Marie: Hello and welcome to *The Parkinson's Research Podcast: New Discoveries in Neuroscience*. I'm your host, Dr. Marie McNeely, and I've partnered with The Michael J. Fox Foundation for Parkinson's Research to bring you to the forefront of the field of neuroscience to discuss the latest advances and discoveries with leading experts.

The Michael J. Fox Foundation created this podcast for researchers, clinicians, and industry professionals with the hope that these conversations and the resources we share will advance your efforts and partnerships to improve brain health. We are welcoming guests with a range of experiences and viewpoints. The views expressed belong to the guests themselves.

And today, we are excited to welcome our guest Dr. Paul Lingor. Listeners, Paul is Professor of Neurology at the Technical University of Munich and head of the outpatient clinics for motor neuron disease and co-head of the outpatient clinics for Parkinson's disease.

Today, we are going to be talking more about his work to identify new biomarkers for neurodegenerative disease using biometals in cerebrospinal fluid and also using tear fluid, as well as his work on novel and combined therapies for Parkinson's disease. So, Paul, welcome to the show today. How are you?

Paul: Thanks a lot, Marie, for having me. I'm very well and hope you are too.

Marie: I'm doing quite well, and I'm excited to have you with us to talk a little bit more about your work. So, perhaps we could start with your background. Can you tell us more about yourself, Paul, and how you found your way to your current position?

Paul: All right. Well, maybe let's start with my clinical training and with my thesis. I started studying medicine in Heidelberg at the University of Heidelberg. And very soon I was really drawn to neuroanatomy and started doing my thesis in neuroanatomy. There was a lab which was strongly involved in research on growth factors. It was the lab of Klaus Unsicker and Kerstin Krieglstein. And there I worked on growth factors on dopaminergic neurons. And that was already a first starting point, the first touch-base with Parkinson's disease. So, that basically kept all my professional life.

And then I went on for my medical training to the University of Göttingen, where I did my residency and did my regular neurology residency, but also focusing on neurodegenerative diseases, on movement disorders. I worked under the supervision of Mathias Baehr and also with colleagues like Inga Zerr, who is very important in the field of dementia, and also with Claudia Trenkwalder, who is a very renowned Parkinson's specialist. And then I was offered a position at the

University of Mainz in 2011, but I stayed in Göttingen because there were very good conditions at that time. And I was a fellow of the Kröner-Fresenius Foundation from 2013 to 2015. That was a very important time where I had more time to focus on research, particularly on our translational studies. And then in 2018, I moved to the Technical University of Munich, where I am now. And here I'm co-heading the outpatient clinics for Parkinson's disease and also the outpatient clinics for motor neuron disease.

Marie: Very cool. And I know you've done some remarkable research looking at metals and the potential role in neurodegenerative disease. So, Paul, to give our listeners a little bit of background here, can you explain the potential role that dyshomostasis of different metals may play in Parkinson's disease?

Paul: This is really an exciting topic. And I would probably say that for a very long time in the 20th century, that was a very prominent topic in Parkinson's disease, but it got a little bit forgotten in the era of alpha-synuclein research or into a second line, I would say. But already in the beginning of the 20th century, it was described that actually in inclusion bodies, which we know now as Lewy bodies, there was an increased content of iron. And also if you quantify brain iron content, then there's specific regions, for example, like the substantia nigra, which have more iron than other brain regions. And in Parkinson's disease particularly. So, there was a long debate and hypothesis of how iron could contribute to Parkinson's disease pathogenesis. And you know, there's also a number of other neurodegenerative diseases which are associated with metal dyshomostasis, for example, Wilson disease or brain-iron accumulation, where iron plays a role, evidently.

So, in Parkinson's disease, but also in other diseases like Alzheimer's disease and amyotrophic lateral sclerosis, there are certain regions where iron seems to accumulate more strongly than in others. And it is really interesting that iron seems also to have some interplay with alpha-synuclein. So, recently it could, for example, be shown that alpha-synuclein is a protein which has specific binding sites for iron, but also other divalent metals like copper and manganese. And in the alpha-synuclein gene, there are iron-responsive elements where if you have an increased content of iron in the cell, there is more alpha-synuclein translation. Also, if you add iron to an alpha-synuclein solution, you can foster aggregation with that. So, there are a number of points which make this interaction between iron and alpha-synuclein quite interesting.

And we have also studied this, for example, by means of synchrotron radiation. So, we studied with x-ray fluorescence, the content of iron in dopaminergic neurons, in the round dopaminergic neurons. And we could basically also reproduce that on a very small scale, what has been described on a large scale, that in dopaminergic neurons, but also even in Lewy bodies, we have an

increased content of iron, but also a decreased content of other metals like copper, for example. We also worked on mouse models where we applied iron to these mice to simulate iron accumulation. And we can see that mice which are treated with iron, they have impaired learning in the rotarod test, for example, or also in novel object recognition tests. And if you treat them with an iron chelator like Deferiprone, that can partially restore this.

So that was very interesting. And we also studied, for example, the role of iron on alpha-synuclein spreading. So, if you inject alpha-synuclein preformed fibrils into mouse brains, then this alters their propagation when mice are pretreated with iron. So, I think in total, although the interest in iron also other metals has somewhat declined in the last years because a lot of research also on alpha-synuclein, there is a lot of interaction. And there has been recently also a study on iron chelation, for example, with Deferiprone headed by David Devos. And this trial studied actually whether you could attenuate Parkinson's disease progression by giving Deferiprone to patients with Parkinson's disease. It was a very important trial. And even though it came out negative, so it did not show any beneficial effect, I think that this story is not completely closed on iron and alpha-synuclein and iron and Parkinson's disease.

Marie: Interesting. And I think beyond iron, there's some data coming out about other elements, things like selenium, strontium, arsenic, et cetera. So, can you comment on these as well?

Paul: We have looked basically in CSF of Parkinson's disease patients for elemental content. And it was interesting because iron and also other metals are deregulated in Parkinson's disease and also in other degenerative diseases. We thought maybe we can explore this in the CSF also as a biomarker. And it's not quite trivial to look for individual elements, by using mass spectrometry, you can identify different elements in the CSF. You need quite larger amounts of CSF. So, it's something you would rather do in a research setting, not in a clinical setting, but you could identify 28 different elements in the CSF. And then you can quantify them. And the elements which you mentioned, selenium, iron, but also arsenide and nickel — they were elements which very nicely separated Parkinson's disease patients from controls where we studied specific subgroups of these patients.

And we could also reproduce this finding in a multicentric setup. So, it seems to be that we have different contents of these metals in Parkinson's disease patients. And then we also explored whether we could use this as a biomarker, which might be used also for disease progression. Because evidently nowadays with alpha-synuclein and alpha-synuclein aggregation, we probably have a biomarker, which is very good to identify Parkinson's disease patients, but we still have not a very good biomarker, which tells us a surrogate of disease

progression. So, this is still something which is really needed. It is also very much needed for clinical trials if you want to assess the efficacy of disease modifying drugs.

So, what we did is we looked at iron, but also other metals, and iron transport, and iron storage proteins like ferritin. And if you look longitudinally — so we had access to a rare collection of CSF samples where we assessed CSF from patients with Parkinson's disease at a time of one year interval. And when we looked at these CSF samples, we could identify that the iron content over the time — over this one year — increased in patients with Parkinson's disease, while the level of ferritin actually decreased. And if you build a ratio between these two, then this was even stronger correlated.

And this was independent of the treatment of these patients. So, actually in one year, we had quite early Parkinson's patients. The medication was adapted. And if you looked at, for example, clinical measures like UPDRS, this didn't change so much because patients obviously got worse, but they were also treated. So, their levodopa equivalent dose was increasing.

But still these markers of iron and ferritin changed over this one year. And it was a small study with only 20 patients. But I think it is interesting, and it should be followed up in a larger cohort.

Marie: Absolutely. And I think your recent analyses looking at multiple elements and different patterns are particularly interesting because there have been some maybe inconsistencies between studies when you're looking at just one element because we're talking about tiny amounts here. Right?

Paul: Yeah, that's true. That's why I think if you probably look at single elements, it will be difficult. First of all, it will be difficult to quantify these elements. So, you need mass spectrometry to do this.

And you need also substantial amounts of CSF. But there might be also differences in environmental exposure to these elements. And if you live in an area, which is, for example, very high on environmental selenium, then also your levels of selenium will be higher in your CSF. And in other areas, this might be different.

So, there is no clear and easy cutoff which you can use for Parkinson's. However, what we learned also from this study, if you look individually on patients from one specific center, which come all from one specific vicinity of the center, then you can train your system and identify a signature which might give you a better differentiation between the groups.

Marie: Absolutely. And I think you're using machine learning models to kind of do these analyses. Can you talk about what goes into these models and how you have confidence in what comes out of them?

Paul: I'm not a bioinformatician, but we're working very closely together with bioinformaticians. So, this is also something which I think in the last years really emerged in the amount of data, which we all experimentally gather is so big that we definitely need professional help from people who are dealing with large data sets. And bioinformaticians have, of course, different tools, different machine learning algorithms where they can feed data and identify patterns in this data. And one of the things which you can do with your data is, for example, to feed your algorithm and train this algorithm to identify a specific condition like Parkinson's disease. And then you give either another data set or you give part of this data set back to the machine and let it re-recognize this pattern. And then it can tell you how well it re-recognizes from a particular subgroup of your samples, your condition. And that gives you some more confidence in whether your pattern is really something you can believe in.

Marie: That makes sense. And Paul, what do you see then for the future of these metal analyses or elemental analyses in Parkinson's disease, whether it's research or the clinic?

Paul: I think that we definitely need to study larger cohorts. And particularly, it would be very interesting to identify methods where we can very securely quantify the elemental content from a small amount of CSF. And then we also need larger cohorts to see whether, for example, this effect on disease progression is really reflected on iron levels or also in levels of other elements.

Why this is interesting, I think, is that if you look at elements, and you look at other biomarker molecules, elements are something which are very, very stable. So, an element basically does not change because of environmental conditions. So, if you take a CSF sample, and you keep your sample, you can heat it, you can cool it, you can leave it on your shelf, but the elemental composition will never change. While if you look at proteins, they can degrade, they can also have different modifications. If you keep them not properly frozen.

So, elements could be really interesting, but I also agree that elements are maybe less specific than proteins. And maybe you need a combination like we had from iron and ferritin to combine elements and also proteins to make a reasonable argument on the use of these as a biomarker.

Marie: That makes sense. And I think one challenge that you alluded to earlier is that when it comes to biomarkers, sample collection can be a problem. So, you mentioned that you need a relatively large sample of CSF to do these analyses.

So, for people maybe who don't want to or can't undergo a spinal tap, it would be really helpful to have other biomarkers that can be collected less invasively. And you, Paul, have been investigating the potential of using tear fluid as a biomarker source in neurodegenerative disease. So, can you tell us more about your research in this area?

Paul: Yeah, that basically also came from exactly this question which you mentioned. If you ask patients if they are willing to donate biomarker samples, then many people would probably agree that blood is okay. Tear fluid is certainly okay, but CSF may be difficult.

So, we were very happy already for this quantification of this group of 20 patients who allowed us to take CSF at one year distance. But if you really want to make larger analyses, then you would like to refer to a biomarker source, which is better accessible. And I think tear fluids are really a very nice example for that. So, you can sample tear fluid using a very simple Schermer test strip. That's something which ophthalmologists use very frequently to determine the running length and to assess dry eye disease. And for the patient, it's a little bit uncomfortable to have this in their eyes for five or 10 minutes, but it's really not more than being uncomfortable.

It's removed right away and patients just have to sit still and wait until the tear fluid is running into these Schermer strips. It can also be done quite frequently. So, you can do it every week, which you certainly do not want to do with CSF sampling. Of course, it's also something which from the point of training of the people who do this research is also important because you can teach, for example, also a student. So, you do not really need to have a profound medical education to sample tear fluid. So, we do this quite a lot. And we have sampled many patients here in Munich with different neurological diseases. And we have also studied, of course, patients with Parkinson's disease. And for Parkinson's disease, this is particularly interesting because the tear fluid is generated in the lacrimal gland.

And the lacrimal gland is innervated very similarly to the salivary gland by nerve fibers, which originate very closely to the brainstem. And as you know, pathology in Parkinson's disease is originating not in the substantia nigra, but very likely we all know the Braak stage hypothesis, very likely other parts of the brain like the brainstem are affected much earlier in disease.

So, we hypothesized that if you have changes which go through the brainstem and then develop further in the brain, that you might also pick up changes in biomarker sources like tear fluid earlier than in other biomarker fluids. And one of the things which we did in the very beginning is we did a proteomic analysis of Parkinson's patients and controls. And we looked at different proteins. And

actually, you can identify more than 500 proteins in the tear fluid at that time. Now we can do even more.

And at that time, we studied the composition of these tear fluids and could really see that in Parkinson's disease, tear fluids, we had a significant reduction of proteins which were involved in immune responses, for example. But others were upregulated like proteins involved in oxidative stress or lipid metabolism.

And then we also followed this up and looked at specific other proteins. And of course, one of the proteins which is very interesting for Parkinson's disease is alpha-synuclein. And alpha-synuclein can actually be detected in tear fluid. And other groups and ours have detected alpha-synuclein with different methods.

We used the SIMOA method, which is a very sensitive type of ELISA, where you can detect proteins at very low amounts. And we could see that in Parkinson's disease tear fluids, you have a slight increase — slight but significant increase — of alpha-synuclein in the tear fluids. It's not enough to differentiate, really, Parkinson's disease from control patients. But on a group level, this is what you can see in tear fluid. But it's also very similar to what you can see, for example, in cerebrospinal fluid or blood. So, in CSF, you also cannot differentiate Parkinson's disease patients by their mere alpha-synuclein level. You have an overall slight decrease of alpha-synuclein in CSF. So, this might be quite similar in tear fluid.

Marie: Very interesting. And you mentioned not just the composition or the protein content of the tear fluid is different in neurodegenerative disease, but the amount of tear fluid as well. So, can you comment on why people with Parkinson's or other neurodegenerative diseases may have reductions?

Paul: This is an interesting finding, which also makes research in this field a little bit more difficult because one of the caveats if you want to work with tear fluid is really that you cannot get it in big amounts.

So, you can basically use your sample for two, three analyses depending on how much sample you have. And particularly in Parkinson's disease, but also in other neurodegenerative diseases, you have a reduction in wetting length. And in Parkinson's disease, this might be quite well-explained by the bradykinesia of Parkinson's patients. The bradykinesia affects not only, of course, limb movements, but it also affects, for example, the blinking rate. And when you watch Parkinson's patients very closely, you will notice that patients have a dramatically reduced blinking frequency. Their eyes are open for a longer time. They have also irritated eyes very often because of that, because the tear film is drying out. And because of that, you have also a reduced wetting length in Parkinson's disease.

It is interesting that it's not only true for Parkinson's disease. So, we have recently looked at a larger cohort of more than 700 patients from our Department of Neurology with all kinds of different neurodegenerative, but also other neurological diseases. And we find this also in other neurological disorders, also, for example, in amyotrophic lateral sclerosis.

Marie: Very interesting. And like you mentioned, this wetting length measure is very easy to get, and it doesn't require, necessarily, a lot of special training for someone to collect it. How do you see this potentially being used in the future?

Paul: Well, this is something which really has a lot of potential, because it is something which you can perform very cost-effectively. You can train people easily to take tear fluid. And at the moment, we are working on a method to not only identify alpha-synuclein in tear fluid, but also aggregated alpha-synuclein. And imagine if you would be able to not only take this alpha-synuclein seeding aggregation assay from CSF, but from tear fluid, that would, I think, be a very interesting option for patients to have a test on alpha-synuclein aggregation from a biosample, which is very easily accessible.

Marie: That makes sense. And Paul, then shifting from biomarkers now to treatments. I know you've done some phenomenal work looking at the drug Fasudil in Parkinson's disease. So, let's talk about this topic. Can you explain what is the evidence to date supporting the maybe symptomatic and also disease-modifying effects of this drug in Parkinson's disease models first?

Paul: We started looking at Fasudil already many, many years ago. So, about 15 years ago. And it started actually with research, which we did back at that time on neuroregeneration. So, you know that axons, as an integral part of the CNS, degenerate in neurodegenerative diseases. And one of the potential treatment options is of course also the regeneration of these axons, if you can keep the nerve cell alive.

And we looked at different molecules, which could actually have both of these properties. So, keep nerve cells — neurons — alive, and also increase regeneration. And this is interesting for different disorders, not only for degenerative disorders like Parkinson's disease or ALS, but also, for example, for spinal cord injury. And one of the molecules or molecule groups, which was interesting to us was the ROCK inhibitors. And ROCK is a kinase, which you find in many cells of the body, but you also find it in neurons.

And if you inhibit ROCK, then you can increase regeneration by the modulation of the actin cytoskeleton. So, because we wanted to work in a translational approach and bring this also to the patient, we were looking for drugs which potentially are already licensed and have a beneficial safety profile. And that's

how we came to Fasudil. Fasudil is actually a small molecule, which was licensed already back in the 90s. And it was licensed for something completely different for the treatment of vasospasms following subarachnoid hemorrhage, so for a certain type of stroke. It was used basically in Japan and in other Asian countries, South Korea, for example.

And there it is still used very similarly to nimodipine, which is used more in the Western Hemisphere. So, it has been used on many, many patients and it has been also shown to be very safe. And we studied this as a Rho kinase inhibitor. And we could see that if you apply Fasudil to neurons, you can protect them from degeneration, but you can also foster regeneration of neurons. So, outgrowth of axons. So, it improves growth cone formation.

And we eventually went from this in vitro work to in vivo work in different animal models. So, we studied first models of Parkinson's disease, for example, toxicity models like the MPTP model or the 6-hydroxydopamine model. But we also looked at Parkinson's models, which are overexpressing alpha-synuclein mutations, like the A53T mouse model. And we also worked with other mouse models for other degenerative diseases like the ALS mouse model, where we, for example, evaluated the use of Fasudil in the SOD1-G93A mouse model, which is a very common model for ALS.

And in all of these models, to a more or less extent, we could see that Fasudil attenuated neurodegeneration, particularly in these Parkinson's models, the degeneration of dopaminergic neurons. But it also increased sprouting, for example, of dopaminergic neurons into the striatum. And in addition, when we looked at these animal models, we also looked at motor function. We looked also in the ALS model, for example, one of the readouts is survival.

So, many of these parameters were positively changed. And in the A53T mouse model, this is a mouse model where we see alpha-synuclein aggregation. And there we could see that alpha-synuclein aggregation was actually attenuated by application of Fasudil, and this was an additional very interesting point because if you want to counteract Parkinson's disease, then alpha-synuclein pathology is at the very center stage. So, this was quite encouraging for us. And therefore, we planned to go further because this drug is a licensed drug, and it has a very beneficial safety profile. That's why we designed also clinical trials both in amyotrophic lateral sclerosis and also in Parkinson's disease.

Marie: Very interesting, and I know with these potentially disease-modifying therapies, obviously the earlier you can treat the better. But Paul, what do we know so far about the effects of Fasudil when given earlier, perhaps in these prodromal phases versus later after substantial symptoms have manifested?

Paul: At the moment, it's really difficult to say, particularly in the real clinical setting because in most cases, we do not have any options to test Fasudil in a preclinical setting. In amyotrophic lateral sclerosis, actually there is a small window where you can include patients who bear specific mutations in which you know are carrying these mutations, and you can treat these patients already at a time where they do not have any clinical symptoms. For Parkinson's disease, this is not as easy, although there is also trials already ongoing treating patients with REM sleep behavior disorder, for example, and looking at conversion of these patients to alpha-synucleinopathies. But it's not as trivial to get exactly these patients.

So, at the moment, we are focusing with our studies on patients who are already clinically manifest and either have manifest amyotrophic lateral sclerosis or have manifest Parkinson's disease. And the study with ALS has been finished, and it's currently submitted for publication. And the clinical trial on Parkinson's disease is currently ongoing. So, this is something which is still recruiting.

Marie: Absolutely, and I'd love to talk about this clinical trial next. Can you tell us more about the trial, and perhaps give us a status update on where you're at right now?

Paul: Yeah, this trial on Parkinson's disease is called the ROCK-PD trial, and it is a multi-center trial running at 15 trial centers in Germany. And it's basically using data, which we also acquired from the ROCK-ALS trial where we treated ALS patients. But in the ROCK-ALS trial, we could only use Fasudil as IV medication. And as you can imagine, treating patients IV over a longer time is really tedious. It's very difficult for these patients. So, the adherence, of course, to such a trial would not be very high. So, we performed a small bioequivalence trial and could show that you can actually have a conversion rate between IV and oral Fasudil.

And in the ROCK-PD trial, which is currently ongoing, we use now the oral formulation of Fasudil, which made the trial much simpler. And it's recruiting currently. We aim for a number of 75 patients. It's a phase II trial. So, it's not one of these big trials where you recruit 400 or 500 patients. Efficacy is not the primary endpoint. Of course, safety and tolerability are the primary readouts. But we also would like, of course, to assess symptomatic efficacy. And because the trial is only very short, we will not be able to look at disease modification with this trial. But it might give us some indication whether it is well-tolerated, whether it is safe, and whether we can really go on with this drug also for a long-term trial in Parkinson's disease.

We also have relatively broad inclusion criteria. So, we include patients with a Hoehn & Yahr stage 1-3 to this trial. And we just want to have patients to be non-fluctuating. And at the moment, we have recruited more than 30 patients. So,

we're already quite happy that we almost have half of the recruitment. And we hope that other centers will also recruit a little bit more in the next weeks so that we might finish recruitment in the beginning of next year and hopefully also have results of this trial at the end of next year.

Marie: Absolutely. And for this Fasudil, specifically, are there any side effects for this drug that people should keep in mind in terms of thinking about the future applications?

Paul: We have a patient population here where we have excellent symptomatic treatments. And therefore, every medication which you want to apply also as a disease-modifying drug should be very, very safe, of course.

So we're actually quite happy that for Fasudil, when we have this experience from the ROCK-ALS trial, and as you might imagine, ALS patients are, of course, even more severely affected than usually Parkinson's patients in an early stage. So, even in this population, we saw only a very small number of adverse events which were unexpected. And it turned out to be a very safe drug.

And we actually did not see any significant differences between the treatment groups and the placebo group in this ALS trial. And yeah, there are some side effects which you definitely should think of. So Fasudil is a drug which is used for the treatment of vasospasms after subarachnoid hemorrhage. So, it is a vasodilator. And being a vasodilator, it can dilate your vessels in different parts of the body. And it has also been studied for different cardiovascular diseases.

And one of the side effects can be that it lowers blood pressure. One of the side effects which has been only seen in studies with aneurysm bleeding is that it can actually increase aneurysm bleeding. But this has so far not been shown for patients who did not have aneurysm bleeding. But this is still something which we exclude in our patients. So, we do not want to include patients with known intracerebral aneurysms. And so far, also the point of blood pressure lowering, this is something which is definitely of interest for patients with Parkinson's disease. But so far, we did not see a significant effect, at least not with these dosages which we're using. So we're quite confident that even in this population, it will turn out to be a safe drug.

Marie: Absolutely. And I think this dosage question is a big one for Parkinson's disease and obviously any other condition. So, how did you go about determining what dose would make the most sense? And what was the data from the animal models that really helped you determine that in humans?

Paul: So, what we did is we looked at what dosage would be the maximal dosage, which we actually can use in our clinical trial because of the license of the drug.

And we started converting this dosage to the animal dosages and treated our animals with these appropriate dosages.

And there we saw the effects which were beneficial in these mouse models. So, if you take this back, that means that we used the maximal licensed dosage in humans to achieve the effects in the animals. And that's why we think that in humans, if we translate it back to humans, we should use this maximal licensed dosage. Now, we do know that Fasudil has been used outside of this label in other clinical trials at even higher dosages. And at the moment, we cannot really say whether even higher dosages might be even more beneficial. But of course, also higher dosages might also be associated with higher side effects. So, this is something which is potentially not completely resolved yet.

Marie: Very interesting. Is there anything else you'd like to share about this ROCK-PD trial before we move on to the next topic?

Paul: Well, it is definitely something which we put quite high stakes on. And if you're interested or any patients are interested to participate, they can also go to our homepage and look up the trial on our web page. And they can contact us and also participate in this trial if they fit the requirements for this trial.

I would also always say that it is a trial. So, I know that hope is something which is very important for all patients with neurodegeneration. And I would encourage all patients who really want to do something about improving treatment of Parkinson's disease to participate in randomized controlled trials. This also means that every one of these trials is usually a placebo-controlled trial. And I know that this is very difficult for patients because there is always the opportunity to be enrolled in a placebo arm.

But it is the only way for us to find out whether a drug is really working. And this is particularly difficult for drugs which are already available as Fasudil. So, don't go and get this drug. If you want to do something about improving Parkinson's disease in the future, participate in a clinical randomized, very well-controlled trial, this will make certainly the most sense. And this is also the safest way to participate in research.

Marie: I think those are very important points. And I know you have a clinical role as well as we mentioned earlier. You lead the outpatient clinics for PD and you've been involved in studies of symptomatic therapies. So, I'd love to talk about, in particular, a recent study that you published looking at the combination of advanced therapy. So, Paul, can you tell us a little bit more about the study and maybe give us some background if people aren't familiar with the term “advanced therapies”?

Paul: When you treat Parkinson's disease patients, in the beginning, this is something which we as doctors have always quite a good hand with. Because in the beginning, we can treat patients quite well. We have literally something like the honeymoon period where we can give medications, and many of the symptoms disappear or are getting better for quite a long time. But this is, like many honeymoons, not endless. So, there is of course also the period where complications of therapy occur.

And at a certain time, particularly when fluctuations start to occur. And we know that this is due to the further degeneration of the dopaminergic system to the decreased storage capacity of our dopaminergic neurons for dopamine and levodopa. Then we are not very good at treating patients with pulsatile medications. But of course, most of our patients are taking their medications several times a day. And we have patients who develop complications, dyskinesia, but also sudden offs.

And this is very debilitating for the patients, and it's severely affecting their quality of life. So, at least at that stage, but preferably even earlier, we should talk to patients about so-called “advanced therapies”. And I emphasize this because we know that talking about advanced therapies is also difficult, both for doctors, but also for patients. Because most of these therapies are more or less invasive, like deep brain stimulation, but also pump-related therapies.

And nowadays, we have really a very big arsenal of these therapies. So, we have levodopa-carbidopa intestinal gel pumps. We have pump formulation where we have, in addition to levodopa/carbidopa, we have entacapone. We have also apomorphine pumps, which are subcutaneous. And we have very recently as an addition, the foslevodopa, which is also applied subcutaneously. And many more are also in development.

But it's very important to talk to patients about these advanced therapies and to explain why it is important to have a continuous application of these dopaminergic medications over time. So, basically this trial, which you mentioned, it came out of our own experience with one of the first patients who we put on an LCIG pump. And that was back in 2005. And actually this patient was put on this intestinal gel pump because this patient had already previously had deep brain stimulation as one of the very first patients in Germany.

And he came to our outpatient department and said, well, I'm in the end of my fifties, I had deep brain stimulation surgery, but that was a couple of years ago. And now I have the impression nothing helps. And other doctors told me, well, we're sorry, but we cannot do anything for you anymore, because this is the final therapy, which we can do for Parkinson's disease. And he was an engineer, and he was really still very excited about doing his projects. And he definitely didn't

want to give up. So, we discussed it very detailed with him. And we said, well, we can try.

And at that time, it was really something which was very new. We can give you a second advanced therapy. And at that time, it was just the time of licensing of the levodopa-carbidopa intestinal gel therapy. And this patient got a pump, and he lived with this pump for many, many years and had a very good quality of life. And that was basically starting our idea to say, well, there must be more patients who are on these advanced therapies, but where after a certain time, the disease still progresses. And these advanced therapies are not sufficiently helping these patients anymore. And it just cannot be that these patients are given the answer that we cannot do anything anymore for them.

But we have to find solutions to either replace this therapy by another advanced therapy, or maybe even add another advanced therapy. And that was the starting point of the CAT-PD trial, which was mainly performed by a very dedicated assistant in our clinic, Dominik Pürner. And Dominik contacted many centers in Germany. And in the end, 22 centers participated and asked them, do you have these patients who have two or even more combined therapies? And at each of these centers, who all are expert centers and treat many of these patients, only a few of these combination therapy patients were available.

So, we summarized this large number over 100 different cases. And using this joint effort of different centers in Germany, we could really say that, yes, first of all, there are these patients who have more than one advanced therapy. And there are some patients who have different combinations, even two or three, and one patient even had four different combinations of advanced therapies. And it is interesting that if you look at the clinical readout, then the first change of these advanced therapies was almost as efficient as the initial initiation of this advanced therapy. So, a patient who had, for example, deep brain stimulation, if you compare the efficacy of deep brain stimulation in the licensing trials, then the first change from deep brain stimulation to, for example, a duodopa pump was almost as good as the initial installation of this advanced therapy.

And that was really an eye-opener for us, because we, for the first time, had the possibility to more systematically, although it was a retrospective analysis, but to more systematically look at a larger group of these patients. And we could also establish that it is really worth thinking about changing one of these therapies or adding one of these therapies. And because this is, of course, a retrospective analysis, we would like to follow this up and currently are developing a registry for patients with advanced Parkinson's disease. And we hope that patients in these advanced stages can then contribute and add data to this registry so we can learn more about the trajectories of these patients when they reach these advanced stages, and also tell a little bit more with more substance on which

therapies are useful for these patients, who should get which therapy, and also when could be the best time point to switch to these therapies.

Marie: Definitely. I think being able to develop the sort of decision tree for the advanced stages of Parkinson's disease would be tremendously beneficial, because I think you hate to just play trial and error like, well, we could try this, but actually having that data that suggests like, okay, if we do this at this point, because you have this already advanced therapy, we can expect these benefits, that is a huge impact for patients.

Paul: Yeah, we think so too. And we hope that we will have enough funding and then get all these centers on board to actually provide this prospective data, which is less biased than a retrospective analysis.

Marie: Very interesting. And you mentioned that for many of these centers, they don't have many of these people who might have tried multiple therapies. So, how do you anticipate that you'll be able to get the volume of data needed to really be able to make a dent in this?

Paul: This is a crucial question. So, we have already basically a very nice data set with the patients who are included in CAT-PD. And of course, as the advanced therapies are evolving, and I just mentioned that even in the last three, four years, we had two more therapies adding to the field. So, there will be more patients actually on treatment. Patients are also getting older, and the awareness for these therapies is also increasing.

So, we can start from what we have in this CAT-PD, but we also anticipate, of course, that centers who are participating will add all of their patients who are on advanced therapies into this registry. And then eventually, you will also pick up patients who will have a second advanced therapy. So, of course, you will need to follow these patients up for a longer time. But we want to start with a core data set of patients who already have these double or even triple therapies.

Marie: Well, that makes sense. I think, Paul, this is a really important area of research. And I think these large data sets are becoming increasingly valuable as we've gotten to the point where we can start to answer some of these bigger questions. So, for you, when you think about whether there's tools or resources or different advances or even particular data sets in neuroscience or other fields, are there particular things that come to your mind that are really having a big impact on advancing or could potentially accelerate Parkinson's research?

Paul: The most important thing is to get data, which is as unbiased as possible. And to get this unbiased data, you really need to have a lot of data. So, I think one of the most important things is, in all types of research, but particularly in research for

such a heterogeneous disease like Parkinson's disease is, big cohorts, is big collections of biomaterial data banks. And therefore, I think all initiatives who are trying to provide these resources to researchers are really important. One possibility is, for example, the PPMI cohort of The Michael J. Fox Foundation.

But also other tools which can be used in addition and can be jointly used by researchers and provided to researchers, for example, like the research tools catalog of The Michael J. Fox Foundation, but also many other collaborative research facilities. For example, in Germany, we have German Centers for Neurodegenerative Diseases. They have a cohort, which is called the Describe cohort, which is a cohort which collects patients with different neurodegenerative disorders, but also Parkinson's disease.

And the purpose of such cohorts is to get large numbers of patients with large data sets with very nicely characterized biomaterials so that researchers can use this jointly together. I think this interactive and collaborative research is very important. And therefore, we also participate, of course, not only in our own home-brewed research in our lab, but also, of course, in local, national, and also international consortia. And of course, the other thing which I think, allow me to be a little bit biased, but we have an interest in translational trials. So, I think really important as a tool or a resource are any type of programs which focus really on translation.

I think it is very important to do basic research. This is our foundation. But in order to translate this, we also need specific tools. And I think we are all, as doctors or as researchers, not sufficiently trained in using these translational tools. And there are some programs which are focusing particularly on translating basic research knowledge to the bedside and where it's not only something which you use as a slogan to describe your program.

So, it's really very important to go back to the researcher and say, okay, I mean, you discovered this now. So, what actually do you need to go to the patient? What are the resources which you are lacking to really apply this for patients? Why is maybe one of your discoveries interesting, but it will never make it to the patient?

And I hope also that there will be funding for investigator-initiated trials because from our own experience, of course, industry is a very important partner. If you want to perform clinical trials, we will never be able to do it without strong industrial partners. But industry is also very focused on specific targets. And there might also be targets which can be very interesting for the disease. And the first one to actually look at these targets might be individual researchers before this really becomes something important to a larger pharmaceutical company. So, I would encourage any funding into investigator-initiated trials.

Marie: Well, Paul, I think you brought up some really important points there. And I'd love to touch on next just what you see as some of the most important future directions or perhaps unanswered questions in Parkinson's disease research today.

Paul: I think it's probably different for every researcher. But I think that one of the questions which we're all driven by at the moment is what the true culprit is in Parkinson's disease. And some of the things we talked about, alpha-synuclein, potentially also metals, potentially also neuroinflammation. But we have a few trials now which have been ongoing and finished with antibodies against alpha-synuclein. And so far, these trials have not been particularly promising.

And I think it is always good and valid to step back, and pause, and think about whether your target is actually the target you want to target. I think there is no doubt from the overwhelming evidence that alpha-synuclein is a very important step in Parkinson's disease and alpha-synuclein aggregation. But one should probably question if alpha-synuclein is the or the only target which we should target with our drugs.

So, I think this is probably, for me, one of the most interesting questions. And what are the steps before alpha-synuclein aggregation? Of course, also the question of a biomarker, which is telling us something about disease progression would be very important. There are other disorders like ALS, for example, where we have biomarkers like neurofilaments, which are even used as biomarkers for the efficacy of drugs. So, this is something which we're still lacking in Parkinson's disease.

Marie: Well, that makes sense. I think these big areas of target identification and biomarkers and making sure that you are using the best possible model are huge areas of opportunity in Parkinson's research. And I know we've covered a lot of ground in our conversation today, but Paul, I'd love to end by just talking a little bit about how your work specifically is bringing us closer to these big goals of finding a cure for Parkinson's or contributing to these improved therapies for people who have Parkinson's today.

Paul: I will be very modest. I don't know if really our work is contributing to that, we will see. But I hope that at least our work on biomarker research could help us a little bit more to understand whether we can quantify disease progression in Parkinson's disease. And by that, maybe also contribute to a biomarker, which can be used in clinical trials. And of course, I have very high hopes that one of the drugs which we're studying, for example, Fasudil could turn out to be a true disease-modifying drug. So yeah, I think this is maybe small steps which we can contribute to Parkinson's disease research.

Marie: Well, Paul, we appreciate all of the work that you're doing in the field of Parkinson's research. And it's been a pleasure to chat with you today about yourself and your work. So, thank you so much for joining us on the show.

Paul: Thank you so much for having me.

Marie: Well Paul, thanks again. And listeners. It's been great to have you here with us as well. If you want to know how The Michael J. Fox Foundation can help your research, please visit MichaelJFox.org/researchresources. And you can find new episodes of this show each month on the MJFF website or on your favorite podcast platform. When you have a moment, please subscribe to our show to make sure you don't miss our outstanding lineup of upcoming episodes. We look forward to connecting with you again in our next episode of *The Parkinson's Research Podcast*.