. Join me in thanking all of our speakers. We're going to take a 15 minute break. We'll reconvene at 2:35. Thank you.

(Recess)

DR. TOGIAS: Might be time to take a seat so that we move to session three. And this session is entitled Biomarkers in Food Allergy and those who were not earlier here today, my name is Alkis Togias from NIAID, and we have a number of speakers, starting with my dear colleague.

SPEAKER: This meeting is being recorded.

DR. TOGIAS: Pam Guerrerio is Director and Chief of the Laboratory of Allergic Diseases in the Division of Intramural Research at NIAID. She graduated the medical scientist training program at Johns Hopkins University and completed medical school and a PhD in human genetics and did her residency and all the good things there. And now she's going to talk to us. The title is food allergy, risk factors and current clinical

endpoints for efficacy. Pam?

DR. GUERRERIO: Thank you, Alkis, and good afternoon, everybody. It's been a great session so far, so thank you to the organizers for the opportunity to present and be part of this is it this.

All right. As Alkis mentioned, I'm going to discuss two topics today. First, known risk factors for food allergy, and then second, clinical endpoints that can be used to assess the efficacy of treatments for food allergy. I have no conflicts of interest.

The tremendous increase in the prevalence of allergic diseases over the last few decades has raised a lot of interest in the factors that account for this. At this point, the pathogenesis of food allergy is thought to involve both a genetic predisposition as well as exposure to triggers in the environment. One way to ask whether genetics contributes to a disease is to ask whether that disease tends to run in families, and twin studies can be especially helpful in this

regard, since twins share many of the same environmental exposures and also the same in utero environment. Several years ago, it was showed that the concordance rate for food allergy among identical twins who share 100 percent of their DNA was 82 percent, compared to only 20 percent for fraternal twins, who only share half of their genes.

Another study found that children who have a parent or a sibling with peanut allergy are seven times more likely to be allergic to peanut than children who have no family history. Overall, the heritability of food allergy is estimated to be about 80 percent. Using a number of different approaches, variants in several genes have been identified and consistently associated with food allergy. These include the HLA genes, which are involved in how food allergens are presented to the immune system; CD14, which encodes the co- receptor for lipopolysaccharide, a component of bacterial cell walls; the Th2 cytokine IL-13 STAT6, which is a transcriptions

factor downstream of the Th2 cytokines; and then variants in filaggrin, SPINK5, and other genes important in the skin barrier function have also been linked to food allergy.

However, it's important to note that all of these studies have only detected associations between these genetic variants and food allergy, and they don't necessarily imply that any of these genetic changes on their own would be sufficient to cause food allergy. The sharp increase in food allergy prevalence over a relatively short period of time suggests that genes are not the whole story. Our gene pool just doesn't change that quickly. And so, there's good evidence that there are several other risk factors for food allergy, including male sex, race, and ethnicity.

Food allergy is about twice as common in males than females, and there's some evidence males have more severe reactions as well. Interestingly, this changes during adolescence when food allergy becomes more common in females. There's also some studies suggesting that

sensitization to food allergens, and perhaps even food allergy itself, is more common among Asian, Black and Hispanic individuals than those of European ancestry.

Epidemiologically, there have been multiple studies suggesting that vitamin D insufficiency is an important risk factor for food allergy. Several groups have shown that infants who are born in the fall and winter months are at higher risk for food allergy than those that are born in the spring and summer when there's greater UV light exposure. Other studies have found that the number of hospital emissions for allergic reactions to food and the number of prescriptions for epinephrine autoinjectors increases the farther you go from the equator, and that relationship held true independent of longitude.

But some of the best evidence actually came out of the HealthNet study from Australia that looked at over 5,000 infants. And here they found that those infants who were low in vitamin D were 12 times more likely to develop peanut

allergy than infants who had normal vitamin D levels. There's also been some interesting data suggesting that the increased use of ant acids, especially during infancy, may be contributing to this rise in food allergy.

Under normal circumstances, stomach acids reduce the allergenicity of food proteins by denaturing the protein and breaking it down. Under normal circumstances, about 2 percent of food proteins are absorbed into the systemic circulation. There was a study done actually several decades ago that showed adults who were treated with these antiulcer medications actually developed new sensitization to food allergens, although the clinical significance of that wasn't clear.

More recently, Ed Mitre and colleagues reported a retrospective cohort study where they looked at over 800,000 infants who were enrolled in the military healthcare system. The study included about an equal number of boys and girls. They reported that 7.6 percent had been prescribed

a histamine-2 receptor antagonist, and 1.7 percent a PPI, a proton pump inhibitor, during their first year of life.

They showed that infants who received either of these medications were over two times more likely to have food allergy than infants who didn't receive these drugs. Now, again, this study is only detecting an association. It doesn't necessarily mean that use of these medications is causing food allergy, but it does suggest that these medications may not be completely benign.

One of the more popular theories to explain the rise in food allergy is called the hygiene hypothesis. And this essentially posits that we are keeping infants too clean and that by not exposing them to germs, their immune system isn't developing properly and they're starting to react against innocuous antigens in the environment, such as food antigens. So, what is the evidence for this? We know that the prevalence of allergic disease is lower in less

developed countries that lack adequate sanitation and a clean water supply. And these countries often experience a concomitant increase in the prevalence of allergic disease as their countries become more westernized. Children who have close contact with animals, either because they grew up on a farm or they have a pet at home, especially a dog, seem to be protected. Children who have multiple older siblings also seem to be at lower risk. And then there are some birth cohort studies, but not all have shown that infants who are born by caesarean section are more likely to have challenged proven food allergy.

One way all those exposures might be influencing the risk of food allergy is by changing the microbiome. There is now really quite a tremendous body of data suggesting that dysbiosis early in life is playing a very important role in the pathogenesis of food allergy. From an epidemiologic standpoint, we know that use of antibiotics during pregnancy or in infants during the first month of life was

associated with a higher risk of cow's milk allergy. Other studies have found that the levels of triclosan, which is a very common antibacterial agent found in hundreds of household products, was higher in the urine of children who were sensitized to food and environmental allergens. And then germ-free mice or mice that have been treated with broad spectrum antibiotics tend to have higher total IgE levels and are also more susceptible to becoming allergic to foods. And this can be prevented if you colonize the mice, only during the neonatal period, with a mixture of bacteria.

There's also been some interesting studies suggesting that allergic infants may be missing bacteria that protects them from the development of food allergy. In this study by Cathy Nagler's group at Northwestern, they took stool specimens from either milk-allergic infants or healthy infants, and they used that to colonize germ-free mice. And they found that the infants who received stool from the food allergic infants

also became allergic to milk and anaphylaxed, while those that received stool from the healthy infants were protected. They went on to show that colonization of the germ-free mice with just a single strain of bacteria was sufficient to confer protection, which of course has very important therapeutic implications.

Another important risk factor also seems to be the timing of solid food introduction. I'm sure most of you in this room are familiar with the LEAP trial where four to eleven-month-old infants at high risk for peanut allergy were randomized to either early peanut introduction or strict peanut avoidance. And as I'm sure you know, the results are very striking. Those in the consumption group had a much lower rate of peanut allergy than those in the avoidance group, and this protection was even seen in those infants who were already sensitized to peanut when they enrolled in the study. However, this protection afforded by early introduction seems to be very allergen specific. These infants were protected

against peanut allergy, but not tree nut or other food allergies.

Overall, the evidence for early introduction really seems to be strongest for peanut and egg, although there may be different windows of opportunity for other foods. For example, there was recently a randomized controlled trial that found that infants who received cow's milk formula in the first one to two months of life were less likely to have milk allergy at age six months compared to infants who strictly avoided milk during that early period.

And then finally, there's a growing body of data that the skin may be an important route for initial sensitization to food antigens. Under normal circumstances, the skin forms this protective barrier both to environmental insults and allergens. However, in children who have eczema or have variants in those genes that are involved in the skin barrier function, such as filaggrin, there is increased penetration of food allergens as well as release of epithelial derived

alarmins that then skew the immune response towards Th2.

Almost a decade ago now, Gideon Lack's group showed that the probability of a child developing peanut allergy was directly proportional to the amount of peanut protein that was present in their household dust and that relationship was even stronger in those infants who had eczema, especially severe eczema.

Our group had recently shown that infants who were either sensitized or allergic to peanut at one year of age, a greater percentage of their peanut specific T cells expressed CLA, which is a homing receptor that directs movement of the T cells towards the skin compared to their non-allergic controls. And these infants had a corresponding reduction in the number of peanut-specific T cells that expressed alpha 4/beta 7, which is a gut homing receptor. So, these data also suggested that sensitized and allergic infants first encounter peanut allergen in the skin.

So, to summarize this first part of my talk, I think there's very strong evidence that genetics plays an important role in the development of food allergy. But there's also convincing data that several early life events, including vitamin D deficiency, exposure to antibiotics, and perhaps antacids, the age of solid food introduction, and dysbiosis also plays a very important role.

All right, for the second half of my talk then, I want to discuss clinical endpoints that can be used to assess the efficacy of treatments for food allergy. And these fall into three main categories, the amount of food the patient tolerates post-treatment compared to pre-treatment, safety outcomes, and then patient and caregiver reported symptoms and quality of life.

The vast majority of food allergy trials to date have relied on the oral food challenge to determine how much food a patient can consume without having an allergic reaction after

treatment, and I think for good reason. The food challenge is the most objective and quantitative assessment we have to determine how much food a patient can tolerate. However, what increase in the amount of food tolerated actually constitutes an effective treatment can be debated, but I think this is a really important question to answer because this will determine whether or not an intervention is deemed to be effective.

Another important question is whether the benefit is sustained once treatment is discontinued. Desensitization refers to this increase in the amount of food that a patient can consume before they have an allergic reaction, but it requires that they continuously are exposed to the food.

Sustained unresponsiveness or remission refers to a lack of clinical reactivity to the food that persists even after they stop treatment. But even here, some level of continued exposure may be necessary, although the dose and the frequency of that exposure is not well defined.

Although food challenges are the gold standard, they certainly do have their limitations, as Alkis alluded to earlier today. There is always a risk the patient will have an allergic reaction, and in some cases those can be severe. The procedure is time consuming, both for families as well as investigators. It requires highly trained personnel and it's expensive to do.

There has been a tremendous amount of heterogeneity across clinical trials in how food challenges have been done. In the case of peanut OIT, the cumulative dose of peanut challenged has ranged anywhere from 1,000 to 10,000 milligrams of peanut protein, and so passing a challenge in one study can mean something very different than passing a challenge in another study. There's also variability in how much time is weighted between doses, anywhere from 15 minutes to 2 hours, depending on the trial, and that can have an impact on what eliciting dose is identified.

In some studies, investigators stop challenges only when the patient has objective

symptoms of a reaction. But in other studies, the challenges are said to have failed that the patient has subjective symptoms in two organ systems and even severe subjective symptoms in just a single organ system. There also isn't any consensus on when challenges should be performed during the course of treatment. In the case of OIT, most desensitization challenges are done after several months on maintenance dosing, but remission challenges have been done anywhere from one week to 12 months off treatment. And as you might expect, the longer patients are off treatment, the more likely they are to regain the reactivity.

Alkis alluded to this as well earlier this morning, but there's also no consistent way that food challenge outcomes are reported. Some studies will report the eliciting dose, the highest dose the patient received, that led to the symptoms that stopped the challenge. Others will report the highest dose that they tolerated, and then other studies will report the cumulative dose

that they either tolerated or that led to a reaction. All of these are absolutely valid approaches, but the inconsistency across studies has made it confusing to compare results across the different trials.

We also don't know the relevance of how much food a patient can tolerate during a food challenge relates to their real life tolerance, where food generally is not eaten in a graded stepwise fashion. We also know that there are other variables, such as exercise, viral infections, even sleep deprivation that can affect an individual's level or threshold of reactivity. Food challenges also are not validated to predict either the frequency or the severity of allergic reactions in the real world, and this can be something that's very challenging to study given the relatively low frequency of accidental exposures and reactions.

The second important clinical outcome, then, is safety, and this can be assessed in a number of different ways, including severe adverse

events, need to use epinephrine, anaphylactic episodes, and non-anaphylactic symptoms. A recent meta-analysis by Chu and colleagues, they looked at 12 different randomized controlled trials for peanut OIT and they found that compared to individuals who were strictly avoiding peanut or who received placebo, those in the active arm of the trials were much more likely to pass an oral food challenge to peanut, with a relative risk of 12. However, they were also more likely to experience anaphylaxis, a greater frequency of anaphylaxis, a greater need to use epinephrine, and more serious adverse events and non-anaphylactic reactions.

And so, I think in discussing or considering any new treatment for food allergy, patients are going to have to balance the risk and the benefits in having those discussions with families. I think it's important to understand what they hope to achieve by undergoing the treatment. In a recent survey by Dunlop and colleagues, they asked 123 caregivers of children

who either were actively receiving immunotherapy or had in the past, mostly for peanut and tree nut allergy. And here 62 percent of the respondents said their primary goal was to reduce the risk that their child would have a severe life threatening reaction; 11 percent wanted to avoid the hassle of strictly avoiding foods; and, only 9 percent wanted to actually incorporate the food into their diet.

But another study, another survey of almost 370 caregivers of children with food allergy found something very different. Here, the vast majority of respondents said their goal was really to be able to eat the food and incorporate it into their diet, and this was especially the case for children who were allergic to egg, milk, wheat, and soy.

I think all of us can agree that any treatment for food allergy needs to lead to meaningful long-term improvements in patient lives. But unfortunately, at this point we have very little data on how our current treatments for

food allergy are doing in that regard. And that meta- analysis by Chu I mentioned earlier, they found no improvement in food allergy quality of life with peanut OIT, but there was very little data to evaluate. There's only been three randomized placebo-controlled trials that have compared post treatment food allergy quality of life between subjects in the active and placebo arms.

I think it's easy to imagine how a treatment for food allergy would improve quality of life, certainly by protecting against any life threatening reactions, obviating the need to read labels and strictly avoid the food, and then certainly being able to incorporate the food into the diet. But I think for some patients there's some treatments that might actually reduce quality of life, especially if it leads to a higher rate of reactions. Although here the reactions may be more predictable in some people that will lead to less anxiety, but I think you also have to consider the burden of the treatment as well.

In the case of OIT, many regimens mandate that patients not exercise, shower, or bathe within 3 hours of taking the dose. They're asked not to take the dose right before bedtime. It can involve frequent clinic visits, especially during the buildup phase. They often need to take the dose every day, which can be an issue if you don't like the way it tastes. And I think we have to assume for many patients, treatment will need to be lifelong.

Part of the issue with discussing the pros and cons of any new treatment for food allergy is that heterogeneity across studies that I alluded to earlier and is just creating confusion for clinicians and patients on how to really consider the results of the various trials. To address this issue, there has been a major effort to try and develop a core outcome set for food allergy that will define specific domains and outcomes within those domains that will be measured in every future food allergy treatment trial.

There are currently two of these initiatives underway. One is being led by the Core Outcome Measures for Food Allergy Consortium and another by the European Academy of Allergy and Clinical Immunology. And the goal of both these efforts is trying to develop some international consensus on core outcomes and the instruments that will be used to measure those outcomes that will be used in all future trials.

So, to summarize the second part of my talk, the main clinical endpoints we have now to determine the efficacy of treatments for food allergy are really the amount of food tolerated, safety, and then patient and caregiver quality of life. The oral food challenge has been the main tool we've used to measure food tolerance, although it certainly has its limitations and better biomarkers are needed. That significant heterogeneity across study has made it very challenging to compare outcomes across trials. But we hope that issue will be alleviated with the development of a core outcome set that ideally

will put more emphasis on patient reported outcomes. Thank you.

DR. TOGIAS: Thank you, Pam. Our next speaker is Dr. Elena Goleva, who's a professor in the department of pediatrics at National Jewish Health in Denver. She received her PhD from the National University of Kyiv, Ukraine, and a postdoc training with Donald Leung and National Jewish Health. Currently, her research is focusing on the epidermal development, keratinocyte biology, skin barrier function, and immune responses in atopic dermatitis and food allergy. And Elena is going to talk to us about, let's see what you're going to talk, the relationship of atopic skin disease to food allergy. Thank you.

DR. GOLEVA: Thank you. I would like to thank the organizers for the opportunity to present at this workshop. So, as you know, since birth, our skin is subject to a number of environmental exposures, and skin is creating a barrier protecting us from variety of

interventions, although the underlying genetics may also be contributing to the skin barrier function.

So, recently, the dual allergen exposure theory has been introduced, suggesting if initial exposure to food allergen occurs through the skin, then this may involve allergic sensitization. However, if the exposure is occurring through the gut, this creates a tolerance. So, there are a number of pathways that are considered to be involved in skin barrier dysfunction that lead to eczema development and later to food allergy, which starts with initial barrier insults through scratching, microbe exposures, allergen, underlying genetic abnormalities, stress and pollution exposures, which release a number of alarmins, TSLP, IL-33, IL-25, and these are involved in regulating dendritic cell function and initiation of type 2 allergic responses in the skin.

So, in a recent study that we published in Science Translational Medicine, we actually

have shown that patients with atopic dermatitis with food allergy have the greatest skin barrier dysfunction. So, looking at the transepidermal water loss in the skin of these patients, we have found that normal non-lesional skin of these patients actually has a higher transepidermal water loss as compared to patients with AD only or healthy controls. And the greatest difference was seen with tape stripping. So, if you perturb the barrier and analyze the water loss as you sample the skin, the tool area under the curve for patients with AGN food allergy was the greatest.

On the molecular side. When we looked at the composition of the skin of these patients, turns out the amount of EOS ceramides, those are highly hydrophobic ceramides that are involved in skin barrier and hydrophobicity was significantly decreased in ADFA patients compared to AD and healthy subjects.

On the other end, if you look at the keratins as a representation of epidermal development and differentiation in a normal

looking skin of ADFA patients, we've seen a greater amount of keratin 5 and keratin 14, which are usually expressed in the deeper layers of the skin, suggesting that their skin has not been fully differentiated. At the same time, we've seen evidence for increased keratin 16 levels in the skin of such subjects, suggesting that there's some evidence of hyperprolific response instead of differentiation occurring in the skin of such individuals.

I would like to point out, so, all these patients in the study, they were allergic to penis, and these patients had a history of anaphylactic reactions. So, therefore, I would like to propose that probably the changes in skin barrier actually, and the changes in tool and changes in skin barrier composition may actually be as a predicting factor for patient either passing or not passing oral food challenge, but this remains to be seen.

So, our group has introduced minimally invasive skin sampling using a tape strip

analysis. So, what is happening? We are using these discs, which have adhesives. They apply to the skin, and then we apply up to 20 discs to the same area, and these discs are stored and could be preserved at -80 for prolonged time prior to analysis. In a number of publications, we have shown that these skin tapes could be used for a variety of applications for RNA seq analysis, lipidomic analysis, proteomic cytokine and metabolic analysis.

So, I would like you to introduce to our work that we had done in the birth cohort study, trying to understand how is skin barrier involved in regulation or predictability of a future allergic response. So, this is a cohort of patients that we have enrolled together with our collaborators in Seoul, South Korea. So, altogether, there were over 100 participants in this study, and these patients were monitored for 24 months since birth. And at 2, 6, 12, and 24 months, the samples were collected from these patients.

At the end of the study, by 24 months, we determined that there were 28 patients with atopic dermatitis. Nine infants develop eczema with food allergy, and nine patients had food allergy only. So, first, we were interested to see whether we were able to predict future eczema development in this cohort. So, what we have done, we took the tape strips from these patients at two months of age and characterized the cytokine profile in these samples. So, what we determined that there was already an increase in TSLP in the skin tape strip samples of kids that developed eczema in the future, at least at 6 months or up to 12 months of age. Also, we've seen an evidence for increased levels of IL-13 in that group.

Profiling the lipid profile in the skin, we have determined that in these patients that are destined to develop eczema in the future, we already see changes in the EOS ceramides that are cross linked to cornified envelopes, so called protein bound ceramides. So, EOS ceramides are

linked to proteins, and this creates a hydrophobic barrier in the skin. So, as you can see, EOS ceramides or protein-bound ceramides were already significantly reduced in skin of kids at two months of age prior to development of eczema, suggesting there were already some molecular processing occurring in the skin that were compromising their skin barrier function.

So, these two lipoxygenases, ALOXE3 and ALOX12B, are involved in a modification of EOS ceramides prior to their cross linking with cornified envelope proteins. So, what we found that these two lipoxygenases are actually under TSLP regulation. And using keratinocyte cultures which were exposed to TSLP, we've shown that both of these lipoxygenases can be inhibited by TSLP. Therefore, we suggest that the type 2 inflammatory response that is already establishing in the skin of these infants in two months may be involved in the regulation of protein-bound ceramide formation.

So, then, using the multivariable

logistic regression analysis, we were trying to estimate the risks of a future eczema development. So, individually, just looking at TSLP alone or the family history of atopy, the odds ratio of future eczema development were at least twofold or sixfold. However, if we are now combining this together with few lipid markers, you see that the odds ratio is starting to increase.

So, here, if we're using TSLP and a protein balanced ceramides, the odds ratio of future eczema prediction is now up to thirtyfold. And the highest combination which was a family history IL-13 and a protein-bound EOS ceramide plus a sphingomyelin gave us an odds ratio of 54 of future eczema prediction.

We also noticed that in this cohort there were a number of children that developed food allergy in the future. Most of these children, they had food allergy to egg. One of the patients was peanut allergic. We also were interested to see whether any predictors of future food allergy development at two months of age.

So, what we found was there were these unique ceramides with monounsaturated fatty acids, 24:1 and 26:1, which were uniquely increased in the skin of the future food allergy kids. And we think these ceramides may also be involved in the regulation of the skin barrier. And this stems from a prior work with artificial lipid membranes where researchers were mixing ceramides with monounsaturated fatty acids or non-unsaturated fatty acids. And when they found that when the membranes are enriched in such monounsaturated ceramides, then the water flux through such membranes is increasing suggesting that mono-unsaturation of fatty acids and ceramides is involved in water regulations with the barrier. Therefore, we also suggest that likely the increased presence of such ceramides in the skin or future food allergy kids may indicate also some barrier deficiency early on in life in such individuals.

On a cytokine perspective, looking at the panel of cytokines in a skin tape samples,

what we found here that uniquely IL-33 was increased in all of the kids that developed food allergy in the future. And again, using a combination of these markers. Individually, they all had some predictability for future food allergy development. However, the greatest effect was achieved if we combined IL-33 and 24:1 and a ceramide with the odds ratio of future food allergy prediction of 100 fold. So, this data will be actually presented at the AI meeting this week.

Using animal model studies, it has been shown that both TSLP and IL-33 have unique role in a future eczema or food allergy development through the epithelial perturbation, these alarmins are released. However, and one of the recent studies by Dr. Jaha's group have shown that if you perturb the skin barrier, you can see elevation of TSLP in IL-33 at the skin site. But when you look at the plasma, actually you only see IL-33 increase in circulation but you do not see TSLP release. But if such animals then are

challenged with ovalbumin, it turns out that this mechanical skill injury promotes food allergy anaphylactic reaction due to IL-33 release from the skin, which then promotes intestinal mast cell expansion.

So, in conclusion, I hope I've provided you some evidence that epicutaneous sensitization may contribute to food allergy development and our birth cohort studies support this theory because there are unique changes that we are observing in the epidermis and epidermal barrier composition that occur in infants at two months prior to development of AD or food allergy. So, we observing an increase in TSLP which is also found in patients with eczema and the increase in TSLP is also found in the future AD infants. On the other hand, we see an increase in IL-33 in the skin of future food allergic individuals.

So, therefore we propose that there's a unique role for TSLP and IL-33 in future AD and FA development. And animal model studies also support some of this notion.

With that, I'd like to thank our funding agency, Atopic Dermatitis Research Network and also Sunbeam ABC. And also, I would like to thank the lab and number of collaborators and Dr. Ahn and Dr. Kim, our collaborators at Samsung Medical Center that enrolled this birth cohort which we're now investigating. Thank you.

DR. TOGIAS: Thank you, Elena. Our next speaker is going to be Alexandra Santos, who is Professor of Pediatric Allergy at King's College London and Attending Physician Pediatric Allergy at the Evelina London Children's Hospital, qualified in Medicine from the University of Coimbra and specialized in allergy and clinical immunology and completed her PhD at King's College. And Alexandra is going to talk to us about basophil activation tests.

DR. SANTOS: Thank you very much, Alkis. I'd like to thank Dr. Rabin and all the organizers for the great opportunity to be here and actively participate in this workshop. It's really a great pleasure to talk about basophil activation test

and whether it's ready for prime time.

So, these are my disclosures and this is the outline of my talk. So, I will take you through the rationale for using the basophil activation test as a biomarker for food allergy. Then, which type of biomarkers I think the basophil activation test constitutes in the context of food allergy, and also some validation, technical and clinical validation that we and others have done and that support the use of this test more widely.

So, the rationale to use the basophil activation test as a biomarker for food allergy is that basophils, together with mast cells, are defector cells of acute allergic reactions. So, as I'm sure you're all very familiar, in an allergen specific immune response, there's a Th2 biased response to the allergen. B cells are commits switch on to IgE production, commit to producing IgE, and differentiating the plasma cells that produce IgE. And this IgE is bound to high affinity receptors on the surface of mast

cells and basophils. And this is sensitization.

But then in allergic individuals who also have IgE, when they are next exposed to the allergen, then the allergen is able to cross link two or more IgE molecules on the surface that are receptor bound on the surface of the mast cells and basophils. And then this triggers cell activation and the granulation with the release of vasoactive mediators that are responsible for the allergic symptoms. So, the basophils are central cells to acute allergic reactions to foods.

Now, the basophil activation test is a flow cytometry based assay. So, we use whole blood in an anticoagulant that needs to be done within 24 hours of blood collection. Then a small volume of blood is aliquoted to different tubes that are then stimulated with different concentrations of the allergen, buffer alone as a negative control, or anti IgE as an IgE mediated positive control, and FMLP or another non-IgE mediated stimulant that is able to activate basophils like FMLP as a non-IgE mediated positive

control. And then we add antibodies sustained for the basophil population. And then within this basophil population, we looked at activation markers on the surface of basophils, and CD63 is one of them, and CD203c is another activation marker that is used.

And so, the typical result for the basophil activation test in an allergic patient is this bell shaped dose response curve, where with increasing concentrations of the allergen, there is an increase in the expression of the activation markers up to a plateau. And then we often refer to basophil reactivity as the proportion of basophils that are activated at a given concentration. And the sort of inflection point of this dose response curve is the maximal reactivity. And then we refer to as basophil sensitivity the amount of allergen or the concentration of allergen that is needed to induce basophil activation. And EC50 would be the concentration of allergen that's needed to induce half maximal reactivity of the basophils. And so,

these are different ways to report the outcomes of the test.

So, the beauty of the basophil activation test is that it is a functional assay that measures the combined effect of different characteristics of IgE. So, it not only detects the presence of IgE, but also whether this IgE is able to convey the activation signal onto the basophils. And I keep citing this work from quite a few years ago now by Kristas and Netal (phonetic). And it's with house-dust mites. So, not really food allergy, but I think it's a very elegant work where they sensitized human basophils with monoclonal antibodies towards their P2 (phonetic), and then they sensitize these basophils with a known repertoire of monoclonal antibodies, and they very elegantly show that, and I'm just giving two

examples here.

So, for specific activity, which is the proportion of IgE, that's allergen specific in relation to total IgE, and the affinity of IgE for allergens. So, they showed that inducing variation of these functional characteristics of IgE would reflect on a different level of basophil activation. And so specifically for specific activity, which is the top graph, they show that the more allergen specific IgE, you add the same amount of IgE. So, just the proportion of IgE that's allergen specific, the greater the proportion of basophils that become activated. So, essentially it changes basophil reactivity.

Whereas if you synthesize the basophils with antibodies that have higher affinity for the allergen, this doesn't significantly change basophil reactivity. So, in terms of the proportion of basophils that become activated, but as you have antibodies of higher affinity, you're able to induce basophils with lower amounts of allergen. And so, this reflects more changes in

basophil sensitivity.

So, we have done some studies in the lab using plasma from patients. So, not monoclonal antibodies. We are not so sophisticated, but we were looking at just plasma from patients that were assessed for possible peanut allergy. So, in red, you see allergic patients, in blue, patients that had IgE but were tolerant. And we measured the levels of specific IgE to peanut, which is what you see in the upper left graph labeled with specificity. Then we calculated the amount of IgE that's allergen specific, that specific activity as a proportion of total IgE. We measured diversity as the number of allergens within peanuts that IgE recognized, and then avidity as the strength of which the collective of IgE binds to the peanut extract.

And as you can see from the graphs, all of these variables, particularly specificity, specific activity and diversity, were directly correlated with basophil and mast cell activation. And so, we then put this in diagnostic models.

So, combining these functional characteristics of IgE. So, what you can see in the graph is the relative importance of the various functional characteristics of IgE to induce basophil and mast cell activation. So, diversity and specific activity were the most important. Titus, which is what we currently used in clinic, was actually the least important.

And then here you can see in the table the diagnostic performance of these models, considering three or four functional characteristics, and then the basophil activation test and the mast cell activation test. So, you can see that the basophil activation test performed best, but also using these functional characteristics performed better than just measuring the levels of IgE to peanut. So, which type of biomarkers can the basophil activation test constitute in the context of food allergy? So, I think it can be a diagnostic biomarker, a prognostic biomarker, a response biomarker and a predictive biomarker. And I'll show you some

evidence of why that is.

So, in terms of using the basophil activation test as a diagnostic biomarker, so we have been conducting studies funded by the Medical Research Council in the U.K., where we invite children that need a challenge for clinical reasons, and then we do double-blind, placebo-controlled food challenges in all children. So, these were studies designed according to the starred guidelines. So, we do double-blind, placebo-controlled food challenges in all children. And then on the day of the challenge, we do skin prick testing and we collect blood samples for igg testing and for the basophil activation test.

So, this is an example of basophil activation test, the egg, for example. So, you can see how we identify basophil. So, in the first row of flow plots, you can see we gate on the lymphocyte monocyte population, then site scatter low CD203c positive, and then CD1 to 3 positive, HLA-DR negative, and that's our basophil

population. Then we gate the negative control, and then we measure whatever is above that with the increasing concentrations of allergen or positive controls. So, we have completed the study for peanuts; we have completed the study for egg; and we are close to completing the study for cow's milk, sesame, and cashew nuts. And the reason for this is that, as you know, the allergy tests are allergen specific, so we have to really to validate them for each individual food allergy.

So, for the basophil activation test to peanut, we have previously shown that this distinguishes quite well between allergic patients there in red and patients that have IgE to peanut but are tolerant in blue. In our initial discovery cohort, the basophil activation test to peanut had both high sensitivity and high specificity. We then validated this in an independent population and applied this cutoff that we had previously identified as the optimal cutoff, and that was for the 100 nanogram per milligram concentration, or the average between 10

and 100, which performed exactly the same.

And this had a lower sensitivity at 83 percent, but a high, very high specificity, so it was 100 percent. In this validation cohort, we then had the opportunity to test with exactly the same method for the basophil activation test to peanut, the children that were coming to the end of the LEAP study, and then one year later at the end of LEAP-On study, and also the peanut allergy and sensitization study.

So, on the day that they came for their peanut allergy assessment, which included food challenges, in the vast majority of cases, we did the basophil activation test. And so, applying the cutoff that we had previously identified to this very large population of very well characterized children from the LEAP and associated studies, again, the sensitivity was lower but the specificity was 99 percent. So, confirming that the basophil activation test is very useful to confirm the presence of peanut allergy if the test is positive. And the ROC

curves in the middle show the relative performance of the basophil activation test in relation to the other tests done in parallel in the initial discovery cohort.

So, more recently, we concluded the study for egg allergy where we included 150 children aged 6 months to 15 years that needed a challenge to egg and 40 percent of those children. So, 60 out of the 150 reacted and the others did not. We had a small proportion of indeterminate challenges. As you can see from the graph in the middle, there was a very good discrimination between the results of the basophil activation test in the allergic children compared to the children that were sensitized but tolerant. The sensitivity of the basophil activation test the egg was 78 percent and the specificity was 77 percent. And on the right-hand side you can see the ROC curves compared to the other tests that were done in parallel.

Now, recently, to inform the new EAACI clinical guidelines for the diagnosis of IgE

mediated food allergy, we have conducted comprehensive systematic review of the literature and meta-analysis of the accuracy of any tests to diagnose any food allergies, so long as the studies included at least a proportion of patients that underwent challenges. We included 149 studies overall and this corresponded to over 24,000 subjects.

In terms of the basophil activation test studies, we included 27 studies and we were able to do meta-analysis for the basophil activation test to peanut and the basophil activation test to sesame because for the other foods we didn't have enough number of studies. These figures on the left-hand side show the risk of bias assessment for the studies included in the meta-analysis, where green means low risk of bias and red high risk of bias. And you can also see the diagnostic performance of the tests as a result of the meta-analysis. So, with the basophil activation test to peanut having about 91 percent sensitivity and about 80 percent specificity, and the basophil

activation test to sesame having 89 percent sensitivity and almost 93 percent specificity, what you can see on the right-hand side is how the basophil activation test performed compared to the other tests in the same studies in the same meta-analysis. So, you can see that for peanut, the basophil activation test and specific IgE to Rh2 (phonetic) using the ImmunoCAP single plex were the best tests. And so for the sesame seed allergy, the basophil activation test was the best test followed by Ses I 1 specific IgE.

We have now completed the guidelines that have recently been published and the basophil activation test was included as a recommended test to support the diagnosis of IgE mediated food allergy with high certainty of evidence. It was a conditional recommendation, mainly for the fact that it's not a test that's widely available to clinicians. And in the diagnostic algorithm, so, in the diagnostic pathway, the basophil activation test figures as a sort of subsequent step in the diagnostic assessment.

So, after patients are submitted to skin pre-test, specific IgE and then specific IgE to individual components for the food that this is informative, and then the basal activation test can be done, particularly for peanut and sesame, which is where he had the strongest evidence. And then oral food challenges only if needed to clarify the allergic status.

So, now some data on the technical validation of the basophil activation test. So, this is a study that we did at King's College London. So, where we assessed the intra assays coefficient of variation, which is what you can see in the left-hand side graph, which was less than 5 percent in experienced hands. We included 102 children that were being assessed for possible peanut allergy in this study, and 72 of these were allergic and 30 were sensitized.

We tested the children using two different methods for the basophil activation test, and this is the correlation plot for these two methods. So, you can see that there was a

strong direct correlation between the two methods for the basophil activation test, but the results were not exactly the same. We had our in house method and then an external method, and this external method had about 4 patients out of 32 that were challenged. So, selecting only the patients that were challenged, there were four that were misdiagnosed compared to the outcomes of challenges.

We also did the same patients, and all these patients we tested in two labs on the same day. So, in our research lab at King's College London, and then in a diagnostic lab. And this is how the result looks like for 100 nanograms per milligram of peanut extract. So, very strong correlation between the two results, which was much better than we expected with a very low variation. So, this was a very nice proof of concept that if the methods are very carefully standardized and performed in experienced hands, then they can be very reliable and reproducible.

Now, the basophil activation test as a

prognostic biomarker. So, there is evidence from studies that the basophil activation test can provide some information about patients that at higher risk of having severe reactions and also of reacting to lower amounts of the allergen. I'm giving you, here, the example of severity for time constraints. So, we and others have shown that the greater the proportion of activated basophils, the higher the risk that patients will experience an allergic reaction during challenges. So, this on the far left, it's the graph of our initial study. So, where we looked only at patients that had a positive challenge, so they had challenge proven food allergy, and then within those we looked at whether they developed a severe reaction or a non-severe reaction. And the basophil activation test discriminated well between the two groups.

In the middle graph, it's looking at the severity of allergic reactions in participants in the LEAP and associated studies. And so here we went a bit further. So, determining cutoffs for

the basophil activation test to identify the patients that have severe reactions, which are the ones that you can see in the middle graph as having red dots. So, these are the patients that had severe reactions on the challenges. And so, the cutoff for the basophil activation test had 100 percent sensitivity and 97 percent specificity to identify the patients that had severe reactions during the challenges.

And then on the far right is a figure from a study by Sharon Chinthrajah and colleagues looking at peanut allergy confirmed by challenge and looking at a variety of different parameters, clinical and immunological, to predict severity of allergic reactions during peanut challenges. And the basophil activation test and two asthma biomarkers were the best predictors of severity and severe outcomes.

We have also done -- apologies, this was meant to be animated, but we have just finished the X study and we did similar analysis looking at severity of allergic reactions. So, the tables

that you cannot read are just a list of parameters that we looked at in terms of how different they are in severe reactors versus non severe reactors. And these are double-blind placebo-controlled food challenges to egg. And there was no statistical significant difference in this long list of parameters except for specific IgE 12 ovomucoid and the basophil activation test to egg at a variety of concentrations.

What you can see in the left-hand side is a representation of the result of the basophil activation test to egg in severe reactors versus non severe reactors. And then in the middle the ROC curves for the basophil activation test in red and the specific IgE 12 ovomucoid in green. And then on the right-hand side, the sensitivity and specificity of the identified cutoffs for the basophil activation test to predict severe reactions to egg during challenges, which was 76 percent sensitivity and 78 percent specificity. Now, the basophil activation test as a predictive biomarker. So, I give the example here of two

peanut oral immunotherapy studies that have shown that the basophil activation test can identify as early as three months into treatment the patients that are going to have sustained and responsiveness versus the patients that are going to have transient desensitization. So, I'd like to highlight here the study by Saritha Patil and colleagues where the basophil activation test to Rh2 as early as three months enabled to distinguish these two different pathways.

So, with patients that had sustained unresponsiveness having a decreased basophil activation to Rh2 that was sustained over the course of treatment and after treatment, and then with patients that had transient desensitization, there was a slight reduction in basophil activation, but this quickly bounced back to levels similar to pretreatment levels. And then this randomized controlled trial of peanut oral immunotherapy had a similar finding.

Now there are a variety of studies in the literature documenting the basophil activation

test as a response biomarker. So, I'm giving here some examples of studies looking at allergen specific immunotherapy in different modalities. So, oral immunotherapy, sublingual immunotherapy, and epicutaneous immunotherapy to do two different allergens. So, peanut and egg. And just taken together, what the studies can show is that there is a decrease in basophil activation to the allergen compared to placebo. And this can be quite early, during -- quite soon after the start of treatment, as early as 12 weeks, for example in the peanut epicutaneous study. And this is not seen in placebo treated individuals or in the egg OIT study that I bring here, which compared to patients undergoing a baked egg diet.

So, now the question is whether the basophil activation test can be used as a surrogate endpoint. So, we've heard a lot about this today and I think I have provided some evidence that there is a clear mechanistic rationale to use the basophil activation test as a possible surrogate endpoint, that if used in a

standardized way and carefully inexperienced hand, it can be a reliable measure. There is evidence from randomized controlled trials that the basophil activation test mirrors the clinical outcome. There's data from observational studies defining very precise cutoffs. And so, I see the basophil activation test as a potential surrogate endpoint both for clinical practice and for clinical trials.

We've heard about the advantages of doing this in food allergy, which would be to reduce double-blind placebo- controlled food challenges, have easier assessment of patients, have less invasive, less risky procedures. So, this would facilitate the feasibility of clinical trials and would also encourage patients to participate in clinical trials.

Now, of course this would be the benefits. Of course there are risks, and I think we can mitigate those risks. For example, for the basophil activation test, there's about 10 percent of subjects that have non responder basophils.

So, for those individuals, we could have an alternative, which could be a mast cell activation test, or could be IgE measurements or a combination.

And also, if we did do a study where the basophil activation test was used as a surrogate endpoint, I think this could be complemented with direct evidence from clinical benefit, and this could be an additional trial looking at oral food challenge outcome or looking at real life benefit in terms of the ability of patients to introduce the food, the reduction in allergic reactions or severity of allergic reactions in the community.

And with this, I would like to conclude with my take-home messages. So, the basophil activation test is a functional assay that includes all elements of acute allergic reactions, as much as we can do in a test tube, and reflects well the clinical phenotype of patients. I think there's clear evidence associating the basophil activation test with the outcome of oral food challenges and whether the patients are allergic

or tolerant, severity of allergic reactions during challenges, clinical response to immunomodulatory treatments, and prediction of response to treatment, whether this is going to be favorable or not. And for this reason, I think the basophil activation test can be used as a diagnostic biomarker, as a prognostic biomarker, as a response biomarker, and as a predictive biomarker.

And lastly, the basic activation test has been recommended as a test to support the diagnosis of IgE mediated food allergy in the new EAACI clinical guidelines. And I think it can be a validated or at least a reasonably likely surrogate endpoint for food allergy.

And I would like to thank all my lab and about two study team, all my colleagues and collaborators and my funders, particularly the Medical Research Council in the U.K. And Immune Tolerance Network and NIH in the U.S.

And thank you for listening.

DR. TOGIAS: Thank you, Alexandra. Thank you very much. Our next speaker is Dr. Hugh

Sampson, who's Kurt Hirschhorn Professor of Pediatrics at the Icahn School of Medicine in New York, and has over 40 years' experience in translational research focusing on food allergy and basic immunologic mechanisms. Need to say he's a past-president of the AAAAI, and most importantly, he was elected at the National Academy of Medicine in 2003. Hugh.

DR. SAMPSON: Thank you, Alkis and like to thank the organizers for inviting me to this meeting. So, I'm going to be speaking about epitope specific antibodies as possible diagnostic, prognostic, and predictive biomarkers. To start, I do want to give my disclosures to let you know that Mount Sinai has licensed the IP for commercial development of this speed based epitope assay that I'll describe to you to allergenics. And I do sit as an uncompensated member on their board.

So, the objectives of my talk in the next 20 minutes is to describe the rationale and technology for mapping allergenic epitopes,

discuss how epitope specific IgE may be used as a diagnostic biomarker, and then discuss some evidence on how epitope specific IgE profiling may be used as a prognostic and predictive biomarker.

So, just to give you a little history on this, this all started over 25 years ago when we asked the pretty naive question about whether or not epitope specific IgE binding to various food allergens could account for the differences we see in allergenic reactivity. And at that time there were a fair number of the allergenic proteins that had been well characterized. We knew the amino acid sequence, there was some information on confirmation, but basically we know that IgE or any antibody can bind to either conformational or linear epitopes. Now, when we do these assays, at that time, the technology was this spots membrane, and basically we are able to generate 10- to 15-mer amino acid peptides. And what we would do is basically take the sequence of the particular protein, we'd generate a series of overlapping amino acid base peptides so that we could identify

where specifically these antibodies bound to the linear portion of the various proteins.

And with that, we started off with ovomucoid and using patient plasma serum. So, this is serum from patients who had undergone oral food challenges to egg. We were able to identify five locations, or epitope regions on the ovomucoid. We then started looking at individual patients, and when we did that, we found that only about 50 percent of them actually bound any of these linear epitopes. So, we tried to figure out what was going on, and we basically took these proteins and we ran them on native gels. And one set was just the native protein, one we reduced and alkylated. So, you've linearized the protein, so you've lost the conformational epitope. And then we also looked at deglycosylation.

And what we saw was that the children who had the persistent allergy, those that did not outgrow their egg allergy were the ones that were binding to the linear epitopes, whereas the children that had transient or would outgrow their

egg allergy, which is about 80 percent of children, did not bind these linear epitopes. So, this got us into the concept of linear and conformational epitopes, but led to the hypothesis that the induction of IgE to these various linear epitopes, which would largely not be unaffected by either processing or digestion, were responsible for the long lasting persistent allergy.

So, then Wayne Shreffler came into the lab, and Wayne was interested in peanut, and peanut had a whole larger number of potential epitopes. And he developed this epoxy glass slide assay where we were able to analyze 210 overlapping peptides at the same time. And we generated this profile of epitopes, or potential binding to peptides, for Ara h 1, 2,and 3.

We then ran into the problem of trying to get consistent epoxy glass slides. So, we had a lot of problems with getting different, lots of slides that would bind these peptides in an equivalent manner. So, then moved on to the Luminex system, where we then started conjugating

the various Luminex beads with the various peptides. And here we took 64 peptides, which seemed to be the most informative from the work that Wayne had done, and put this into this assay system, this Luminex assay system, where we could then get mean fluorescent outputs, intensity outputs, on each of these separate peptides with a very high throughput system.

And then shown down here is basically looking at the kind of map you might get from a whole group of patients. But basically, this fluorescent intensity reflected IgE levels to each of these different peptides. The other advantage of the Luminex system when we compare it to the microarray is that we are able to use much smaller amounts of serum or plasma, that its high throughput can be done in much less time than we could do with the slide method. And then also the variation in reactivity to the various peptides done on different days, different amounts, was much tighter using the Luminex bead system.

And then this is just giving you

basically a heat map readout of a number of patients that had been analyzed for binding. And what you see along the y-axis is, or the horizontal that goes across the y-axis there is the individual patients, and each column represents a different peptide. And you can see that there are major differences in the different patients. And with that, you can basically sort individuals out into those who are, in this case, we're calling them allergic, but these are the reactors from the nonreactors.

So, then we wanted to look at whether or not this assay, where we're looking specifically at different epitope binding, could be more accurate in the diagnostic arena than what we have currently. So, we were able to get 133 subjects from the LEAP trial, and these were individuals that were in the avoider group. And then analyzed them for epitope binding. And basically, what we were able to find after going through various machine-learning algorithms were two epitopes that seemed to be most specific in the diagnosis of

peanut allergy, as shown by oral food challenge.

So, we took this algorithm and then we applied it to two other study cohorts, the CoFAR2 cohort that we had. So, this was 82 patients under the age of or at about five years of age, and then 84 patients who were in the Poise trial at Stanford, looking at peanut oral immunotherapy and taking baseline samples. And then this slide just shows you the different sensitivity specificity of all the different methods of looking at peanut allergy. And the ones, I think that are most important to us as clinicians is the accuracy of diagnosis. And what you can see are the accuracy of diagnosis for skin test, for specific IgE to peanut and the various components.

Looking then, though, at the use of these two specific peptides, you see that the accuracy rate is significantly better than what we see with the standard methods in use. And then if we want to combine all three together, we can actually get a little bit better accuracy. And so, looking at this and comparing the different

groups, we see that the concordance with the oral food challenge was about 93 percent using this bead-based epitope assay, compared to prediction with the prick skin test, which was in about 84 percent, and then ImmunoCAP IgE and Ara h 2 were significantly lower. So, overall, this assay did seem to have best concordance with the outcome of oral food challenge in this group.

The next thing then was to look and see was it possible to use this profiling to get a better idea of how much peanut individuals would be able to tolerate before developing a reaction. And there was evidence, again, from some work that Wayne had done looking with the microarray system, that the more diverse epitope binding you had, the more likely you were to have a reaction or a different reaction rate.

So, we basically were able to take samples from two studies for the discovery cohort, the BOPI trial from London and then the OPIA trial from Australia, and use those to develop the algorithm based on the different challenge levels.

And you see the different amounts of protein that these individuals ingested, which would then give us a broad range of different levels of reactivity. And then we were able to use three other trials, Cafeteria trial, CoFAR6, and POPEETS trial, along with some samples from the first two, to validate this system.

So, looking at this, this is now just a heat map representation of what IgE looks like. The red is the higher levels of mean fluorescent intensity, the blue are the lower. And what we can see is that there is a nice inverse correlation between the cumulative tolerated dose and the diversity of IgE binding to these various epitopes. But the question was, and again, using machine learning, whether we could cut that down so you can use this in a more effective way. And we found that in this case, there were two peptides that did seem to be most representative, and these were one from Ara h 2 and one from Ara h 3.

In looking at the predictive models,

there was a strong suggestion that we would, in fact, be able to get some idea putting people into three different buckets, low, moderate, and high tolerance to the peanut protein. And from this, we have this result where we were able to put people into these different areas, either low, moderate, or high threshold levels. And when we run these algorithms, then what we can see is, for example, if you happen to be in the low threshold group, you have about a 50 percent chance of tolerating a cumulative dose of 44 milligrams of protein, or you would react after the 30 milligram dose, the 100 milligram dose. Whereas if you're in the middle group, you would have about an 80 percent tolerance or probability of tolerating that amount. If you're in that high group, you actually have 95 percent. So, most likely, you would tolerate that with no difficulty.

Looking at higher dose levels. So, now, this would be somebody who would respond after the 300 milligram dose. And what you see is in that low group, only 10 percent would tolerate that,

whereas in the moderate group, about a third, and in that high tolerance group, you actually have about 75 percent of them, could tolerate this dose with no problem.

So, we then went on and looked at some other things. One is, could we predict early on in children whether or not they were going to develop peanut allergy? And this comes from 293 subjects out of our CoFAR2 Natural History study, where we looked at epitope profiling, using the Epitope plus peanut specific IgE, or using the standard peanut specific IgE in the Ara h 1, 2, and 3. And what we wanted to see was whether or not we could predict in this group by either 3 to 15 months of age, which is when they entered the trial, or 2 years of age, what the outcome would be at 5 years of age. And basically, what you see from this is that if you combine the epitope specific IgE with peanut specific IgE, you could predict at about 95 percent accuracy, who, in fact, would end up with peanut allergy at the 5-year challenge.

We then also had the opportunity to look at another group of patients, and this was from a cohort of patients from Turkey, where Dr. Sackensen had evaluated these milk allergic children with different forms of milk protein. And we know that about 80 percent of children will outgrow milk allergy. We know that that group can often tolerate baked milk products. But also, as they develop their tolerance, they are able to tolerate less denatured protein.

So, she had challenged these patients first to baked the baked milk products. So, a muffin. Those that tolerated that got challenged to a Greek yogurt, which has a fair amount of denaturation of the protein, and then to whole milk. And divided those into the three groups. And we wanted to know, could we profile these and be able to determine who, in fact, would fall into each of these groups.

And so, looking at this group, then again, this is a heat map representing that. And as you see, as you go on the heat map from right

to left, the baked milk reactive group. So, these are the group that probably are not going to outgrow their milk allergy, had the most IgE binding to a diverse number of epitopes. And as you go across, you see much less in the way of binding. And the figure on the right there just shows you that correlation.

And what you can see from this is that, in fact, as you go right to left, there is less IgE binding. But the question was, could we actually pigeonhole them into their particular group? And this is just showing you the outcome of that comparison. And you can see that we are able to determine with about 95 percent accuracy which group each of these patients would fall into based on this algorithm generated with the epitope profiling. And so, you have ability to phenotype these children at about 86 percent with a high sensitivity and specificity, the area under the curve being 0.89.

And then finally, one of the things we wanted to see was whether or not we could predict

in this trial of milk oral immunotherapy, who would end up developing sustained unresponsiveness. And what was done here was taking the children from this Milk OIT trial. This was one where we used Milk OIT plus or minus omalizumab. In this case, omalizumab had no effect on outcome, great effect on reducing adverse reactions, but no real difference in immunologic response.

But again, what we wanted to know could we see who, in fact, was going to develop the sustained unresponsiveness, who was not going to get a good response, and who would only be desensitized? So, we had serra (phonetic) available from 47 of the 55 children that were in this, who had completed this trial. 94 percent of them passed the 10-gram milk oral food challenge at the end of the trial. Of that group, they then went off all immunotherapy for eight weeks and then were re-challenged to 10 grams. And half of the children were able to tolerate that.

So, we then evaluated the IgE and IgG4

binding to 66 different milk epitopes. This is using the algorithm for this, we found that there were six epitopes that seemed to give us the best predictive value. And what you see here is with these six, we had an area under the curve of about 0.95. This breaks down the whole cohort, but basically we have sensitivity about 87 percent, specificity of 86 percent, and an accuracy about 86 percent. So, most patients, as you can see there, were in the correct, or were correctly predicted. This, then all has to be validated. The last two are really things that have just been done under discovery.

So, in conclusion, the bead-based epitope assay has been validated as a potential diagnostic biomarker for peanut allergy. This is commercially available from a CLIA certified lab in Pennsylvania, and there's work ongoing to try to do similar validation with milk, egg, wheat, and sesame. The bead-based epitope assay has also been validated as a potential prognostic biomarker for a range threshold of reactivity or

accumulative tolerated dose. And again, this is also available from the same lab in Pennsylvania.

We then have, at least have discovery phase data suggesting that the bead-based epitope assay may also be useful in predicting the degrees of milk and egg sensitivity. In other words, will they tolerate the unbaked form, cooked form, et cetera? Also, may be very useful for the early identification of infants at risk of developing persistent peanut allergy, milk, or egg. And I think this is especially critical now because there's a lot of evidence suggesting that early intervention in those first few years of life can be very critical.

And then also for identifying milk, peanut allergic patients who are more likely to achieve this sustained unresponsiveness or remission compared to those who were likely only to end up with desensitization. And I think this becomes important when you're having those discussions with the patients about going into some form of immunotherapy. And then finally,

early studies suggest that the bead-based epitope assay profiling may provide some predictive information regarding the peanut allergic patient's potential for severity of reaction following an accidental ingestion.

With that, I just want to acknowledge the people that did a lot of the work, especially our statisticians who do all the machine learning, Mayte Suarez-Farinas and Maria Suprun. A lot of assistance from Bob Getz (phonetic) and Paul Kearny, who were at AllerGenis. And then thanking all the investigators from these various trials who provided us with patient samples to evaluate.

Thank you.

DR. TOGIAS: Thank you, Hugh. So, our next speaker is Eric Wambre. Now, I will have to apologize to Eric only because your biosketch was not transcribed on time. So, all I can say --

DR. WAMBRE: But you know me.

DR. TOGIAS: -- is that I know you very well from when you were at Benaroya. But I do know that you're the Associate Director of the

Jaffe Food Allergy Institute at the Department of Pediatrics at Mount Sinai. And, of course, that you have a long history of working on T cells and food allergy. So, if that's enough, go ahead.

DR. WAMBRE: That's perfect. Thank you. So, good afternoon. So, I would like to thanks Dr. Rabin and also the organizer for giving me this opportunity to talk about prognostic biomarker in food allergy.

And I would like to start because it's, I think, one of the last presentations today that I think overall, we can see biomarker as kind of an art, an art of forecasting individual clinical outcome. And a year ago, I had the pleasure to visit Istanbul, where I discovered that over there you can see some people that are reading at the pattern of your coffee cup and to see, to predict your future. And actually, when I was there, I was really impressed about this, and I realized that I was almost doing the same thing, and I was kind of surprised. But at least I would like to highlight one things, one key differences between

what is done in Istanbul and what we are doing is clearly that what distinguished the mystical readings of the coffee ground from our biomarker discovery is not just the tools that we employed, but the rigorous validation process we add there, too. And by this, I mean how the extensive clinical research and data analysis we are doing to validate those biomarker.

And I would like to start with this, the limitation right now, and at least this is my point of view, but in food allergy, clearly right now, the main limitation is the difficult access to the biological samples for food allergy, and mainly to cover the broad spectrum of the disease severity, because most of the time when you get access to those patients, you want to make sure they are truly food allergic. And the only way right now is food challenged. And this is not easy to get access to all those blood samples covering again the broad spectrum of disease diversity.

So, the goal is really to encourage

efficient monitoring during the clinical trial. That's where we usually have access to all those samples, and that will head also to have those biomarker validation. So, overall, I think we need to have an increased collaboration between the industry, academia and also the FDA.

The other limitation I see is not about the discovery of the biomarker, but mainly about the weakness about financial support to develop the clinical grade prototype. Once we found a potential biomarker, then we need to standardize the assay and we need also to do a lot of technical performance. Looking at the reproducibility sensitivity, this is not really fun to do, but that's very important when we want to validate a biomarker.

And finally, the last things that kind of summarize everything, that's also to ensure the transparency and integrity of the road that are used to generate sophisticated analysis. This point mainly came from COVID when we saw a lot of tsunami of data from COVID research. But as

system biology rely increasingly on the complex model and graphics, there is a risk that the quality of the underlying raw data is overlooked. And this really emphasized the need for rigorous data validation to prevent incorrect conclusion.

So, as prognostic biomarker might also influence, can be also influenced by therapeutic intervention, the distinction between predictive and prognostic marker can be ambiguous. So, overall, we can say that prognostic biomarker really try to forecast disease outcome, while the predictive biomarkers will try to forecast the treatment outcome. And in food allergy, prognostic biomarker can inform about the progression of the disease irrespective of the treatment, specifically trying to predict the likelihood of outgrowing the allergy, the development of tolerance, but also the risk, how severe could be the reaction after an accidental exposure. It also can help to identify the persistence at the severity and whether -- how dangerous could be the food challenge in those

patients. And finally, those prognostic biomarker can help clinician to make informed decision about the prevention strategy, disease management, and treatment option.

So, currently there is several prognostic biomarkers that have been identified that hold promised and as the previous two talk earlier we showed that the basophil activation test is one the diverse IgE repertoire as well, and this usually reflects the severe on the type. We also observed that the high specific IgE titer could be also used as a prognostic biomarker. The size of the wheel from the skin prick test, also the component testing, the diversity of the allergen that could be involved. And also, finally, there is some report about increased T cells repertoire.

Overall, that's not the only one. We have also the allergen specific T cells. A few weeks ago, there was also a very nice paper about the B cells as a potential biomarker to predict the severity. We have gene expression. We have

the microbiome metabolome proteome that can really represent putative on the type axis which require however further investigation.

So, prognosis biomarker for food allergy are likely to be involved in the disease pathogenesis or the tolerance induction. And as T cells activation and commitment to Th2 lineage precede the main effector phase of the allergic disease, they may represent potentially a very good prognostic biomarker that will inform about the disease progression. This is what I tried to do in the last decade. So, I tried to see whether there was a relationship between the allergen specific T cells and the clinical outcome.

And as I said, food allergy is a very (inaudible) disease. It involves kids, teenager, adults, you have different symptoms, the severity vary, the level of IgE. So, that's not an easy task. And as I mentioned earlier, the access of blood samples was really the

limitation of investigating this link between T cells and clinical outcome. So, a few years ago, we had this idea of providing immune monitoring as a service to get access to those samples, to those clinicals.

So, the goal is really to provide immune monitoring solution tailored for company or organization conducting clinical trial in food allergy. And the goal was to provide them services for basophil activation test or T cell assay or any assay they were looking for. And right now, we are structuring this platform into a non-profit academic research organization within the Mount Sinai. And the goal really is to serve as a central laboratory for mechanistic studies.

And overall, we want to take advantage of the biological samples collecting from industry or government sponsored trial, and taking advantage of the patient with clear clinical outcome, and to gain insight into the mechanisms

underlying the immune mediated disease.

We also want to discover and validate new biomarker and translate this to clinical and drug development. We hope that this will enhance our ability to match the right patient with the right medicine, accelerate the development of the therapy. And also, our goal is to harmonize the methodology to ensure the repository across varied cohort and trial.

So, what we've learned from this, so the next couple of slides, it's really a summary of what we observe in blood samples from five different clinical trial. And so, all those patients are most likely your patient and they were all challenged, so they all react to maximum 500 milligrams during a screened visit.

And the first things we tried to looked was whether T cells could be used as a potential biomarker. And we focus on the Th2A cells. And this is just representative data showing you what we have when we looked at the allergen specific T cells in a non-allergic patient versus a peanut

allergic patient. And you can see, looking at the CRTH2 expression which clearly define the Th2A phenotype, as well as the ST2 expression, that if you are non-allergic, if you don't have any peanut allergy at all, you don't have any expression of CRTH2 or ST2 within your peanut reactive T cells. However, if you are allergic, you will have more cells, about 10 to 50 fold higher frequency, and you will have expression of ST2 within the peanut reactive T cells. So, peanut specific Th2A cells are restricted to the peanut allergic individual.

The other things is we wanted to see whether the Th2A cells may play a role during the food challenge. So, on figure A, you have an example of a patient that received a whole food challenge and there is only 10 days apart from these two assessments. So, pre-challenge, you see a clear Th2A phenotype on this patient characterized by expression of CRTH2. And if you focus on CD38, this is used as a natural activation marker. And you see that before the challenge, the cells, we only observed 16 percent

in this example of activation. However, 10 days after the challenged, now the cells, the T cells were highly activated with 60 percent of the activation. And we also observed a dramatic increase of the frequency in the periphery of those peanut reactive T cells.

So, yes, the Th2A cells are, those data suggest that they are involved in the food allergic pathogenesis. Interestingly, looking at the overall Th2A cells, that's on figure D, we also observed that we can see some proof of activation of these subsets. And here we compared the conventional Th2 versus Th2A pre and post. And you see that most of the activation was observed within the Th2A cells.

So, then we looked at the heterogeneity of the patient, because one things that strike us is, yes, all the patients were challenged. Yes, all they reacted. However, you see that when you looked at CCR6 and CRTH2, it's not a yes and no response. You have patients that don't have a lot of CRTH2 expression within their peanut reactive T

cells. And interestingly, we made the same observation for CCR6. And actually, we observed that the patient with high level of CRTH2 were usually the one with a low level of CCR6, which could be associated with a Th17 like response.

So, then when we looked at the peanut specific T cell response, we observed that these cells mainly fall in three T cell subset, the Th2A, the Th2 conventional, but also the Th17 cells. And when we looked at the -- we decided to classify the patient based on the level of Th2A cells. So, we defined the patient with a low Th2A level, meaning less than 20 percent Th2A cells as the Th2A low peanut allergic patient. And in contrast, the patient that have more than 20 percent of Th2A will be the Th2A high patient.

And when we looked at the characteristics of Th2A high versus Th2A low peanut allergic immunotype, we first observed that Th2A high patient have statistically higher frequency of circulating peanut reactive T cells. Interestingly, they also have a statistical

difference in term of the peanut specific IgE and IgG4, suggesting a potential functional connection in B cells derived shift from IgE to IgG4. However, we did not observe any differences by looking at the skin pretest.

When you talk about biomarker, it's very important to make sure this is stable in a short period of time. So, to do this, you have here an example of a Th2A high peanut allergic patient with a lot of CRTH2 positive cells. And below you have a Th2A low peanut allergic patient with almost no Th2A cells. And you see on the bottom, this is called a river plot. You see, just focus on the red river that depict the Th2A cells. You see that this is stable over time in a short period of time. By short period of time, I mean six to two years. You see that in the toddlers there was no variation over time whether you are Th2A high or Th2A low. And same thing in adult, if we define someone as a Th2A high peanut allergic patient that's maintained. And this is important because we want to make sure we can also

use this as a way to have a predictive biomarker during therapy.

However, when we plot all the data from the different patient we received, there was differences, we observed differences between age. So, the question, and it's also related to this morning talk, was, is pediatric food allergic patient similar to teenager or even adult? Because you can see that the younger population tend to be Th2A low, while when you are a teenager you tend to be more Th2A high patient. So, then the next question was, is drugs working similarly? And you will see on the next couple of slides.

So then how the Th2A cells are impacted during immunotherapy. So, this is just, I wanted to show you a real life in my lab. So, when we receive samples, so we need about 10 milligrams of blood to perform this assay. And this is now within the peanut reactive T cells. So, we received the first samples and you see here about 83 percent of Th2A cells characterized by expression of CRTH2 and 161.

And then when we receive, at the end of the escalation during Palisad (phonetic) trial, we observe that now the frequency of Th2A cells percentage go down to 23 percent. And at the end we had these samples, we only have 3 percent. And our goal was to predict whether it was at least an active or placebo. And we predicted it was an active patient. And actually, we were right. And just to make sure this is two different pictures, that's not a side effect.

So, this is the statistic, the statistic behind that. We had a clear decrease of the peanut specific Th2A cells during Patforia (phonetic) trial while the Th17 seems to plateau. And we did the same thing. And once we receive a placebo, usually that's what we have. You have about the same level of Th2A cells, 73 at baseline, 67, 74 at the endpoint. So, we conclude it was a placebo.

Actually, we give that to immune. So,

this is the statistics. And they say, oh, that's pretty good. After we were unblinded, they say, oh, you're pretty good, but you have one patient, you're wrong. One of the patient was in the active group and I was just, okay. I mean, that's science. But actually, we carefully looked at this patient and first of all, yes, we were wrong, but this patient was also technically a non-responder. You see, that's the one I highlighted in red. This patient, at baseline and the maximum tolerated dose was 10 milligrams. At the exist visit it was 30 milligrams. Didn't reach the primary, neither the secondary endpoint. And when you looked at the raw data, you see that this patient remained a Th2A high patient. So, I'm not sure what happened on this patient because you also see that the IgE level increased dramatically and not the IgG4. But at least we were predictive, also. So, we can also predict who were the non- responder here.

The next was about the impact trial. So, now looking at younger patients, those

patients were age one to three years old. They receive high dose immunotherapy. And again, we observe this strong correlation between the frequency of peanut specific Th2A cells and IgE level, even at that young age. And here I plot different immune characteristics. We can see that the Th2A high patient were again linked with high level high frequency of peanut reactive T cells. CD154-plus the TH2A high patient were also the one with a high level of peanut IgE, also with high level of peanut IgG4. However, there was no relationship with the maximum dose. But keep in mind that this dose was up to 500 milligrams.

What we observed during this trial is then consistent with the entire study was, so, the conclusion of this trial overall, was the lower baseline peanut specific IgE were predictive of sustained unresponsiveness and age also was a factor. The younger the better. The younger patient were the one with a sustained in responsiveness. And when we looked at the Th2A phenotype, we observed that there was an increased

likelihood of resistance to tolerance induction by OIT in the Th2A high peanut allergic, meaning that if you are defined as a Th2A high peanut allergic at baseline, you have a benefit, you will have a benefit, but you will be desensitized. However, you won't have remission, meaning that you will lose your benefit when you stop the therapy.

You see that in blue. The blue curve reflect the patient that were desensitized and remission sustained benefit. And they were the one that started with the lowest frequency of peanut and the lowest proportion of Th2A while the patient that started that had remission or sorry, they were desensitized. No remission or remission. They always started with the highest level of Th2A.

Finally, recently we also looked at a known extract based immunotherapy. This is data from Aravax that are using now epitope to help to desensitize or even induce tolerance in peanut allergy and overall, so this product is called PVX108, and it covers seven synthetic peptide from

RH1 and RH2. And the goal of peptide immunotherapy is to bypass the immune mediated response and to directly target the T cells. And so, this trial focused on the efficacy. That was a phase one. And first of all, it's a very safe approach. You see that even the basophil, there was no basophil activation ex vivo using PVX108. There was no adverse events during the trial.

However, what we observed is a strong decrease of the peanut specific Th2A response. And interestingly, this response even went down after the therapy stopped. So, this therapy stopped after week 21 and they followed those patients after 18 months and we see the Th2A cells decrease. However, the question I would like to, it's an open question that I would like to address was, do the changes in upstream biomarker can occur before they are observable measured clinical benefit? Here you specifically target the T cells and we don't know what will be the impact on the IgE and IgG4, because we bypass that. So, can modulation of the T cell response can predict

before the benefit of this therapy? So, this is an open question. We will learn soon. But it means that sometime you should also wait a little bit. If you see a change in the T cells and no clinical benefit, it's probably because of the kinetic, the half-life of the IgE or how the IgG4 will be elicited. So, open question.

So, I will stop here. Just my conclusion. Overall, it's clearly the next few years will be critical times to further evaluate prognostic biomarker currently showing promise, while continuing to utilize advance in high throughput technologies and computational biology to help optimize the most promising biomarker. And by working together with patients, physicians, scientists, industry, FDA, NIH, we can envision the discovery and all the confirmation of several biomarkers in the near future.

I would like to thanks my previous team from the Benaroya Research Institute that generates most of the data that I just show you here. I also would like to thanks funding support

from the NIH, ITN, and the Food Allergy Research and Education. Also, the access to those precious samples from clinical trial, from those different companies that run those clinical trial. And finally, I would like to thank my new team at Sinai, people from the Jaffe Food Allergy Institute, and also the people from the Immune Monitoring Center and the new academic research organization that we will launch in two months called OCAM (phonetic) Immune. And thank you, everyone.

DR. GUERRERIO: Thank you, Eric. So, we're going to move to our last speaker, who is Wayne Shreffler. He's going to talk about cellular biomarkers for response to AIT for food allergies. And few words about Wayne. He's the Chief of Pediatric Allergy and Immunology and Director of the Food Allergy Center at Massachusetts General Hospital. He's also an investigator at the Center for Immunology and Inflammatory Disease and the Food Allergy Science Initiative. Received his MD and PhD from New York

University, and he did his fellowship in allergy at Mount Sinai.

DR. SHREFFLER: Thanks, Alkis. Great to be part of this symposium. Thanks, Dr. Rabin and all the organizers for inviting me and actually just for the vision of doing this, I think it's an incredibly important thing, and it's great to see FDA interested in engaging on this topic. And the need for biomarkers, I think, is, as someone specifically focused on food allergy, but not limited to that, certainly, really important, all the way from clinical outcomes to better predictors.

So, when I got the first sort of note of the title and it said cellular biomarkers, I thought, oh, great, I'll talk about basophils. I used to do some basophil work, and then I saw Alex was on the program, and I thought, okay, well, that's clearly not going to be my topic for today. And then I thought, well, I'll talk about Th2A and peTh2 T cells. And I saw Eric was on the program, I said, oh, God, okay, that won't be the tack I

should take, either.

So, what I'm going to try to do is, this is last and least, because this is an area, I think, in the biology and food allergy right now, where we are furthest from a validation of the kinds of things that I'm going to talk about, although I will talk about peTh2s. But that is, I think, an important aspect also of biomarker research. And for those of us who have condemned ourselves or didn't have the wit to do high-level immunology in really cutting and informative models, we relegate ourselves to trying to understand and get insight into the biology by looking at the cells that we can access from our patients in the settings of interventions, as you all know.

So, disclosures here, none particularly directly relevant. Just a quick note on the methodology that I won't have time to go into. And so, a lot of this is going to get glossed over, but we can talk about it afterward. There's a lot of different ways already some of the data

that you've seen have attempted to interrogate something about antigen specific T cells, ranging from tetramer selection. We haven't seen too much of that in today's session, to a lot of activation and enriching for putatively antigen-specific T cells on the basis of their activation state post either in vitro most often, or sometimes in vivo exposure to antigen. And that's a good method in many ways. It's the method that we have available. It's been refined a lot over time, but it is subject to a lot of, and a lot of bystander activation that we have to really keep in mind at all times.

So, by way of overview, I am going to obviously limit my scope for time and just because it's what I'm most familiar with on T cell subsets, and I'm going to talk in the context of OIT trial data. I'm going to talk about the CD4 subsets that I see sort of the most convergence in the literature around that I think that we're getting kind of on the way toward within the adaptive compartment, understanding their

plausible role in immune pathogenesis. So, potentially someday actually surrogates, but more immediately correlative observational data. And so, their potential for either diagnostic or monitoring biomarkers.

So, these include both dynamic it induced changes such as the ones we've just seen from Eric in the Th2A or peTh2 subset, as well as potential baseline differences. And I'm going in order of kind of the strength of the evidence to the weaker evidence as we go.

And finally, I'll throw in a teaser of unpublished data and try to promote my fellow who'll be presenting later in the meeting for those of you that are attending that as well. I think looking at, I think really interesting, again, in the spirit of the discovery part of why we look at biomarkers and the potential for that as a biomarker for iatrogenic eosinophilic gastrointestinal disease.

So, T cells of course exit in their naive state after education and then progress

after antigen engagement through a series of differentiating steps and lose multi potential and acquire more and more specific effect reflection, function rather. And as they do that, their homing capacity changes. So, it goes from primary lymphoid homing to homing to peripheral sites. And that can affect our ability, again, for those who've condemned ourselves to looking at a lot of peripheral blood in humans, what we can actually see. And along the way they also have changes to their surface markers some of which are directly indicative of differentiation, others of which are indicative of function.

I'm highlighting CD27 because we know that's important as a marker of terminal differentiated effector cells such as those Th2As. And these are the subsets of cells that I'm going to talk about, the Tfh13s, the Th2A's, these Th17-like cells, and Tregs and especially type 2 deviated Tregs.

So, starting with this Tfh subset. So, we know that follicular helpers are obviously

adapted to provide B cell help for antibody production. We've heard already from others about that. Tfh13 are a subset that are uniquely required in a couple of mouse models, at least, using very sophisticated techniques of lineage-specific deletion for the generation of high affinity IgE and consequent food anaphylaxis in those models. And they're also strongly associated with human disease. And I'll show a little bit of data on that.

Their expression of 21 is a little bit lower than non-type 2 Tfhs and their really, sort of, signature hallmark is high production of IL-13, but also other Th2 cytokines, although IL-5 is generally lower. Here's data from Stephanie Eisenberg's paper actually showing this population of PD-1 high CXCR5 positive cells, and comparing them in an LPS induced inflammatory state in mouse with Alternaria. And you can see this emergence of this IL-13, IL-4 positive population within that Tfh gate that is in the top-right panel, obviously markedly enhanced and induced in the

Alternaria model. And you can see things such as in the middle bottom the differences in IL-21 production, or in the right their expression of GATA3.

We can see these also in a population of patients with peanut allergy at baseline undergoing about to undergo peanut OIT, that is oral immunotherapy among the CD154 positive. This is 20-hour in vitro stimulated. Again, getting back to that bystander issue where we could in fact observe in this UMAP distribution some segregation of different Th2 subsets, including these Tfh13s in the top, highlighted there, and in fact show that the correlation between serum IgE from those patients to IL-4 production in those cells, but not, for example, by comparison in Th2A subsets in the bottom two panels here, or other CD4 subsets that we looked at were correlated. So, really correlative data supporting in humans that that is the subset producing IL-4 that's important for driving IgE production.

Th2As, I'll be brief because you've just

heard so much about them, and I think we're already supposed to be in our panel discussion. These are circulating terminally differentiated effector memory cells. They're really highly differentiated, low proliferative potential, presumably, although not a lot of direct evidence around that, strongly associated with seasonal hay fever as well as IgE food allergy, by Eric. They are CCR6 negative, as he just showed us, CD161 positive, 49D positive.

Interestingly, they have, actually, a lot of features of tissue resident memory cells, which if we have time, we'll talk a little bit more about. But really, in terms of their functional characteristic, what is impressive, I think, is their high multipotent production of Th2 cytokines, including IL-4, IL-13 and IL-5, and in human IL-9, probably not so in mouse, where it seems like these are really the equivalent of high IL-5 CCR8 positive effectors in most Murine Models of Allergic Inflammation, by my read of the literature at least, and I think maybe lacking

actually in some food allergy models.

They also acquire a really interesting innate like capacity to be sensitive to alarmins through upregulation of IL-25 receptor and IL-33 receptor, and have a really interesting, and this is one of the kind of features that is reminiscent of resident memory cells, especially in the skin that have been described, this kind of upregulation of cassette of (phonetic) genes that provides for them capacity to both sense and metabolize lipids, including hematopoietic PGD synthase, rather, et cetera.

So, Eric has already shown us this, but greatly expanded in circulation among patients with hay fever, activated and expanded in season of exposure and activated post in the case of peanut allergic patients, a challenge 10 days later, as he's already shown us. This phenotype is remarkably similar, and Eric made pains to point that out in his first publication in 2017 to work by Cal Prussin when he was still at NIH, characterizing this population of what he called

pathogenic effector, Th2s expanded and associated with the eosinophilic gastrointestinal disorders.

These features in this table, I kind of challenge anyone to really find things that differentiate circulating Th2As, as we've come to coin and term them, from these tissue resident peTh2 cells. They're highly similar, including, really, their pattern of chemokine receptor expression, their transcription factor profile, maybe some differences in Eomes (phonetic), but really a lot of overlap in phenotype. And this, and, sorry -- in Calman's work, what he emphasized was really, it was repetitive TCR stimulation. He could replicate this in vitro under Th2 polarizing conditions. That repetitive engagement of TCR was sufficient to drive the phenotype of these cells in vitro, and suggested that the nature of allergic inflammation in the setting of eosinophilic gastrointestinal disease, or in asthma, or in nasal polyposis, or in atopic dermatitis, all of contexts where these cells have been observed, lent itself, perhaps, to chronic

stimulation and the acquisition of this phenotype.

However, I think the other strain in the literature that's really interesting when thinking about these cells comes from labs that had studied early on, so called Th9 cells. And these are cells actually, that in several labs can be induced by co-culture with IL-4 and TGF. And the model that those groups, kind of, have been pursuing is that this is a phenotype that is acquired with tissue residents and actually may not be so dependent upon repetitive TCR stimulation as the model of peTh2s and yet have a lot of phenotypic overlap.

And so, I think, again, trying to understand the biology from what we can observe suggests that there may be multiple pathways to acquire at least some of these phenotypic attributes in the context of allergy. And one of the first observations actually before, back in 2014, although people in asthma were describing these cells, it wasn't really on the food allergy landscape, at least not to my mind or

recollection.

But this paper by Helen Brough and Gideon Lack's group compared using something called a microarray, which I thought was going to land really well until Hugh pulled out like the spots data and glass microarray slides. So, using this older technique, a bulk, bear in mind, technique, nevertheless, the really differentially expressed genes kind of have these features, certainly suggestive and characteristic of Th2A, peTh2 cells, including hematopoietic PGD synthase and high levels of IL-9, IL-5, 13, even in excess or above IL-4. Just comparing atopic but non-allergic to peanut allergic patients after stimulation in vitro and sorting of CD154, CD69 double positive cells.

And Cecilia Berin actually began, along with other labs showing kind of clinical correlation not in the context of OIT but just at baseline between Th2 effector function and things like eliciting dose at baseline. So, suggesting that, remember, this is not the population of

cells per se, at least to the extent to which you accept IL-4 percentage in surrogate for Th2As. Buyer beware, there would be Tfhs in here as well, but they're very rare. So, to the extent that you sort of accept that as a marker of that phenotype, a correlation between that and clinical sensitivity with something seemingly as crude as just a graded food challenge.

And our lab has been really interested in this as well, but initially really, kind of, went into it with trepidation, that sort of stratifying patients on something like what threshold of allergen they react at and looking for non-IgE dependent reasons for that. Certainly, there's lots of understanding, and you've heard from Alex indirectly through the basophil and Hugh directly through studies of IgE epitope binding pattern differences in diversification, some of that explanation.

But this suggests to us, and I'll show a little bit more on why I think so, that there are IgE independent variables that influence that

sensitivity as well. Another example of the kind of clinical prognostic biomarker utility of an assay like this when comparing baseline baked egg challenge patients, all egg allergic but differentiated by their reactivity to baked egg versus tolerance to that, a well-known way of stratifying phenotype and food allergy for those in the audience that don't sort of live and breathe food allergy stuff.

And so, we also showed in a threshold type study comparing patients that react at low versus high threshold, these differences in 154 reactivity and a phenotype shown here by bulk RNA seq. Again, with highlights of this peTh2 phenotype. Our friend hematopoietic PGD synthase PPARgamma, IL-9, IL-5. But also, markers of a Th17-like phenotype, certainly well represented within that 154 positive reactive population and upregulated in the low dose reactive patients versus the hyporeactive patients. If you put them in culture and stimulate them with autologous monocytes, they certainly will make Th2 antibody

nicely, including IL-9 in an antigen specific sort of way with equal, no difference between the groups, if you polyclonally stimulate.

And although they have these transcriptional features that look IL-17-like, we didn't get really any IL-17 protein secreted to speak of, or IL 22, which was also transcriptionally upregulated. So, suggesting that they have some of this sort of, maybe not a fully licensed phenotype in terms of their Th of 17 characteristic.

But the other thing we did in this paper, and I think this is something we haven't heard too much of yet today, is to use TCR sequencing as a means of trying to hone in and to some extent overcome the bystander problem that one has when you sort of don't want to use tetramers, don't want to bias yourself or limit yourself to certain HLA restriction and are stimulating with whole antigens. And that is that we compared by bulk TCR sequencing the sequences that were enriched in the 154 reactive cells

versus the resting cells and met a statistical threshold for that enrichment and then used those sequences as a way to interrogate the rest of the bulk data.

Very briefly, if you do that, these sequences indeed look meaningfully enriched for motifs suggestive of specificity, and they are overabundant as clonotypes, now in the reactive versus the hyporeactive, correlating with what you see if you just look at CD154. Now we also, from the same patients just sorted out effectors in Tregs, show that they have good contrasting phenotype, including evidence of functional suppression, and then use the sort of validated enriched for specificity clonotypic information to look at those compartments and see, whoops, sorry, that the reactive patients, that the difference, what distinguishes them is the expansion of that effector population, not differences in their underlying regulatory population.

So, again, this is in the category of prognostic biomarker as something that suggests

low-level reactivity among patients. And here to me is one of the important punchlines. It has no correlation with IgE titer. So, this is independent of what's driving IgE. It's not about the Tfhs. It's a different mechanism suggested. We hypothesize that it has to do with how these cells can drive mastocytosis and influence gut barrier.

So, Tregs, quickly, allergen specific FOXP3 play a critical role, we know, in oral tolerance, certainly in animal models and from human mutations, accidents of nature as well. There are different flavors of regulatory cells, the follicular regulatory, that really effectively suppress IgE in a number of model systems. RORgamma-T that can suppress Th2 inflammation at vector sites, and GATA3 positive, which Talal Chatila showed really nicely some years ago, that can exacerbate, in fact, Th2 inflammation.

CD137 is a marker that will somewhat enrich for Treg. It depends a little bit on how long the activation is in in-vitro and what the

context is. But a marker of note in kind of this discussion of biomarkers. This is from Talal's paper, really. All I've shown here is the human data, where he shows really nicely that there's not only a reduction in overall FOXP3 CD25s, but there's a bias, an increase, actually in this phenotype of IL-4 GATA3 positive Tregs in disease versus healthy controls.

So, along with that, some evidence from milk specific, but I just want to highlight that this is milk specific that have been expanded in vitro for several days. And I'll come back to why I think that's important. We see again, within the Th2 module score population, a sizable Treg-like population as well, smaller in these roughly 10-year-olds to Eric's age dependent thing, not two-year-olds. So, already a good complement of Tfhs, as well as Th2As. These are basically all Th2A high patients, but also this regulatory population.

And interestingly, if you look at their TCR repertoire, because this is single cell RNA

seq data with matched TCR, they're really much more polyclonal and there's no overlap in their repertoire with other CD4 compartments. Cecilia Berin, I think, has done as much to address the paradox of why we see bulk Treg increases in multiple studies of OIT and yet have failed to see antigen specific signature increases by showing actually that there are a couple populations of FOXP3 positive cells, some with more functional activity, suppressive activity, some with less, but high levels of FOXP3 expression that are emerge in a bystander like fashion dependent on IL-2. And lastly, the Th17 population, which we think corresponds to the CCR6 positive group.

So, to sort of sum up this part, the response biomarkers I think that we can really begin to have some confidence in, are limited to really this profound suppression of Th2A that Eric has just shown us. There is evidence also for effector cell exhaustion anergy. I think that essentially is where some of these cells go. There is some global expansion of Treg from

several papers, but we don't really see an expansion of antigen specific Tregs, and we don't see suppression of Tfh13s.

At baseline in terms of predictive. So, Eric has shown you the low Th2A story. Cecilia Berin has similar data. We have similar data that it's really the treatment responders that have strong suppression of Th2A. And Cecilia's data on Tregs, which I won't have time to go into, shows this global expansion. This is our data showing within the CD154 positive or 137, not shown here. There's not really an OIT induced difference. There's a little bit of an expansion, an IL-10 signature within these Tfh-like Tregs, but it's not significant in this small population. It's something to look at more.

So, the predictive phenotype, is there one? We have a little bit of data to suggest that there are not well mapped to these specific CD4 subsets, but an inflammatory signature, a T cell activation signature, and one other thing, which was high levels of GPR15 expression among patients

who failed to have treatment response in OIT.

That's really caught us our eye, because we have a story emerging about GPR15 in the setting of EOE. GPR15 is associated with peTh2 cells that are really unique to EOE, active versus remission. If we take advantage of Eric's observation of CD38 upregulation, and this is what Caitlin will show on Saturday, we can see, in fact, that there is an upregulation of CD38 positive GPR15 positive cells within active EOE, and that these markers, sort of, exploiting this phenotype, begin to approach some fairly impressive AUCs when discriminating active versus no EOE, and even when discriminating between active and remission disease state.

And I'll leave it at that, just with your thanks. And the point that biomarker work is also sort of about discovering those new biomarkers.

Thank you.

DR. TOGIAS: Thank you, Wayne. Why don't you stay here? We have, unfortunately, only

15 minutes.

DR. RABIN: We have a little bit longer.

DR. TOGIAS: A little bit longer. That's wonderful.

DR. RABIN: Because our speaker will be here at about 5:15 or so.

DR. TOGIAS: So please, all speakers come here, and we don't have enough seats, so --

DR. RABIN: We'll bring one up.

DR. TOGIAS: -- grab a chair and we'll be fine. But before we start this discussion, we had a discussion with the FAB Alliance Group that has a lot of stakeholders related to biomarkers in the food allergy arena. And what I'd like to do, and we've had an agreement to do that, is ask one of their representatives in this case, is, I think, Kari Brown to join us and actually give us a couple of minutes of some thoughts about where they're coming from when it comes to the biomarkers of food allergies. So, Kari? Yeah. Please.

DR. RABIN: Excuse me. Right before you

do that, a number of people online and here have asked whether or not slides are going to be available or the recording is going to be available. The answer to slides is no. The answer to the recording is yes. It'll be available in about two and a half to three weeks. My guess is if it's like we do with our advisory committee and such, it will be on the same webpage that you use to register for the meeting.

DR. BROWN: Great. Great. Thanks for giving us a chance to speak real quick. So, I'm Kari Brown. I'm with Revelo Therapeutics, but I'm speaking today as a member of the FAB Alliance. So, that's the Food Allergy Biomarker Alliance. So, this alliance was actually formed quite organically from multiple people in this room who identified the issue with having limited options for determining efficacy in food allergy clinical trials.

So, the Alliance is a group of individuals across academia. So, researchers, clinicians, people in industry, in clinical

development groups, and also in different biomarker approaches, also people from patient advocacy groups. So, anyone is able to join who is interested. We are approach agnostic. We recognize that approaches in terms of identifying viable (phonetic) biomarkers for determining efficacy in food allergy clinical trials, could be algorithmic. They could integrate multiple biomarkers, and they also could potentially just decrease the need of the volume of double-blind placebo-controlled food challenges, and not necessarily eliminate the need.

So, really, our goal is to come together across, I would say, silos. Sometimes it tends to be right in academia and in funding. Government funding in academia may have more communication in their research industry and regulators may have more communication in their silos. So, we're really working to bring groups together across these walls and have communication across the groups as possible to move this forward.

So, if this is of interest to anybody,

you're welcome to speak with me or Thomas Wilmers has been doing a lot of the coordination of this as well. So, if you would like to join the effort, we are volunteer, non-funded, but we are all trying to move this forward. So, this, today is a big step in terms of bringing multiple groups together in the room together. So, I think the Alliance really appreciates that initiative and thanks for giving us the chance to speak.

DR. TOGIAS: Thank you, Kari. Why don't you take a seat?

DR. BROWN: Thank you.

DR. TOGIAS: So, thank you, everybody. These were great talks, great presentations, and so we probably should have some discussion. But before we start, we are at this stage where a lot of great data have been presented, a lot of things are moving forward. What's in my mind, and I'll ask the first question is, okay, where do we go from here in terms of bringing those things together, those observations together? Do you, in your mind, have some thoughts about this as

opposed to just doing the wonderful research you are doing? What will bring this research together in a way that will produce the basis for moving forward with biomarkers? So, who wants to take this? Erik, go for it.

DR. WAMBRE: As I mentioned earlier, the way to really push those biomarker is having people running clinical trial. I mean, I'm thinking about the industry or government pushing those biomarker. Not forcing, but at least having some mechanistic study along with the clinical studies. I think that will help pushing the basophil test or diversity of IgE repertoire. The T cells, all the T cells. And much more. There is much more biomarker. If they are always associated with clinical samples with very clear patient outcome and clinical data, I think that will help.

And the other things, as I mentioned, and actually, Alexandra Santos highlighted that, is, when you have a biomarker, you need to do a lot of validation, sensitivity, robustness, et

cetera, et cetera. This take time and money. And I agree, it's not fun. You have to do it sometimes 10 times the assay to make sure it's stable, and you have the same conclusion, but usually nobody want to sponsor that. And I think that's one issue.

DR. TOGIAS: Yeah. Thanks, Erik. Somebody else wants to add to this?

DR. SAMPSON: I'll just second what Erik said, but also, if there was some way to get some kind of a repository of all the different samples that have been around for these various clinical trials, it would be a great way to be able to do a lot of validation on some of these assays.

DR. TOGIAS: It's a good point. Okay, well, that's my question, so let's see what the audience has to ask. Steve?

DR. TILLES: Since nobody else is going to ask a question. Steve Tilles working at Aimmune Therapeutics, and this is actually, I think, primarily for Dr. Santos, but be interested in the rest of the panel's thoughts as well. And

Dr. Guerrerio talked about the unmet need of food allergy. And we've sort of had a consensus in this group that double- blind, placebo-controlled food challenges aren't necessarily a wonderful thing to have in every trial.

I will say that at the podium at many meetings, a lot of thought-leaders think there's no problem with that. And we give epinephrine fairly routinely during these challenges. So, I really feel like this is a big, almost existential threat in some ways to innovation reaching the masses. And so, my question is, with this elegant presentation and you proclaiming that this would be transferable at experienced sites, how far are we from this being either a tabletop option or at least widespread so it can be used as a surrogate outcome in pivotal trials?

DR. SANTOS: Thank you very much for your question. It's difficult to know how far we are, because I think there's a lot of boxes to tick and people to convince, I think. But I think from a scientific evidence point of view, and

maybe one of the things, and adding to the previous, actually answering the previous question, maybe we can look at, looking at the evidence we have and synthesizing this evidence to see whether it answers the requirements to actually be able to use the basophil activation test, for example, as a surrogate endpoint for a clinical trial.

The oral food challenges are wonderful in many ways, and they are safe and they are important for some patients, but they do, could create selection bias in the studies, not only from the clinician's point of view, because there are patients that we wouldn't dare challenge, but from the patients as well, because some patients, which are not necessarily the most severe, don't want to undergo a challenge for one reason or another. So, I think that it would be really helpful for other reasons as well.

I think that it would be important to maybe synthesize the evidence that we have. Of course, it's not a perfect, and it's not the same

as a clinical outcome, and that's absolutely fine. And I think we can make a list of and be conscious of the limitations and address them and see whether there is good enough to be able to do more trials, and then also to increase the applicability of using these treatments in clinical practice, because in clinical trials, challenges have a lot of challenges and create selection bias into clinical studies and so on. But I think it's much more difficult to convince a patient to do a treatment in real life and see whether treatment is working by doing repeated challenges. So, I think this is a bigger problem, and I think we need to be pragmatic as well, and make it easier for our patients to actually have solutions for them.

DR. TOGIAS: Ron?

DR. RABIN: So, question from the crowd and from me as well, about the BAT. Dr. Santos, first of all, why do you think that you get the differences that you get depending upon the food allergen, peanut versus egg, for example? So,

that's question number one. Why don't you answer that and then I'll go on.

DR. SANTOS: Thank you. So, it's interesting because from the existing literature on IgE tests, for example, the cutoffs that have been identified over time are different for different foods. And because patients are allergic to one food and not the other, I still believe that diagnostic tests are allergen specific. Having said that, and having applied the same method for the basophil activation test to different foods for peanut, egg, and I can disclose preliminary analysis we've done for milk, the optimal concentration is 100 nanograms per ML. I don't know why that is. If there's any brilliant mind in the room. So, the optimal concentration to be able to. So, this is the concentration at which the test distinguishes best between allergic and non-allergic. So, there are some similarities that I don't fully understand. This is why we are doing separate studies.

In the end, we may possibly come to a

conclusion that actually you need the same concentration of allergen, and the cutoffs are not that different. So, we can extrapolate to other foods, which I think, from a practical standpoint, would be very beneficial, because doing all these studies take years and we need to diagnose patients today to different foods. So, that would be very helpful. But we have been doing these studies separately on the assumption that allergens perform differently and the diagnostic tests need to be validated separately.

So, far, as I said, the concentration, the optimal concentration, is the same for the studies so far. The cut offs are not exactly the same, but not that different. So, we'll see.

DR. RABIN: All right, my second question has to do with how you perform the assay, because, as I understand it, there are two different ways that one could perform the assay, which is using the patient or the subject's own basophils, which is most of the data that you presented, is that correct?

DR. SANTOS: Yes, that's correct.

DR. RABIN: Or with a cell line. Okay. And that when you use the patient's basophils, the attraction is that you're kind of integrating the cellular responsiveness. But if you're using a cell line, for example, then really you're looking at affinity, avidity, and those sorts of things. And I can't remember. I think that you did some comparison studies with the same sera, and you still found that using the patient's basophils was better?

DR. SANTOS: Yes.

DR. RABIN: Gave you better ROCs. Is that correct?

DR. SANTOS: Yes, that's correct. So, I think in an ideal world, if we can have both, I think the basophil activation test is still closer to the clinical phenotype than any passive sensitization assay, because it uses the patient untouched blood that contains defector cells and the antibodies in the same amount and affinity, all the characteristics that are in the patient.

So, that is, in my opinion, and in my experience, it's best than using any passive sensitization assay, which is dependent on the cellular system, which is not from the patient. And, yeah, as you said, it's a functional test of the antibodies rather than the whole integrated system.

We have compared, for peanut only, we have directly compared the basophil activation test with the mast cell activation test, and we have seen that they are both in the specificity is similar. So, when a test is positive, it predicts peanut allergy with a similar specificity. But the basophil activation test is much more sensitive, so you have a lot more false negatives in the mast cell activation test. The mast cell activation test, however, has one advantage, which is to provide results for patients with non-responding basophils. So, these patients that have the IgE mediated pathway sort of shut down, then if we transfer those serum onto another cell, they can elicit a response. But still, if I have to choose, I would choose a basophil activation

test for a biomarker for food allergy.

DR. RABIN: Okay. Thank you.

DR. TOGIAS: Corinne? Oh, I'm sorry. There was somebody. Okay, you guys decide.

DR. KEET: I guess my question is, if we're thinking about biomarkers of response for immunotherapy and thinking about sort of what was discussed this morning about how we think it may be in the causal pathway, whether we think there needs to be coherence, especially if we're looking at blood biomarkers between the different methods of immunotherapy in terms of the predictors of response to therapy. Given that, maybe we think the endpoint of desensitization may be similar, or whether you think that actually that's not the case and that we should be pursuing OIT biomarkers and SLIT biomarkers and EPIT biomarkers and whatever else we discover.

DR. TOGIAS: It's a good question. Who wants to take it?

DR. SAMPSON: Go ahead.

DR. WAMBRE: At least just one. Just

one comment. I think it depends where in the cascade the biomarker is. As I mentioned, if you use DNA, vaccine, or peptide immunotherapy, probably your best readout will be the T cells. However, if you want to block, if you want to use IgG4 as a new therapy, I think you should look at IgE and the basophil test. So, I think it really depends of what is your main target, I think.

DR. TOGIAS: Yeah.

DR. SAMPSON: Yeah. I mean, very preliminary data with the peptide assay, you do see different responses to different epitopes when you look at OIT versus epic versus slit. But that's all preliminary, so we'll have to see what pans out.

DR. TOGIAS: Yeah. For response to treatment, I can see that different biomarkers will be more appropriate. But for diagnosis, prognosis of disease, natural history kind of thing, then maybe we should have, hopefully, some common biomarkers that will allow us to predict a few things. Question for Wayne, since you haven't

gotten into the discussion. When it comes to T cells, I've always been concerned, especially since we're doing a lot of pediatric research about blood volumes and what are the prospects of being able to conduct this work with much less blood volume?

DR. SHREFFLER: I don't know what the big deal is. Kids have a lot of blood. You know, I mean, Eric has shown elegantly, Cecilia, others, that you can to some extent take advantage of the higher lymphocyte counts in young pediatric patients to your advantage and do meaningful assays with feasible amounts of blood. Certainly, 10 MLs is great, but you can go below that for many of these readouts.

I think when you get into some of the things that -- you know, I'm really interested in, the TCR repertoire space.

DR. TOGIAS: Mm-hmm?

DR. SHREFFLER: And there, you generally need depth that's harder to achieve with less than at least 20/30 mils, and the more the better,

frankly.

DR. TOGIAS: Yeah.

DR. SHREFFLER: However, I do think that we see in the case of peanut, in the case of milk, where the data sets are becoming substantial, there are some really dominant public TCRs that, when located in a particular CD4 subset, I think may well have biomarker utility and that are abundant enough that there's always going to be sampling error and that's going to be a major limitation on sensitivity. But I think that an assay like that ought to probably able to achieve high specificity.

DR. TOGIAS: Before you run, there was somebody waiting back. I'm sorry, I don't know your name.

DR. SORELLE: Yeah. No, please. I'm Jeff Sorelle. I'm from UT Southwestern. I, in addition to doing basic science research, also am a pathologist, run the clinical lab test that hopefully we could use one of these someday. So, when we evaluate a new test, and as the FDA

mentioned this morning, they have to look at several different factors. So, I wondered if we can maybe just do a show of hands. Since you've all shown good biological applicability, who all has now done testing on samples in over three clinical trials with the assay you presented? Andre, I think you've done over.

UNIDENTIFIED: Yeah.

DR. SORELLE: And then who has done testing on at least 100 patients? And who has done all of this on like over 400 or 300? Someone there. And then who has locked down a cut off and then done a validation cohort study? So, we obviously have a lot of good data here to move forward with trying to figure out what these cutoffs need to be or what are our goalposts, and probably we'll have a conversation with regulation going forward to say which people have met or which tests have met these criteria and can we move forward with. So, that's all that I sort of had to say. Thank you very much for all the really great data.

DR. TOGIAS: Somebody wants to respond to that or you're all in agreement?

DR. SHREFFLER: Yeah, I think these kinds of alliances with industry, because it's about the funding, right? It's about doing the boring work that's not going to get a competitive RO1, you know what I mean? I mean, it's work that needs to be done, but it's very yeoman's practical kind of stuff. It's what I get bored with personally. Right. But it's really important if we're ever going to translate this to something that's useful.

DR. TOGIAS: Ron?

DR. RABIN: Yeah, I had a question for Hugh about the bead assay because I think there was some data published about it. But as I remember, you didn't discuss IgG and IgG binding to the peptides, particularly during OIT, and whether or not that was predictive. Can you give us a few short words on that?

DR. SAMPSON: Yes. Basically, all the data I showed you is published somewhere, but I

guess you're not going to give out the slides, so they're not going to know where. But anyway.

DR. RABIN: Well, I can have everybody email you if you want.

DR. SAMPSON: No, no. That's okay. I get enough emails. Actually, surprisingly, the reason we're not using it, when we use these machine-learning algorithms and identify what are the most predictive epitopes and IgG and IgG4 don't rank up there. And that really surprised me, especially with the immunotherapy trials. We always think that that's one of the important factors, but they don't weigh in. They're down the list. But we have looked at them.

DR. VOLLMERS: All right, Thomas Vollmers again, AllerGenis and the Food Allergy Biomarker Alliance. My question is, how do you all think we should leverage the momentum gained from today, the excitement perhaps in the room, about biomarkers and response markers? And where do we take this meeting? Maybe it's Duran (phonetic) as well. And where do

we go from here? How do we continue the conversation as academia, the FDA, the NIH, industry?

DR. SHREFFLER: Send us money, Thomas.

DR. VOLLMERS: Believe me, don't have any.

MR. RABIN: Well, are you asking me?

DR. VOLLMERS: Yeah, you too. Yeah. Yeah.

MR. RABIN: Well, I mean, I think we learned a lot today and I think we have to have a lot of internal discussion. I think eventually, as these things come out, what happens is, I think the way things evolve is that if things have real impact, they would probably go before an advisory committee at some point. And whether, how far we are from that stage, I would not begin to predict, certainly. I think for obvious reasons, we're very careful about revealing what we think in the moment. So, this has been very revealing to us. We've learned a lot about biomarkers. We've

certainly established our objectives here. We hope that the morning talks have informed industry and those of you who are interested in this about what you need to do to bring things to us. And how fast things will evolve, I couldn't predict. But that's the process.

DR. ORTEGA: Just one comment. I don't try to impose any work to those who are sitting there, but I work years ago at NHLBI, and often we used to generate some proceedings. Have a publication out of that, because you are very familiar to that.

MR. RABIN: It's going to happen.

DR. ORTEGA: Maybe if it's that on the books, that will be first step, too.

DR. TOGIAS: I think Ron has already initiated the process.

DR. ORTEGA: Perfect. Yeah.

COURT REPORTER: Could you state your name, please.

DR. ORTEGA: Hector Ortega, San Diego.

COURT REPORTER: Thank you, sir.

DR. TOGIAS: All right, unless somebody has something else to say, I have the privilege to close the meeting. Ron, do you have to do some closing arguments? No, he doesn't. Okay. Well, thank you very much and we'll see you soon at the Academy.

(Whereupon, at 5:11 p.m., the PROCEEDINGS were adjourned.)

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CERTIFICATE OF NOTARY PUBLIC

STATE OF MARYLAND

I, Mark Mahoney, notary public in and for the State of Maryland, do hereby certify that the forgoing PROCEEDING was duly recorded and thereafter reduced to print under my direction; that the witnesses were sworn to tell the truth under penalty of perjury; that said transcript is a true record of the testimony given by witnesses; that I am neither counsel for, related to, nor employed by any of the parties to the action in which this proceeding was called; and, furthermore, that I am not a relative or employee of any attorney or counsel employed by the parties hereto, nor financially or otherwise interested in the outcome of this action.

Mark Mahoney

Notary Public, in and for the State of Maryland

My Commission Expires: June 7, 2023