Early Detection of Parkinson's Disease: The Challenges and Potential of New Biomarkers

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Dr. Todd Sherer: Hello and welcome to this webinar brought to you by the journal *Science* and *Science Translational Medicine* in association with the Michael J. Fox Foundation. My name is Todd Sherer and I'm the chief program officer at the Fox Foundation. The topic for today's webinar is "Early Detection of Parkinson's Disease: The Challenges and Potential of New Biomarkers."

It gives me great pleasure to introduce our speakers for today: Dr. Norbert Schuff from the University of California and VA Medical Center in San Francisco, Dr. Michael Schlossmacher from the University of Ottawa, Dr. Andrew Siderowf from the University of Pennsylvania School of Medicine, and finally Dr. Ken Marek from Institute for Neurodegenerative Disorders in New Haven, Connecticut. A warm welcome to our speakers and I want to thank them for participating and I want to thank everybody for taking the time out of their day to participate in this webinar on what we think is an important topic for Parkinson's disease research.

Each of our speakers today is going to give a short presentation after which, we will have time for a question and answer session, during which the panel will address the questions submitted by you the live audience. A note to our viewers that you can resize or hide any of the windows in your viewing console. The widgets at the bottom control what you see. Click on these to see the speaker biographies or to download a PDF copy of the slides that are presented during the webinar today.

If you're joining us live, you can submit a question to the panel at any time by typing it into the box on the left of your viewing console and clicking the submit button. If you can't see this box, click the red Q&A widget at the bottom of your screen. Please do remember to keep your questions short and to the point. That will give us the best chance of selecting them today and having them discussed by the panel. You can also log in to your Facebook, Twitter, or LinkedIn accounts during the webinar to post updates or send any information about the event.

Prior to going to the formal presentations of the first speakers, I just wanted to remind everyone also that I had written a perspective article in Science Translational Medicine on Biomarkers and Parkinson's Disease, which provides a lot of background information that will be relevant to the discussions today. So, I please encourage people to go to that article for more information.

I'm going to start the discussion today with a short introduction on Parkinson's disease and the need for biomarkers and then turn it over to our speakers for more of the in-depth discussion.

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So, just to get everyone who's participating today on the same page, I wanted to just provide some overview information on Parkinson's disease. As most of you probably know, Parkinson's disease is a progressive, neurodegenerative disorder that's predominantly marked by motor symptoms, although there are many non-motor symptoms that are also present and can be debilitating for Parkinson's patients. Pathologically, Parkinson's disease is characterized by a selective loss of nigrostriatal dopaminergic neurons and the presence of alpha-synuclein positive aggregates known as Lewy bodies.

Current treatments for Parkinson's disease mostly focus on replacing the dopamine that's lost in the disease through the loss of the nigrostriatal neurons. These treatments are able to treat some of the motor symptoms of the disease; however, they lose effectiveness over time and are marked by significant side effects really resulting in a need for really innovative and transformative treatments for Parkinson's patients, particularly those related to affecting the underlying disease course known as disease-modifying therapies.

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Biomarkers are critical in our ability to develop disease-modifying therapies for Parkinson's disease. The goal is to develop disease-modifying therapeutics that target the underlying disease process and alter the progression of the disease, either by slowing that progression or ultimately reversing that progression.

Our current ability to test these disease-modifying therapies is really limited without the tools of biomarkers to help us in that evaluation. Current clinical trial designs require large sample sizes,

long duration, and rely on subjective clinical outcomes that are often influenced by the medications that I mentioned and can be quite variable across patients.

Parkinson's disease biomarkers would greatly accelerate our ability to develop new therapies for Parkinson's patients in that they can help us identify patients at the earliest stages of disease, improve patient selection for clinical trials, an example using DATscan, which we'll talk about a little bit later for example in helping us select patients for clinical trials, assess the efficacy of new treatments and therapies, and also monitor disease progression. And we'll touch on all of these issues throughout the webinar today.

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Now, I'm going to just give a brief summary sort of the format for today. We have four speakers who I mentioned during the introduction. Dr. Siderowf will talk about studying individuals at risk for Parkinson's disease. Dr. Schlossmacher will provide an overview of promising biological markers of Parkinson's disease. Dr. Schuff will talk about the latest in neuroimaging methods for Parkinson's biomarkers and Dr. Marek will summarize and talk a lot about the challenges in developing Parkinson's biomarkers, the impact these biomarkers can have on clinical testing, and provide information on the recently launched Parkinson's Progression Marker Initiative study.

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So, with that information, I'm now going to turn it over to the first speaker who is Dr. Andrew Siderowf and Dr. Siderowf is going to talk about efforts to study individuals at risk for developing Parkinson's disease. So, Dr. Siderowf.

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Dr. Andrew Siderowf: Thank you, Todd, and thank you to Science Translational Medicine for the opportunity to participate in this webinar. For the next few minutes, I'm going to discuss the potential use of biomarkers as screening tools for Parkinson's disease and I'm going to do this in the context of the PARS study, which I will describe in more detail in a moment.

In the course of this talk, I would like to make a few key points. First, prevention, as you can see on the top of this slide.

Prevention is the ultimate goal of therapy throughout medicine and treatment of high blood pressure to avoid outcomes like heart attack and stroke as a ubiquitous example of this paradigm. Our hope is that Parkinson's disease can be approached in a similar manner. Biomarkers have an obvious role in disease prevention since at the time of screening, there are no clinical manifestations of disease and biomarkers may be the only manifestation of underlying pathology.

The second key point I wanted to make is that because Parkinson's disease is not frequent in the general population. It's obviously an important condition, but in terms of the numbers, it's about probably 1% of people over 60. Screening for PD requires the identification of large numbers of subjects to find a relatively small number of cases. And most subjects that get screened will have normal screening results. As a result, highly specific tests are crucial for screening for Parkinson's disease.

The third key point I want to make during this talk is that efficiency is also crucial and that this can be achieved by targeting high-risk groups and reserving expensive tests or invasive tests only for those at highest risk.

So, returning to the slide and to describe the PARS study a little bit, PARS stands for Parkinson Disease At-Risk Syndrome. The PARS study is a multicentered study using olfactory testing, which is smell testing, followed by DAT imaging to identify individuals with abnormally reduced DAT findings similar to those seen in patients with Parkinson's disease who are likely to be at risk for clinical Parkinson's disease. PARS takes advantage of the fact that up to 90% of PD patients have olfactory deficits and that these deficits are present at the time of diagnosis.

The goals of Parkinson's disease are shown on this slide. The first is determine the feasibility of screening for Parkinson’s disease using this two-stage paradigm. The second is to assess clinical and biological features of pre-motor Parkinson's disease and then third and importantly is to begin to develop a pre-motor cohort of people who are at high risk for developing Parkinson's disease who would be eligible for preventive interventions.

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The next slide illustrates my second key point, which is that screening for PD requires large numbers of individuals. In PARS,

over 10,000 individuals were initially identified and just under 5000 completed smell tests, which was the first phase of the screening strategy. The other components of the first phase were questionnaires about early non-motor features of PD including autonomic dysfunction, sleep disturbance, mood disturbance, and motor complaints, which you could think of as being similar to the memory complaint in mild cognitive impairment. We also looked at epidemiologic factors associated with Parkinson's disease. These are illustrated in the box directly under the heading phase I. And the numbers in the study you can see on the right-hand side of this slide in the flow diagram.

The second part of PARS focused on dopaminergic imaging, but also included detailed motor and cognitive examinations and collection of biosamples for genetics and fluid-based biomarkers.

As you can see, a substantially smaller group of subjects were in phase II and they were selected based on olfactory status. Because so many people need to be screened, there is a potential for a very high number of false-positive tests. As a result, there is a great premium on the specificity of the overall test screening strategy. This is a key concept and I will repeat it again in the next few minutes.

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In PARS, this is mostly accomplished by DAT imaging, which where the existing evidence strongly suggest that individuals with normal scans do not currently have a Parkinsonian disorder. This does not entirely rule out the fact that they could develop one in the future, but probably not in the next few years.

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The next slide begins to show how targeting high-risk individuals even early in the screening process can improve the efficiency of the screening program. In PARS, we tested whether questionnaires that assessed pre-motor features of PD could be used to increase the likelihood of identifying individuals with an impaired sense of smell, which we call hyposmia. Osmia is smell and hypo is low so hyposmics are those that have a poor sense of smell and as you'll see throughout the next couple of minutes normosmics are people with a normal sense of smell.

As is shown on the left side of the slide, you can see that the odds of having any one of these non-motor features among hyposmics

is increased, but not dramatically so. By contrast though, individuals with multiple non-motor features, which is shown on the right-hand side of the slide, are at substantially higher risk for hyposmia. In this way, simple tests can be used to begin to enrich the population, which will be considered for confirmatory testing.

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The next slide, which is my last slide, further illustrates my final key point that using a two-stage process and reserving the highly specific tests for a relatively small number of cases improves efficiency and reduces costs. In the case of PARS, this means that the number of subjects that need to have DAT imaging, which is moderately expensive and requires a minimal amount of radiation exposure, can be avoided in the vast majority of people that are likely to have a normal test.

Referring to the figure, among normosmics, only 1 person out of 100 had an abnormal DAT imaging study defined as a DAT finding of 65% or less of the expected value for age and gender. This is illustrated just above the shaded line in the table. By contrast, among hyposmics, which means people that have an abnormally bad sense of smell, 11% had definitely abnormal scans. Intermediate scans in the 65% to 80% range are also greatly overrepresented among hyposmic individuals. Thus, using a relatively available and inexpensive pretest, that is, olfactory testing, the number of confirmatory tests that are required can be reduced by 80% to 90%. And as long as the first phase test, in this case olfactory testing, is reasonably specific and highly sensitive, few real cases will be missed.

So, in summary, the PARS study shows that screening for PD is possible using biomarkers implemented in theories. Further advances in biomarker technology will hopefully make this process more efficient and accurate. And ultimately, screening can be linked with neuroprotective therapies to realize the goal of preventing Parkinson's disease.

Dr. Todd Sherer: Thank you, Andrew. It's very interesting and it's a very important study I think the PARS study and we could get more details probably through the question and answer session.

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The next speaker for today is Michael Schlossmacher who's going to provide an overview on promising biological markers of

Parkinson's and also provide a little bit more detail on the impact of having these biomarkers on clinical testing.

Dr. Michael Schlossmacher: Thank you very much, Todd. And thank you to Dr. Orla Smith and her team at Science Translational Medicine for asking me to participate.

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And following up on Andrew Siderowf's presentation, I have to say that when I think about biomarkers I'm personally concerned, as many of you in the audience will be, about the heterogeneity of Parkinson's disease. In other words, that when neurologists identify a group of patients as suffering from typical Parkinson's disease, we now know that the clinical syndrome of Parkinson's disease may have a number of different pathological and etiological backgrounds.

And this is highlighted in this first slide that has a listed example of patient enrollment in past PD trials without biomarkers. This is not summarizing one specific trial in the past, but it's kind of an overview that helps to illustrate one of the issue why maybe some of the trials weren't as successful to achieve what Todd mentioned earlier, namely to induce a disease-modifying effect.

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And this whole notion that I want to convey to you or talk about is the issue of stratification. Stratification is a very important process that we employ when we follow the literature of clinical trials as being conducted in breast cancer trials or in coronary heart disease or in congestive heart failure. And we have not yet done this in Parkinson's disease and I think it's time that we employ some of the tools that we already have to do that.

In this class, essentially in the left-hand slide, you see that typically in the past, we screen to have patients with typical PD that met all the clinical criteria based on subjective rating scales to enroll 800 people into treatment arm and for instance 800 people into placebo arm.

And if you looked in the middle of this slide, what we now know is that there are four boxes and those four boxes represent different types of Parkinson's, some are very typical, others are typical. And in the middle, the third one is actually the orange box that is probably the most common. We do know it's in 75% of the cases, these patient suffer from an alpha-synuclein associated pathology

that forms inclusions. But underneath it, there's a group that's less than 10% that may look for all intents and purposes exactly like the group in the orange box, but these patients suffer from an inclusion-negative or a synuclein-negative phenotype. And at the top two, the pink box and the yellow box indicate that the males of these subjects without any evidence of dopaminergic deficit and people, clinical lookalikes such as multiple system atrophy and currently we don't have good tools to differentiate them.

But if you conduct a clinical trial and you bring all four into your treatment arm and you expect that the drug only engages your target at a 20% rate, ultimately as you'll see at the bottom right in yellow, your P value may not be significant enough to see the few people that you selected that you treated because of the low response rate, and in addition because you confused the population and it was not a pure enriched cohort.

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So, when one thinks about how can we do this differently in the future, two things come to mind. Number one is what John Growdon referred to in the past when he spoke about biomarker development in Alzheimer's disease. He said there are markers of trait such as a DNA mutation, markers of state for disease, which reflects the disease has developed, a marker of rate such as the progress by which speed the disease develops and carries on, and a marker of fate, meaning what marker can tell us what the outcome is. And there's mostly likely not going to be one marker that fits all these criteria in PD.

And the second aspect is that there's a marker spectrum, if you will, some are more proximal to the cause and some are more linked to the phenotype with all the clinical deficiencies, and that's often referred to as a surrogate marker. So, when we look currently at the landscape of what are -- this is the slide that you see next that listed marker candidates. Essentially some of the markers that are listed here are either very proximal to the cause and have been delineated to genetics or proteomic studies.

And because alpha-synuclein has such a prominent role in the development of the disease, people have started to develop various assays to quantify synuclein, either the entire concentration of it or oligomeric forms. And in the future, we'll see papers that address post-translationally modified forms.

And then there's work that comes out of the genetics field where sequenced variations are being investigated. And I will refer to it briefly on the next slide then, the GBA1 mutation discovery that's quite interesting. Other people have looked at sequence variance and synuclein that's the SNCA gene and LRRK2. And we've also seen work just as the metabolome interrogation in plasma, transcriptome changes in peripheral blood essentially to try to inform us as to what disease process is going on in the central nervous system.

And at the bottom here listed is the exploration of dementia-associated proteins such as tau and a beta, which Andrew Siderowf has done recently, to try to inform us as to whether a subset of Parkinson's disease patients that later on developed cognitive changes can be identified.

And on the right-hand side of that panel, you see two figures that I've taken from a recent paper that we published that just gives you a small snapshot as to what we and many others in the field are doing. In the upper panel, you can see quantification of alpha-synuclein in the spinal fluid from five different cohorts, the left one being Alzheimer's disease and the fourth one to the right is Parkinson's disease and the middle one is dementia with Lewy bodies. It's another disease linked to alpha-synuclein misprocessing. And you can see there's a gradient and it appears that at least for Parkinson's and in DLB and to the very right the MSA group shows slightly lower levels than other neurological patients.

[0:20:15]

And at the bottom you see in the same patients the tau quantification and as expected, tau is elevated in the AD group on the left and has an intermediate value in dementia with Lewy body disease patients. So, in the future as we do in Alzheimer's disease, it may be that we use CSF parameters to interrogate what a patient's biochemical process is like in the central nervous system because obviously we can't take a brain biopsy from it.

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And many of these studies have to be validated and studied further in larger groups so this is very much at the beginning of it. Now, if you were to ask me what is the most tantalizing or most intriguing biomarker at this juncture to inform us as to what's going on in the brain, I would say the recent work in that summarized paper here in this next slide regarding the GBA1 gene

is really interesting for two reasons. Number one, as led by a number of geneticists around the world headed by Ellen Sidransky and recently published in New England Journal of Medicine, which is listed in the upper left panel here, multicenter study has shown that around the world about 8% to 20% of people with typical Parkinson's disease carry a single mutation in the GBA1 gene. That gene has previously been linked to Gaucher disease.

Why is it important? It's important because three teams independently, they're listed by names in the upper left portion of the slide, have shown that every single person who suffers from Parkinson's disease and carries one of these mutations actually has a full-blown typical synuclein inclusion related illness. In other words, that gene does something that's very close to the disease that affects synuclein processing and, therefore, helps to initiate that disease. It's a very important susceptibility gene.

And on the right-hand side of that panel, you see what this gene does to synuclein. All these bars represent synuclein quantification in the cell model of Parkinson's. And we've confirmed that in a mouse model that every time you have a mutation in this GBA1 gene, your synuclein concentration rises.

And at the left lower portion of the panel, you can see from many different genetic studies we've learned that the more synuclein you produce, the more likely you will get Parkinson's disease and related problems, that's the triangle here and the red circle illustrates the contribution of the GBA gene mutation. So, the GBA1 gene maybe a very important marker to inform us as to what the disease process is all about that affects a person with typical Parkinson's disease.

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And then going to the last slide, how do we then incorporate some of the existing information and potentially future findings with respect to validated biomarkers into better, cheaper, and hopefully more effective clinical trial. And that scenario is summarized in this last slide and essentially, I want to draw your attention similar to what Andy said earlier about a multi-step or two-step process. That the process that I envision through stratification essentially begins with a similar first step as previously on the left-hand slide and then when we move to the middle bar or the middle portion of it, again there are these four types of people with typical Parkinson's disease clinically.

But this time, we employ biological tools to eliminate the ones that we don't think are the right candidates for the drug. And we focus on the third one, which is in orange again and we try to pick out by employing neurophysiological studies, biomarker studies from smell test for instance or some of the things you've heard about a moment ago, or for instance in combination with genetic testing to look for those people who carry a GBA1 mutation, and differentiate it from the people at the bottom, the fourth one that don't have anything that indicates that this is a synuclein related disease. And we exclude those because they have a normal smell test or they don't carry a GBA1 gene mutation or they have an autosomal recessive Parkinson's disease mutation.

And then when you look at this orange slide, it says here we only pick 300 out of 600 patients that match that genotype and with those 300 or a similar number, we then proceed in the clinical trial and the treatment arm. Furthermore, we hopefully will have tools in the future such as if there's a drug that we want to test that affect alpha-synuclein metabolism, that we can actually in peripheral blood or in spinal fluid or in the saliva or in the urine, monitor the effect of that drug in our patients as to what it does on the alpha-synuclein concentration.

And if you follow that stream of thought and you arrive at the right corner, the right bottom corner, you get the same response rate of your drug of interest in the patient cohort, let's say 20%, 60 out of 300 patients will show a positive effect, and you will be able to monitor that effect in vivo. And that, therefore, markedly increases your chance to be successful with the clinical trial to show efficacy or not and to monitor the engagement of the target and hopefully do this at a fraction of the cost.

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So, this is kind of a snapshot, an overview of how I envision we can go in the future when we employ biomarkers and I will stop right here.

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Dr. Todd Sherer: Thank you, Michael. The next speaker for today is Dr. Norbert Schuff who's going to give us a similar perspective, but focused on neuroimaging methods and where are we with neuroimaging biomarkers for Parkinson's disease.

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Dr. Norbert Schuff: Thank you very much and it's a pleasure for me being on this Science webinar. I just briefly want to give you an overview of the potential neuroimaging techniques that can be used for the challenging problem of identifying Parkinson's disease very early, measuring progression of the disease, as well as hopefully helping with identifying drugs with disease-modifying interventions.

So, to just relate to my previous speaker that MRI and imaging in general I think we see as a marker of the state of this disease where we see the imprint of the effects on the brain as providing us with potential information about, lots of information about the disease conditions. And our approach usually is two-fold, which I'm showing on my first slide. We are investigating functional changes related to the disease as well as morphological changes.

In functional changes, certainly we have made some progress in this area by measuring the newer dopamine transporter imaging method to really identify dopamine depletion in the Parkinson's brain as a major progress. We have also techniques to measure in general brain activity by measuring cerebral metabolism, blood flow. And this functional MRI, we can also look at the brain functional networks so the regions of the brain that are engaged while you're doing a task.

On the morphological side, we can measure brain volume, iron content, tissue microstructure with Diffusion Tensor Imaging, a new variant of MRI, which I will explain in a moment. We can also measure brain connectivity with this technique and we have additional tools to measure amyloid beta, a prominent marker in the context of Alzheimer's disease.

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So, I have divided my talk into existing methods, which I consider definitely dopamine transporter SPECT, which has been FDA approved now for diagnosis of Parkinson's disease. And in the slide, you'll see on the left panel, there's a difference between a normal volunteer having a very bright in the striatum of dopamine, which is then diminished in the case of univariate Parkinson's patients and bilateral affected Parkinson's patients. So, this is very impressive. This technique still has challenges to differentiate idiopathic from atypical Parkinson's disease as well. We don't know yet whether we can measure reliably the progression of the disease.

On the right, I've shown some examples from MRI where we're measuring the volumes of the striatum and the basal ganglia in general, which gives us some indication to separate Parkinson's disease from MSA for example as you see in my presentation. Also in the terms of measuring brain iron, which is the highlighted red areas, you see on the far right column, again, there are a lot of overlaps with normal values and so this technique is not yet reliable for diagnostic use.

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We have a number of emerging imaging techniques, which I think have really a great promise to drive this field forward toward identifying a really reliable biomarker for Parkinson's disease. One of them is Diffusion Tensor Imaging, which I'm showing you on the right panel together with this structural MRI. So, Diffusion Tensor Imaging measures the microstructural integrity of brain tissue specifically in the white matter, it gives us a great contrast. In the right row of the MRI sections, you'll see for example on the left the structural MRI showing you the tip of the substantia nigra and red nucleus, the brain stem. On the left, you see that DTI provides much more contrast and additional information than what we see in regular MRI.

[0:30:09]

And in addition to that, we have the opportunity of course to look beyond the dopaminergic neural system, which is very likely affected in Parkinson's disease. So here, I'm showing you a dopaminergic scan with FDOPA on the top row and the bottom row a ligand, the PET ligand, which measures acetylcholine in the brain, another neurotransmitter system. So, we can combine those two neurotransmitter systems and see how they correlate in Parkinson's disease.

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So to come to my conclusion, we have existing methods certainly for diagnosis of PD… with dopamine transporter SPECT. We have also MRI to measure major brain changes like brain atrophy or vascular disease that can be used to screen potentially patients who have confounding diseases. And then, there's an emerging range of methods, which include PET with different ligands looking at different nutrients with those systems. Beta-amyloid PET, which has been very successful now, explored for the fetal Alzheimer's disease. DTI is a potential measure for studying the impact of PD on white matter and connectivity of the brain. And

finally, resting state functional MRI, which can measure the function connectivity of the brain.

So, with those markers together, we really hope that they can provide really novel information towards the goal of identifying disease-modifying interventions. And I'll leave it at that point.

Dr. Todd Sherer: Thank you, Dr. Schuff.

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Our fourth and final speaker for today's webinar is Dr. Ken Marek. Dr. Marek is going to talk about addressing the challenges in developing Parkinson's biomarkers, the impact these biomarkers could have on clinical testing, and give more details on the recently launched Parkinson's Progression Marker Initiative Study. Dr. Marek.

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Dr. Kenneth Marek: Well, thank you very much, Todd, and thank you for the opportunity to be part of this webinar.

So, as the final speaker, I want to very briefly sort of highlight some of the points raised by the other speakers and summarize the utility of biomarkers, particularly as they relate to clinical trials. And then again, briefly just introduce the audience to the Parkinson Progression Marker Initiative, an initiative in which we hope to identify and help to validate biomarkers for Parkinson's disease.

So, we've heard already about the critical need and utility of biomarkers as tools that will help us to both understand, diagnose, and identify drugs for Parkinson's disease. But in this slide, we see some of the key issues really highlighting the utility of biomarkers in clinical trials for Parkinson's disease to identify disease mechanism, drug mechanism, identify dosage, utility for study eligibility, I'll discuss this in a moment with the PPMI study. Andrew has already discussed pre-motor diagnosis. Certainly, monitoring disease progression is another critical utility for biomarkers. Michael has focused on stratification of the PD sub-types, again another really important use of biomarkers.

I did want to, as many of the other speakers have already, focus on disease-modifying PD therapeutics as a major unmet need in Parkinson's disease in an area where biomarkers have particular

utility. Here, we would expect that if we had objective biomarkers that could be utilized in these studies, they might be helpful to potentially shorten disease duration, reduce study sample size, and limit study costs.

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In the next slide, I've also wanted to point out that when individuals ask me certainly about biomarkers, I'm always asking them a biomarker for what. And this case we're talking about what stage of Parkinson's disease, in this slide, which is a cartoon of the progression of Parkinson's disease. And you can see that we've already discussed on this call the PARS study, which focuses on pre-motor disease. I'm going to talk about the PPMI study and there is yet another initiative as well as others that are not listed here focusing on later disease. So, I think different biomarkers for different stages. Again, as Michael indicated, we need more than one biomarker to define Parkinson's disease.

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And with that in mind, I wanted to in the last couple of minutes just introduce everyone to the Parkinson Progression Marker Initiative or the PPMI study. The PPMI study is as biomarker focused Parkinson's disease progression study. It is very comprehensive. It's a longitudinal study and it involves the cooperation of multiple constituencies including the Michael Fox Foundation, which has been a leader in sponsoring this study, a variety of industrial partners, the government, academics, and of course patients and families.

The PPMI study really rests on three pillars illustrated here. One is to develop a specific dataset focused on biomarker validation and that really consists of both Parkinson's disease patients and healthy subjects to ensure that all of the data that we acquire in this study is standardized so that we can develop these biomarker assessments in multicenter studies for this study as well as future studies. And a key feature is that all of the data in the PPMI study will be accessible to researchers in the field. And I would urge all of you on the call to access the PPMI website as listed here, www.ppmi-info.org, and it will be possible to access PPMI data from that website following the instructions that are there.

So, one of the key issues here is to make these data available so that individuals in the community can contribute to this endeavor,

and also to make the biosamples that are collected in the PPMI study available through an application process on the website as well.

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In the final slide, I just wanted to give a little bit more detail on the PPMI study in this study synopsis. It is a study of about 600 subjects. It is a comprehensive study involving motor and non-motor assessments as well imaging. Some of the imaging modalities that Norbert has discussed, the DATscan as well as MRI including DTI are components of the study. There will be very extensive biosampling including CSF sampling and again some of the biological biomarkers that Michael has already identified are scheduled to be evaluated in the PPMI study as well as others that hopefully will be identified within this study and by the community.

So, we expect the study to go on for about five years. The study has begun, and I will just close by saying that we have just enrolled our hundredth subject as of last week.

I'm going to stop there so we have time for questions and turn it back to Todd. Thanks so much.

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Dr. Todd Sherer: Thanks, Ken. I want to thank all our speakers for their excellent presentations and particularly for sticking to their allotted time, which is not always the case. So thanks very much for that.

We're now going to move over to the question and answer section, particularly move to questions being submitted by our online viewers.

A quick reminder to those watching us live that you can still submit your questions by typing them into the text box and clicking the submit button. If you don't see the box on your screen, click the red Q&A icon and it should appear. And we already have a number of questions so I'm going to jump right into the question and answer session.

One of the first questions that came in really relates to I think one of the themes that we were addressing during the webinar is how can Parkinson's disease biomarkers help in the design and execution of clinical trials? Ken, I know I you touched on this in

your remarks, but maybe you want to expand on this and then we can open this to the panel to start some of the discussion.

Dr. Kenneth Marek: Sure. This is Ken. I'd be happy to do that, Todd. I think that many of us in the Parkinson field have been involved in numerous studies during the past several years, particularly disease-modifying studies that unfortunately have failed to show an effect, it failed to show efficacy. And one of the problems I think in those studies is the lack of an objective biomarker to help us in order to make that case.

So, I think a key utility for biomarkers in clinical studies is to help us to assess the progression of disease as well as to monitor whether we can detect changes that are in disease-modifying trials that would be, you know, positive and give us clues that these drugs really, really are efficacious.

I think that corollary to that is that we also want to be sure that individuals who are participating in those studies actually have Parkinson's disease. And here again, I think biomarkers are critical in ensuring that those subjects who are enrolled in the study actually have Parkinson's disease. And I think biomarkers, particularly imaging biomarkers, have great utility in that regard and have been utilized and will be utilized in the PPMI study to help us answer those questions.

[0:40:11]

Dr. Todd Sherer: Great. We have a number of questions about specific biomarkers that I thought would be worth addressing because we didn't get a chance to go into detail on some of these during the webinar. The first question relates to cardiac neuroimaging. In Parkinson's disease, olfactory dysfunction, REM behavior disorder, and orthostatic hypotension have all been seen in Parkinson's and all seem to be associated with cardiac sympathetic denervation. All three of these non-motor manifestations may actually predate the onset of the movement disorder. Why is 123I-MIBG scanning, which is being used for cardiac sympathetic neuroimaging, not being more routinely used in biomarker research in Parkinson's disease and is this an avenue worthy of further investigation? Andrew and Ken maybe have some thoughts on this.

Dr. Andrew Siderowf: So, Todd, this is Andrew. Maybe --

Dr. Todd Sherer: Sure.

Dr. Andrew Siderowf: -- I'll take a stab at it first. I think this is an excellent point and MIBG imaging true has I think been under utilized in PD research especially in the United States. I think outside of the US, especially in Japan, you see more studies using cardiac sympathetic imaging to assess Parkinson's disease and Parkinson's risk.

A couple of key points: One is that as opposed to the DAT imaging, which as Norbert suggested, doesn't differentiate Parkinson's disease from other Parkinsonian syndromes like PSP or MSA actually. That by contrast, the MIBG imaging actually is specific to Parkinson's disease and dementia with Lewy bodies can actually differentiate PD from non-PD, Parkinsonian syndromes better than looking at the brain. So this is an area in diagnosis where it has a potential use and hasn't been used that much. And as Michael was suggesting earlier, this could improve the efficiency of clinical trials to get people that actually have PD and have a real synucleinopathy to be in the studies.

The other I think interesting part about MIBG has to do with Parkinsonian subtypes. As the question alluded, there's a link between MIBG abnormalities, smell abnormalities, and REM sleep behavior disorder. And in fact, the MIBG abnormality maybe more striking to people with Parkinson's plus RBD compared to people that have Parkinson's, but don't have RBD. RBD, which is REM-sleep behavior disorder, occurs probably in about 30% to 40% perhaps, probably about 30% of people with Parkinson's disease. So MIBG imaging may offer an opportunity not only to identify Parkinson's disease versus other Parkinsonian syndrome, it should actually identify a subtype of Parkinson's disease, which includes these other non-motor features, which may have a specific molecular or biological signature and may have a specific therapy.

Dr. Todd Sherer: I don't know if anyone had anything else to add to that, but I do want to move on to similar questions because we really have a lot of these and I want to make sure we get to as many as possible.

Norbert, the next question I think is relevant to your background and expertise. The questions asks whether there have been any advances in proton and phosphorus MRS in terms of diagnosing early Parkinson's disease.

Dr. Norbert Schuff: Yeah, I'm very glad this question has been raised because I didn't specifically address spectroscopy in my slide. There has been little

progress actually in this field and the reason is probably two-fold. One is certainly from the biological point of view, the metabolites that the MRS can see has very high concentrations. They're not only concentrations of neurotransmitters several magnitudes higher. And the other is related to the technical limitations of MRI being low sensitivity and the problem of qualifications of those metabolites.

Having said that, there has been some interesting improvements and advancements recently at the very high end of the magnetic fields at 7 tesla for example where people have started to look at glutathione. As you may know, glutathione is a metabolite very strongly involved in the whole oxidative stress issue. So, glutathione can be detected with MRS again with the caveat that the sensitivities are really low, but there has been progress made in this direction and I think in the future, we will see more results with respect to glutathione.

[0:45:18]

Dr. Todd Sherer: Thanks. Thank you for addressing that. Andrew and Ken, there are a number of questions related to the PARS study so I'm going to try to give you a few of them so you can address this. One question relates to why is there the hypothesis that a decrease in DAT binding would occur in early pre-symptomatic Parkinson's patients? If dopamine neurons are lost, would we expect that there may be early compensation and an actual increase in dopamine binding in these people? I don't know, Ken, if you want to take a shot at that one.

Dr. Kenneth Marek: Sure. This is Ken. I'll try to address that. I think it's a really interesting question and I think that it speaks to an issue that is relevant to most of the imaging studies that are done is that is to what extent are we imaging changes in disease and to what extent are we imaging changes in compensatory mechanisms that may occur in individuals, particularly early in disease.

With regard to dopamine transporter imaging, I think that the reason we see this profound loss in dopamine transporter even in early disease is that these individuals really have probably had a very longstanding change over time and there may have been very early increased dopamine transporter imaging, but that might have occurred 10 or 15 or 20 years earlier. And then there is this progressive loss that occurs over time that overwhelms that compensation. So, we're seeing that and I think it's profound

enough that of course it does overwhelm the compensation reliably over time. But I think it would be quite interesting to sort of understand that at the very earliest phase if we could.

Dr. Todd Sherer: Andrew, also in the PARS study, how do you go about controlling for smoking knowing the impact that smoking can have on the risk for Parkinson's? And are there any differences in smoking in the normosmic and hyposmic populations?

Dr. Andrew Siderowf: That's a good question. I had mentioned earlier, we were interested in both smoking and caffeine as well as other epidemiologic risk factors for Parkinson's disease like pesticides and heavy metals in PARS. And the short answer is that we don't see an effect in PARS of smoking on either hyposmia or on DAT imaging, and there's a very modest effect of caffeine consumption on hyposmia and actually none on imaging. And the reasons for this are unclear. It may have to do with sample selection. It may have to do with the fact that actually people who volunteer for PARS don't smoke very much at all. And it could also have to do with changing smoking habits for people whether people are just smoking less so you just see less effect of this.

Although, it's well established that specially smoking, there's an inverse relationship between increased smoking and decreased risk of Parkinson's disease, in the PARS study for whatever reason, we haven't detected this effect.

Dr. Todd Sherer: The last question on this study and also related to PPMI, our last for now, could you provide a little more detail on the biological samples that are being collected and whether samples are available for researchers to do analysis on? So I think maybe, Ken, you could just talk about what's being collected for PARS and what's being collected for PPMI and how investigators could try to seek access to those samples.

Dr. Kenneth Marek: Sure. Yeah, I would be delighted to do that. This is really a critical and a key part of the PPMI study and the PARS study. And we want to make it clear to the community that biological samples will be available for people to utilize for their own clinical studies.

Within the PPMI study, we are very much focused on the collection of biosamples. In particular, in that study, individuals will undergo blood, CSF, and urine sampling. Blood sampling occurs every three months in the first year and every six months

thereafter. And importantly, CSF sampling occurs at baseline, 6 months, 12 months, and every year thereafter. So in this study we have really focused a great deal of attention on CSF collection, understanding the value of CSF that we've learned from Alzheimer's disease and now we're learning in Parkinson's disease. And we have really been committed to ensuring that individuals undergo these tests.

[0:50:21]

All of these samples are stored in a bio repository and again, it is possible for anyone to initiate an application to access these samples through the PPMI website. And then this application would be reviewed by an independent biosampler review committee that would make a decision with regard to whether to provide those samples to the applicants. So, we would encourage everyone who has an interest to review what's available and apply for these samples.

With regard to the PARS study, that study also is collecting blood and serum samples, and now in part as a result of the enthusiastic response from the PPMI study has began to collect CSF samples as well. Those samples also are available to the community through an application process and we would again urge everyone to in this case contact either Andrew or myself to access those samples.

Dr. Todd Sherer: Okay. In terms of having those samples, and Michael, I'm going to bring you into the discussion now because there are a couple of questions related to our capability to detect alpha-synuclein in CSF. Are there standardized assays? What's the influence of different antibody combinations in terms of the reliability of the results? Is anything being investigated or done to sort of bring clarity to that? And in terms of alpha-synuclein, what are we actually measuring in the CSF? Is it total alpha-synuclein, is it oligomer form? I'm combining a number of questions here, but I think you'll be able to do good job answering that.

Dr. Michael Schlossmacher: Thank you, Todd. Yeah, Michael here. So, this is an emerging field of biomarker research and I'll say two things upfront. Number one is it's still in its infancy when compared for instance to what has happened already in the field of Alzheimer's disease and biomarker development there. And the second thing I just want to preface it that I have a potential conflict of interest because I work on this and we've pursued a commercial application too.

So, the field has seen I think a total of nine or ten studies currently that have explored quantification of alpha-synuclein in the cerebrospinal fluid. And six of these studies seem to go along, but four studies clearly don't agree with it, don't agree with the results of the others. When one looks at the quantification of total alpha-synuclein in cerebrospinal fluid, one has to say that the amount is miniscule in the cerebrospinal fluid when compared to how much is inside neurons. So, it's 1 in every 1000 molecules for alpha-synuclein as far as we can quantify it, taking the spinal fluid and then measuring it from the same donor at autopsy to see how much actually gets outside or doing the neuronal cultures. So, it's a minuscule amount that makes it outside.

Current technology has either measured the total concentration without any respect to posttranslational modifications. So, there's much more to be done in the interrogation of what species of alpha-synuclein are present in spinal fluid. And there's only one report out there so far by Omar El-Agnaf and his Japanese colleagues that has employed a sandwich assay that can detect oligomeric alpha-synuclein, but does not quantify the total. An intriguing data that his team has published in neurology last year is that it is potentially a ratio of the oligomeric form versus the total concentration of alpha-synuclein that maybe most informative with respect to who has Parkinson's disease.

But we don't know yet whether it's a good marker of progression. We don't know yet whether it also picks up who has dementia with Lewy bodies or multiple system atrophy. So there's much more work to be done.

And answering the part from the beginning of your question, what's being done to streamline these or systematically interrogate these different assays; as Ira Shoulson likes to say, currently, people have the boutique assays and we really need to provide something to the community to run their samples, whether they come from DATATOP or from PARS or from PPMI in the future. We need to investigate and compare the existing assays. And so, there's an effort that the Michael J. Fox Foundation together with John Trojanowski and Leslie Shaw is currently organizing, where some of these teams, currently I think it's four or five teams, their assays are being compared with blinded samples of CSF, blinded samples of recombinant protein to see how much congruence, how much concordance and consistent these data are versus how much do they differ the

results when you examine the same specimens by different assays.

[0:55:14]

Dr. Todd Sherer: Thanks, Michael.

Another question came in to follow up on a biomarker that was presented at the recent AAN meeting in Honolulu. I unfortunately was not able to attend that meeting in Honolulu, but maybe some people in the call were. There was a presentation that showed that compounds exhaled in the breath may predict developing Parkinson's disease. Does anyone on the panel know about this research or have any comments? It was presented by a Dr. Schlesinger from Israel.

Dr. Michael Schlossmacher: This is Michael here. Actually, it's interesting, I learned about this from a patient. I was not in Honolulu either, but there's a patient who brought this to my attention. And I've not read any specifics, but there's a number of diseases such as diabetes, certain forms of pneumonia, and even some forms of lung cancer that apparently have a key metabolome signature in the exhaled air by which very sophisticated mass spectrometry techniques can then pick up particular metabolites or compounds.

Apparently, that technique was applied to people with PD and I think the sampling was relatively small, but it's definitely a very different approach to interrogate a biological specimen. It's not even fluid, it's exhaled air. And one just has to see this in the published literature to further comment on it.

The second thing that's interesting, Dr. Zhang from the University of Washington Seattle has just published a paper I think in brain in which he shows that alpha-synuclein is not just present in the blood and in spinal fluid, but is also present in human saliva. And he showed this by mass spectrometry and started to now quantify alpha-synuclein in saliva to see whether that could -- so in spit and in saliva and then also I think in nasal secretion. So, he's trying to find out now whether that would be something applicable to informing us as to who has some problems systematically with alpha-synuclein processing.

Dr. Todd Sherer: We have time for a couple more questions. This one goes to Dr. Marek. At a recent conference, I think you presented in Barcelona about the early cognitive decline in Parkinson's and I mentioned

during the introduction that Parkinson's is more than just a motor disorder. In that presentation, you discussed the link between low dopamine as measured by the dopamine imaging and these cognitive deficits in Parkinson's patients. Could you provide a little more detail on what we mean by cognitive decline in Parkinson's patients and whether these deficits in the dopamine biomarker could actually predict the onset of dementia in some of these individuals?

Dr. Kenneth Marek: Sure. There are a couple of answers to this question. One just generally as I think you pointed out, Todd, of course cognitive impairment has now become a well-recognized characteristic of Parkinson's disease. Unfortunately, most individuals with Parkinson's disease will ultimately develop cognitive impairment as their disease progresses. As we've learned more about it, I think it's even clearer that it is possible to detect cognitive impairment at an earlier stage of illness. And one of the challenges is for us to be able to understand are there biomarkers that can predict who will be more likely to develop that earlier or who will develop more severe cognitive impairment or are there many different kinds of cognitive impairment.

So, two bits of data then: One comes from the PARS study that Andrew did not have time to really report is that one of the interesting phenomenon is that in those individuals in the PARS studies identified solely by their hyposmia and reduced dopamine transporter density, we also see that those individuals do less well on cognitive testing than individuals who don't have hyposmia and dopamine transporter deficits. Suggesting that even in individuals in this sort of prediagnostic category, there may be some evidence of cognitive impairment.

The other bit of data comes actually out of the LABS-PD study I mentioned briefly. In that study, there were individuals who underwent dopamine transport imaging on day of find and were followed for many years. And now five or six years later, what is becoming clear is that individuals who had reductions in their dopamine transporter density at baseline compared to other individuals were much more likely to develop cognitive impairment and actually frank dementia over this five to six-year period.

[1:00:01]

Now, we don't know whether the dopamine transporter density loss predicts cognitive impairment or whether it is just correlated

with this. But, you know, again, this is an area where I think we will be seeing lots of additional effort as we try to understand and get smarter at being able to predict who might develop cognitive impairment in Parkinson's disease and when.

Dr. Todd Sherer: Great. I think this next question will be the last question and Michael, I think this will go to you. We've talked a lot about using neuroimaging to monitor loss of dopamine neurons in the nigrostriatal system and even in the cardiac system or sympathetic neurons in the cardiac system. What about neurochemical biomarkers of loss dopamine and norepinephrine in the cerebrospinal fluid, what's been done with that? Where are we? Are there certain specifications in terms of CSF sampling that's required? What's kind of the status when looking at those biochemical markers of catecholamines for Parkinson's disease?

Dr. Michael Schlossmacher: Yeah, thank you, Todd. Michael, here. So, this is a very important aspect as we know and also thanks to Heiko Braak's findings. You know, we have an evolution of neuropathology that encompasses substantia nigra cell loss and locus coeruleus cell loss so we should see and we do see a reduction in norepinephrine and dopamine concentrations in vivo and untreated people. I think the first interrogation or the first effort to systematically study this was either in the late '70s or early '80s to try to use those as early biomarkers. And I think the very first compound studied was homovanillic acid in CSF and that's markedly reduced as metanephrine as a breakdown of catecholamines in people with PD and seems to be more specifically altered versus the multiple system atrophy or some other atypical Parkinsonian disorders.

I have not done extensive research in this so I'm going to answer this with a little bit of a caveat. From what I understand, the ability to monitor and quantify them precisely is still requiring a certain amount of infrastructure setup through HPLC. So it's quantification, which is really what we want and what we need for a metabolite such as HVA, homovanillic acid or DOPAC is still more labor and cost-intensive than we want it to be. But I think that should certainly be overcome.

And the second thing and I think from a practical perspective, this is the much important thing in this. When we treat our patients ultimately with a MAO inhibitors or with dopamine replacement strategies, these metabolites of endogenous catecholamine metabolism will be altered obviously. And then it's very difficult to

separate and differentiate what are truly endogenous metabolites that are abnormal versus what is induced by pharmacotherapy. And as we know from a number of studies, the effect in vivo of dopamine replacement therapy lasts certainly longer than two weeks. So then we have this conundrum as to how do we then sample these specimens in people who have been treated previously.

So, the way I see it, I think it would only be really applicable to people who have not yet been treated and that's certainly a minority of the patients.

Dr. Todd Sherer: Thank you.

Unfortunately, we're out of time for this webinar. And I'd really like to take the time to thank our speakers for being with us today sharing their thoughts and insights on this important topic and providing thoughtful answers to the questions that came in. Again, our speakers were Dr. Norbert Schuff from the University of California and VA Medical Center, Dr. Michael Schlossmacher from the University of Ottawa, Dr. Andrew Siderowf from the University of Pennsylvania School of Medicine, and Dr. Ken Marek from Institute for Neurodegenerative Disorders.

I do want to reiterate one of the things that Ken said and that all the data for the Parkinson's Progression Markers Initiative is being made available for download at the website, www.ppmi.info.org. So anyone on this call that's interested and wants to dig down and take a look at that data, please go ahead and do so.

Many thanks to all our viewers for the excellent questions that they submitted and we apologize that we did not have time to answer all of those questions.

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at webinar@aaas.org so we can take that feedback to improve this in the future.

Again, thank you to our panel for generously giving their time and knowledge for us to learn more about this important subject that has important relevance to developing new treatments for Parkinson's disease patients.

Goodbye and I wish everyone a happy afternoon and a good rest of the week.

[1:05:44] **End of Audio**