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 that we share will advance your efforts and partnerships to improve brain health. We're welcoming guests with a range of experiences and viewpoints. The views expressed belong to the guests themselves.

And today we are thrilled to be welcoming our guest, Dr. Birgitt Schüle. Listeners, Birgitt is an Associate Professor in the Department of Pathology at Stanford University School of Medicine and Co-director of the Alzheimer's Disease Research Center, Neuropathology, and Induced Pluripotent Stem Cell (iPSC) Core. Today, we're going to talk more about her work developing alpha-synuclein cell lines and some considerations surrounding open science in the field. So, Birgitt, welcome to the show, and thank you so much for joining me.

Birgitt: Well, thanks for inviting me. I'm really so happy to be here today with you, Marie.

Marie: Well, we are excited to get to know more about you and your work. And perhaps to help our listeners get to know you a little bit better, Birgitt, can you tell us a little bit more about your career path and your academic and medical background?

Birgitt: Well, absolutely. Thanks for that opportunity, Marie. I grew up in a small town in the middle of Germany. And as early as in my sophomore year in high school, I knew I wanted to pursue a career in medicine. I was also fascinated by science documentaries, which really fueled my excitement for science and research. So, I went on to study medicine at the University of Göttingen in Germany, which is less than 40 miles away from my hometown. And I then completed my clinical training at the Medical University of Lübeck by the Baltic Sea.

In Germany, in order to earn the title of Doctor of Medicine, you have to conduct a research project, write and defend a medical thesis, all while you're juggling your clinical training. So, I started my medical thesis as early as in the fifth semester of studying medicine, and I was in the Department of Physiology, focusing on a calcium channel in the olfactory bulb of frog tadpoles using patch clamping. This is a very challenging technique, and it lets you study ion channels themselves.

So, during my time in the lab under my mentor, Dr. Detlev Schild, a physician and physicist, which was great. I felt like a real scientist. So, experimenting and

testing your ideas really grew my passion for research, although I felt Dr. Schild had new ideas every other week, which could become also very overwhelming. So, I think that was really a turning point for me when I realized I was more drawn towards research than practicing medicine full time. And as if studying medicine and working on my thesis wasn't enough, I wrapped up my last year of medical school and clinical training while welcoming my first son. So, here you have women in science and in their professional career. So, that's hard to juggle both — family and career.

So then during my clinical training in Lubeck, I had the incredible opportunity to spend a year in the lab under the mentorship of Dr. Christine Klein. She's one of my role models for women in science. And you might remember her from the earlier episode. You interviewed her as well in this podcast. So, right from my first week in her lab, Christine encouraged me to start planning a post-doctoral fellowship in the U.S. At that time, I thought that was a little early, but she was absolutely right. Finding the right lab and the right mentor really takes time and effort.

So, her lab at that time was small, but really buzzing with excitement. And we screened for Parkin gene copy number variants in patients and tumor samples, establishing that copy number variants make up about 50% of mutations in the Parkin gene, which is quite important for neurogenetics. And it's in another project we described, for the first time, a loss of an epigenetic imprinting mechanism that leads to myoclonus-dystonia or DYT11 syndrome. So then, in terms of my post-doctoral career, in the summer of 2002, I toured five labs in the U.S. for post-doc interviews.

And people in the field might know these folks, Michael Schlossmacher and Xandra Breakefield's lab at Harvard, Stefan Pulst at UCLA at that time, the late Tony Fink's lab at UC Santa Cruz, and then Uta Francke's lab at Stanford. And my family and I were camping along the way, so it was a bit surreal to step out of a tent, change into a dress, grab my laptop, and then head off to an interview.

Marie: Oh, wow.

Birgitt: But it was a really cool and exciting trip. And both also my husband and I ended up landing post-doc positions at Stanford. So then getting there. So, after wrapping up clinical responsibilities in Lübeck, and I also secured a German research fellowship, I made it to the U.S. half a year later in January 2003 to start my post-doctoral position at Stanford in the Department of Genetics. And I worked in Uta Francke's lab. She is a really prominent clinical geneticist who has become my second role model and I'd call it "sister in science". My research in her lab focused on your developmental syndromes, Rett syndrome, Prader-Willi syndrome, and Roberts syndrome.

And what I also did in parallel, I went through a subspecialty training to become a board-certified clinical molecular geneticist. I wanted to stay connected to the clinical side of things and honestly, I wanted to have a backup plan in case things didn't work out. And not to mention, I had my second son during all of this too, so looking back sometimes, I think, "Oh my goodness, how did I manage that?" But I have to say, I couldn't have done this without a supporting partner and husband by my side.

So then, the next stage was the Parkinson's Institute. So from 2005 to 2019, I spent at the Parkinson's Institute and Clinical Center. It all started in late 2005 when I came across an ad for a staff position in genetics there. And for those who might not be familiar, the Parkinson's Institute was founded in the late 1980s by Dr. Bill Langston, who is the scientist who first identified that MPTP causes Parkinsonism. And he was also one of the founding scientific advisors to The Michael J. Fox Foundation. So, the vision of the Parkinson's Institute was to combine research and clinical care under one roof, fostering close collaborations between scientists, clinicians, and also patients, making translational science and clinical research a reality. And I have to say, it really worked at the Parkinson's Institute.

At the Institute, I led three research programs. The first one was a genetics and biomarker program that enrolled over 200 families with Parkinson's disease and 1,500 patients and healthy controls, with the goal to include every patient from our clinical center. In 2009, I initiated one of the first induced pluripotent stem cell programs for Parkinson's disease at that time, funded by the California Institute for Regenerative Medicine or CIRM.

And this program eventually developed over 100 cell lines from patients with genetic forms of Parkinson's disease. I also served as the Brain Bank director from 2015 to 2019. I was overseeing and conducting brain autopsies for the Institute's Brain Bank, which had over 350 brain specimens. So, as you can see, the Parkinson's Institute was a very special place. It was really full of people who were all in to make a difference for those living with Parkinson's. From the warm greetings of the late Lori Goldstein at the front desk to really the dedication of all the medical, research, administrative, and finance team. Everyone really played a crucial role.

And I really feel so lucky that I worked with some amazing scientists like Carlie Tanner, Sam Goldman, Dino Di Monte, Maryka Quik, Amy Manning-Bog, and Jeremy Nichols. However, being a small nonprofit right in the middle of Silicon Valley with Stanford just a few miles away as competition, it was really tough to keep both research and clinical care going from a financial standpoint. And the Institute had to close its doors on August 1st, 2020.

During this challenging time, there were some decisions made at the top regarding monetization of research resources like cell lines, tissues, and other valuable assets that had been created through taxpayer-funded research and generous donations. And instead of embracing open science principles and sharing these resources to advance research, as was actually mandated by the research contracts, there was a shift toward trading these assets with third parties instead of making them widely available to researchers and the scientific community.

So, leaving the Parkinson's Institute in January 2019 after almost 13 years was really bittersweet for me. I had experienced both the highs and lows of a nonprofit organization and saw firsthand how a lack of resources and funding can challenge ethical integrity, honesty, and transparency. And due to these circumstances, I was unable to access these cell lines in my lab that we derived over a decade. But I have to say thanks to the support of The Michael J. Fox Foundation and a personal lawyer, we navigated the obstacles that prevented those resources from being shared with the scientific community.

Almost four years of legal back and forth was needed, but then the stem cell lines were rescued and finally arrived in my lab at Stanford in the spring of 2023. That was a huge victory for me, and the time, money, and hours we spent strategizing, drafting letters was worth it. I went through this for the science and also to honor the promise I made to every participant who we consented to donate their skin samples for stem cell research. Their contributions would be used and shared to advance science and help in Parkinson's disease. So, you can tell, Marie, it's very emotional for me.

Marie: It's an emotional thing. Yeah, absolutely.

Birgitt: But I have to say fighting for those lines wasn't always easy, and I had amazing supporters in my lab members who believed in me and understood why this battle was so important for me. It was a challenging time with moments of doubt, exhaustion, and even shame. But at all times I felt I was doing the right thing.

Although many, many of those cryoboxes of cells were unfortunately destroyed, the good news is that cells in culture can divide and multiply, so we can now expand and grow many of these lines again. Thanks to the support of The Michael J. Fox Foundation, these lines are now being made available to the scientific communities in the true spirit of open science.

So, we've already deposited several lines with the American Tissue and Cell Collection or ATCC, and we'll dive into that in more detail shortly. We're also collaborating with other repositories like Wi-Cell on additional lines. Plus, we've

banked other IPS lines with the NIH at the National Human Cell Repository and the National Centralized Repository for Alzheimer's Disease and Related Dementias. So far, we've deposited and banked over 60 lines with alpha-synuclein, GBA, LRRK2, ApoE variants, and also corresponding control lines. And we're not stopping here. We plan to share more of these genetic lines, along with isogenic matched controls, over the next two years.

Marie: Well, Birgitt, I think you've had a remarkable journey over the years. And you've definitely shown persistence and perseverance and have had to overcome some enormous challenges. And you mentioned open science, and I think this is such an important topic to discuss. So, for you, Berghitte, why do you believe open science and sharing scientific resources are so crucial?

Birgitt: I think open science and sharing of these patient-derived stem cell resources are essential for a number of reasons. So, first is the high cost and time associated with generating research tools such as IPS lines that make resource sharing economically critical. For instance, it costs between $10,000 and $15,000 to generate, characterize, and bank a single cell line. And then you have an additional $15,000 to $20,000 required to create genetically-engineered isogenic lines for a mutation for a donor.

And in addition, the process also is very time-consuming. So, it takes between six and nine months for the derivation of an IPS line and four to six months for the gene editing. And second, sharing resources enhances reproducibility. So, ensuring that the research findings can be consistently validated across different laboratories makes it so. Finally, it advances yield by providing researchers with a better understanding of the characteristics of available research tools, ultimately accelerating progress in scientific discovery. Open science is so important and I'm glad that we researchers are beginning to understand that working in silos and being cagey is not the future of science. I'm grateful that The Michael J. Fox Foundation is endorsing this and encouraging these open science principles.

Marie: Definitely. And I really like the point that you made about accelerating scientific discovery. So Birgitt, can you share your thoughts in more detail on how open science can impact the speed of progress in science?

Birgitt: I believe that open science can really help speed up scientific progress. I also experienced for many years the silos in which science is happening. Working in silos limits the potential for collaboration, innovation, and learning. It decreases morale and leads to scientific burnout.

But let me break down how this works. So first off, open science encourages collaboration across different disciplines, and researchers can build on each other's work more effectively, in my opinion. When research is conducted out in

the open, it's easier for other scientists, maybe with diverse perspectives, to replicate studies, verify results, and build on them. And this helps to identify and correct any potential errors.

And then there's the fact that open science practices like publishing in open access journals or on pre-print servers get research finding out to the global scientific community and the public much faster. And this helps new knowledge get applied sooner. But of course, the speed of scientific progress also depends on other factors like funding, infrastructure, the complexity of research, and the need for thorough testing, and, of course, peer review. But open science is powerful and can really accelerate progress. But it's one piece of the puzzle in advancing science.

Marie: I agree. I think open science has many advantages. But what do you see as the drawbacks, if any, of open science?

Birgitt: Like anything, open science has also its challenges, but a lot of it depends on how the scientific community values and embraces it. So for example, open science often encourages sharing of research readings early, like through pre-print servers. And this can create some pressure to publish quickly, sometimes before the research has been peer-reviewed. And this might lead to sharing incomplete and less robust results. Another concern is that sharing data, methods, and findings openly might lead to someone else using your ideas to publish similar work first. However, I think with the rise of pre-prints, it's becoming much easier to spot when someone's copying your idea. And really, that's frankly quite embarrassing.

Marie: Right.

Birgitt: So, there's also the issue of credit and recognition. So, in traditional academic settings, credit often comes from publishing in high-impact journals, and open science emphasizes collaboration/sharing, which might not always align on how researchers are evaluated, unfortunately. But I hope that's changing.

And then it's clear that the culture of open science is still evolving. Not all institutions, senior researchers are fully on board yet. So, we might face some resistance from mentors or peers who are more used to the traditional research practices. So although open science is gaining traction, not all funding bodies or institutions provide the necessary support for the extra work involved, like making data sets available on databases or covering open access publication fees.

But on the bright side, it's changing. And I'm really thankful that The Michael J. Fox Foundation now covers open access publication fees. I think the field is slowly but surely changing. And I remember when I started my postdoc in 2003,

the public library of science launched. And I felt that was the start of something that is going to have a big impact. And I think that's what we're seeing now 20 years later.

Marie: Definitely. I think seeing these shifts in the field is really encouraging. And we've talked a little bit about how open science works and some of the pros and some of the cons. But, Birgitt, let's talk next a little bit more about these cell lines that you deposited with the American Tissue and Cell Collection, or ATCC, that was supported by MJFF funding. So, can you tell us more about these cell lines?

Birgitt: So, the idea for these IPS lines actually goes back to 2012. Back then, we created the first zinc finger edited lines for the most common LRRK2 mutation, the G2019S. And that got me thinking, what if we also generated a gene dosage model for alpha-synuclein? However, that was a little early to propose, as the reviewers didn't really believe it was feasible to generate these cell lines.

But a couple years later in 2015, we tried again, and we received funding from MJFF to start developing this model. So, our goal was to create a model with varying copies of alpha-synuclein to answer the critical question, how much alpha-synuclein is good for you? Since alpha-synuclein is a key therapeutic target in Parkinson's, understanding its optimal levels could really significantly impact the development of treatments, whether through gene targeting, antisense oligos, small molecules, or immunotherapies.

Marie: Birgitt, that makes sense. And can you describe a little bit what were the steps for characterizing these cell lines before depositing them at ATCC?

Birgitt: We started with a cell line derived from a donor with an SNCA genomic triplication. We then used CRISPR editing to engineer these cells to harbor alpha-synuclein frameshift mutations in the first coding axon of the alpha-synuclein gene, creating functional knockouts for the alpha-synuclein protein. So, when we selected clones that underwent CRISPR editing, we also selected clones that did not incorporate the edit. So, this ensures that all cell lines that we have in this panel will be at the same passage number and have experienced the same subcloning process in culture.

So, I like to compare these unedited clones to a saline or a vehicle control in other types of experiments. So, these IPS cells have been highly characterized in our lab and then authenticated by ATCC. Before we deposited these lines, we performed extensive characterization according to NIH guidelines, such as morphology, pluripotency testing, and more. And we developed a special genotyping assay to detect these frameshift mutations and also used bionano optical genome mapping to ensure the genetic integrity of the cell lines.

The lines, as I said, were derived from a patient who was a descendant of the Iowa Kindred, a multi-generational family from Iowa. And this family has been followed at the Mayo Clinic since the 1920s due to their inherited very severe form of autosomal dominant Parkinson's disease. Members of this family with roots in both English and German heritage typically experience a very unusual early onset of disease with symptoms starting as early as the age of 34. And disease progression is pretty rapidly with patients often presenting dementia and dying within two to 12 years after symptoms first appear. It's very, very early.

In 2003, Singleton and collaborators identified the genetic cause of the Iowa Kindred, which is the triplication of the alpha-synuclein genomic locus. And this triplication spans 1.7 megabases, including seventeen genes, including alpha-synuclein. And the triplication is resulting in a two-fold increase of alpha-synuclein protein in many tissues of the human body.

When you're looking at post-mortem analysis of affected individuals, it reveals a significant degeneration of cells in the substantia nigra, extensive Lewy Body resins in both subcortical and cortical regions, cortical vacuolation, nerve cell loss, and gliosis in the hippocampus. So, the pathology can even be more severe than the most pronounced cases of dementia with Lewy bodies.

So, this discovery underscores the critical role that elevated expression of wild-type of alpha-synuclein can play in triggering the neurodegenerative condition, which is remarkably similar to idiopathic Parkinson's disease. Bill Langston even described these cases as "Parkinson's on steroids" due to the rapid progression and severity of neuropathological changes. So, these IPSTs were then differentiated into neurons and showed alterations in oxidative stress, mitochondrial health, and even in the process of neuronal differentiation when we compared them to control cultures from an unaffected sibling.

Marie: Well, I think these results are really interesting. Can you maybe go into some of the detail on what are some of the key characteristics, perhaps, or the benefits of these cell lines?

Birgitt: We successfully generated and characterized multiple clones, each harboring distinct frameshift mutations within the alpha-synuclein gene. So, these human CRISPR-engineered isogenic IPS lines, in which alpha-synuclein is now titrated from four functional copies to zero functional copies, allow researchers to test the varying protein levels of alpha-synuclein using cellular biology, -omics approaches, also the establishment of screening assays, which then could facilitate drug development. So, I think the system enables the exploration of various ways from molecular pathways to potential therapeutic interventions. And to ensure rigor and reproducibilities, we deliberately created multiple clones for each SNCA gene dosage variant, each carrying, as I said earlier, distinct

frameshift mutations. So, this strategic approach provides great flexibility for the experimenter when planning and conducting experiments.

Marie: Well, that makes sense. So, Birgitt, can you talk next about how these cell lines then might be used by other researchers in the field?

Birgitt: The accumulation of alpha-synuclein protein is a key event in Parkinson's disease, leading to formation of Lewy bodies, neural impairment, and the death of dopaminergic neurons, ultimately. So, targeting alpha-synuclein to modify the disease progression has become a prime focus for developing new therapies. And there's some consensus in the field that reducing alpha-synuclein or eliminating its toxic forms could be crucial in slowing, reversing, or even preventing Parkinson's disease or other related alpha-synucleinopathies.

However, the optimal level of synuclein reduction for neural protection remains uncertain. And I believe these cell lines can be used as a great in vitro tool to model alpha-synuclein pathology in the dish and understanding what level is good for you and what level we should be reducing.

Marie: Definitely. And I think in science, things that sound very straightforward in hindsight often aren't. And I think a years-long process can sometimes end up distilled into just half a sentence in the method section of a paper. So, let's talk perhaps a little bit more about the process, Birgit. What was this process actually like for you, working with ATCC and MJFF to make these cell lines more widely available for everybody?

Birgitt: I have to say the process to bank these lines once we had them was easy and smooth, and it involved some stages. So first, we had to clarify the use and distribution rights, which included navigating ethical consent forms, patent protection, obtaining various institutional approvals that probably took the longest time. But without these, ATCC couldn't have shared those cell lines with other researchers. But once that was sorted out, we provided lab protocols and sent the cell lines to ATCC.

And ATCC performed their detailed authentication tests, purity of lines, really making sure that they’re not contaminated with other non-human cells that you can often find. And they had really some specialized IPSC characterization tests, such as the PluriTest assay, flow cytometry. So, they're very, very thorough making sure these lines will be really good once they're made available. So, ATCC really has a rigorous quality control system in place to make sure these are the true cells we say they are, and they're pure. And I can really just highly recommend obtaining these cell lines from the ATCC for your research.

Marie: Definitely. I think these quality control steps are critical. Like you said, if you're distributing these to people all over the world, making sure they know what they're getting and the purity is not in question. I think it's not easy to get to this point. So, Birgitt, what were some of the challenges that you faced in developing these cell lines that ultimately passed all the tests and checked all the boxes for ATCC?

Birgitt: Besides the institutional and legal obstacles in the background that I mentioned before, overall working with ATCC was a great experience. We had regular communication to address questions and keep things on track. And I'm also very thankful to MJFF to sponsor banking these lines. And now we have 14 of these CRISPR clones available with various functional copies of both synuclein, and I'm really proud of that.

One challenge is that ATCC currently doesn't ship these cell lines internationally. So, interested parties can contact me directly, and we're providing them directly from my lab under a Universal MTA (Material Transfer Agreement) to interested parties outside the US. But I hope this bottleneck will be resolved soon.

Marie: Well, Birgitt, I'm really glad that you found a way to at least work around it for now to share these cell lines internationally. You explained some of the important benefits of this particular model, but what are some of the limitations?

Birgitt: As we often quote, “Every model is wrong, but some are useful.” This gene dosage model is excellent for studying the effects of alpha-synuclein gene dosage as if it were a germline mutation present at birth. The model is also interesting in determining the effect of deletions of the alpha-synuclein gene. So, really thinking about gene dosage and what level is good for you.

So, for example, we've identified cases of deletions of the alpha-synuclein locus, particularly in children with intellectual disabilities and autism spectrum disorder. And genetically large mutations and multiplications are caused by non-allelic homologous recombination. But the impact of losing alpha-synuclein at the cellular level in human neurons is still unclear. And I think this is a great model to use this for. So, with the SNCA CRISPR model, one can, for example, compare the effect of an alpha-synuclein deletion in vitro and define the effects of the loss of the protein at the cellular level.

But you asked about limitations. So, one aspect we cannot evaluate with this CRISPR model is the acute therapeutic reduction of alpha-synuclein in mature cells. So, in neurons or non-neuronal cells in the CNS. And that's something that you would do with a therapeutic approach in patients. For that, it would be great to have an inducible functional CRISPR inhibition model where you can, at any time point you wish, down-regulate levels of alpha-synuclein and not have it from

the get-go. But we are working on such a model, and we hope to have something like that soon.

Marie: Well, we'll have to stay tuned, Birgitt. I think that's really exciting. And I really appreciate you sharing more about your work with us today. And as we wrap up our conversation, can you describe how your work as a whole is bringing us closer to a cure or better treatments for people with Parkinson's disease?

Birgitt: Well, that's a big question, Marie. So I mean, we all hope to make a meaningful impact with the work we're doing. Becoming a stem cell scientist, or maybe I call myself “IPS modeler”, really wasn't my original career plan. I initially saw myself as a geneticist given my training background, but my journey into human IPS reprogramming began in 2009 when NIH reviewers pointed out that my application lacked an authentic model of Parkinson's disease.

Around the same time, Yamanaka's groundbreaking work on nuclear reprogramming was making headlines. And, at that time, somewhat naively, I thought I could incorporate human IPS modeling in my revision. But it has taken a bit more time and effort and many, many groups across multiple disciplines to develop these models and bring them to fruition. And I think there's still more to do.

However, all work in developing IPS models, I think, represents a step forward for modeling Parkinson's disease on a human background, which could present a predictive translational model. And that's what I hope will bring us closer to better treatments for the disease. So, by creating highly characterized and validated IPS resources, we're establishing a foundation that allows other scientists to bypass this time-consuming process of reprogramming, characterization, CRISPR-engineering to get isogenic lines. And I think this can save labs months, if not years of work, enabling them to really focus on the biological questions that are important and the techniques they excel on, diving really straight into disease mechanisms and testing treatments for Parkinson's disease. I think that's our impact.

Marie: Birgitt, I think you were doing remarkable work. And I love this story you shared. I think in some cases, that reviewer number two that we always complain about — they can sometimes give you just that push that you need to explore a new area to open up a new line of research. And it sounds like that was the case here. But I think there are still so many unanswered questions in the field of Parkinson's. And I know your lab, Birgitt, is working on multiple different projects beyond just these cell lines. So, can you tell us briefly just about some of the future directions for your research or areas of opportunity that you're particularly excited about?

Birgitt: As you said, I primarily focused today on the human IPS resources we're developing. And I wanted to make it a big point that these models are essential for studying disease mechanisms. And as you've heard, there's still a lot more to uncover about alpha-synuclein, both in health and disease.

But my lab is also working on a gene therapy targeting alpha-synuclein funded, by MJFF. So, we began testing this approach in our human stem cell models and have since moved on to evaluating one of the lead vectors in large preclinical models. So if you're interested, we could definitely explore that more in the future.

Marie: Absolutely. Well, Birgitt, thank you so much for your time today. We really appreciate you sharing your insights and expertise with all of us today.

Birgitt: Thank you. It was my pleasure to be here today and to share my story and my research. Thank you, Marie.

Marie: Well, thanks again, and listeners. It was wonderful 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.*