

**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 so excited to welcome our guest Dr. Kalpana Merchant.

Listeners, Kalpana is President and Chief Scientific Officer at TransThera Consulting, which provides guidance on drug discovery and translational strategies to biopharma startup companies. She is also an Adjunct Professor at Northwestern University's Feinberg School of Medicine, a member of the Oregon Innovation Council, a Scientific Advisor at The Michael J. Fox Foundation, and she serves on advisory panels at the National Institutes of Health.

And today, Kalpana, we're excited to talk more about your work in drug discovery and developing therapies for Parkinson's disease and other neurodegenerative conditions. So, welcome to the show today. How are you?

**Kalpana:** I'm doing really well. Thank you. It's always good on a Monday to start out a day like this.

**Marie:** Well, we are thrilled to chat with you, and I'm looking forward to learning more about you and the amazing work that you've been doing. But can you first tell us a little bit more about your background and how you found your way to your current positions?

**Kalpana:** So, I'm a neuropharmacologist/neurobiologist by training. And let me start backwards. Currently, I'm working with a number of small startup companies as an advisor or on their board in order to help them guide drug discovery and development, much of it on neurodegeneration diseases, including Parkinson's disease. And simultaneously, my other big hat that I wear is an advisory role to The Michael J. Fox Foundation.

Between the two, I'm kind of full-time busy in my retirement, and the retirement is from big pharma. So, I had the opportunity to retire from Eli Lilly and Company, where I was the Chief Scientific Officer for Translational Neurosciences or Tailored Therapeutics back in 2014. So, in March, it'll be exactly 10 years since I left Eli Lilly and Company. And prior to that, I was for about 10 years plus, I was

at a company that started out as the Upjohn Company that became Pharmacia, Pharmacia & Upjohn, and then ultimately, Pfizer, where I primarily did neuroscience drug discovery, including on Parkinson's disease, but not solely on that.

And that transition to the industry, which started out, as I mentioned with the Upjohn Company, was from academia. So, I was at University of Washington as a faculty in a clinical department, Department of Psychiatry. And the transition actually was quite accidental. I wasn't looking for a role in the industry, but was at a conference and ran into someone after my talk who had just left the Upjohn Company. And based on the topic of my talk, he wondered if I would be interested in considering to join the Upjohn Company, where he had just left and his position was still open. So, it was quite by accident. And I ended up interviewing with them. And then the rest is history. I joined them shortly thereafter. And as I said, I was there for 10 years.

The only reason I ended up at Eli Lilly was that the campus I was at was closed down by Pfizer as a way of restructuring. And that's when I landed at Lilly, joined the Neuroscience Department. So, it closes the circle of my last, well, since 1993 that I've been in the industry until now. So, it's been a different path than what many people do as an intentional career-setting path. And I have to say, I don't think I would change anything. Looking back through the history, I would still perhaps do the same things that I've done.

**Marie:** Well Kalpana, I love it. And I'm very curious to hear where your connection — where The Michael J. Fox Foundation fits into your story. So, can you tell us how did you originally get connected with MJFF? And what has your experience been like? As we mentioned in our introduction, you are one of the Scientific Advisors for the organization.

**Kalpana:** As you know, The Fox Foundation was launched back in 2010 with the clear intent of curing Parkinson's disease. And at the time, the goal was to cure it within 10 years.

Of course, this is now 2024. But because of their intended goal, The Fox Foundation decided it was better to engage the industry (pharmaceutical industry) from the get-go, because ultimately they wanted to cure the disease through medications. So, it was a couple years after their launch that they brought together executives from various companies in the large pharma industry, basically to ask them, what are the major obstacles to drug discovery and development, specifically related to disease-modifying therapies, which would slow or prevent the disease. And what they heard very clearly from the executives, which is still true, is that finding the right target for a given drug,

which is connected to how the disease originates, is one of the most important steps.

Of course, if you don't target your drug at that target that's connected to disease biology, no matter how good the drug properties are, you're still likely to fail in terms of inducing greater clinical benefits or sufficient clinical benefits in patients, be that Parkinson's patients or any other disease. So, that was one clear message they gave us — that The Fox Foundation, if they could de-risk drug development by ensuring that the right kind of translational science was being done in order to pick the right target that the industry could work on. And much of that work happens, even today, in the academia. That kind of basic research that's required to connect the target to disease biology happens or initiates in the academia, which is what The Fox Foundation was initially geared towards in terms of their funding priorities.

The second message they heard was having the right kind of tools and models, animal models included, was also critical in order to then progress this target and drug through the right kind of model systems to find something that was human-worthy, something that looks and smells like a drug. So, at the end of that meeting, when the Vice President of Neuroscience at the time at Eli Lilly, who was Gerald Shepp, came back, and he was kind of filling me on the gist of the meeting and a couple months later, The Fox Foundation wanted to launch a workshop on animal models of Parkinson's disease. How do they make them more translational? What can they do in order to improve the predictive validity of the animal models?

And that's when my VP asked me if I would be interested in going to that workshop as a participant. So, that was the beginning, I would say, back in 2012 or so, that I attended one of their first workshops. And that was the start of my interactions with The Fox Foundation. And I really enjoyed it and hope I continue to work with them on the various initiatives.

**Marie:** Oh, very interesting. And Kalpana, as we describe your background, you have worked now for 20 plus years in drug discovery and development, particularly in this area of neurotherapeutics. And you've actually been able to see the process all the way from the perspective of really small biopharmaceutical companies through to these large companies, we mentioned specifically Eli Lilly. So, how has this process of drug discovery and development really changed over the past 20 years or so from your perspective and your experiences?

**Kalpana:** Someday, I'm going to write a book on this when I really, really retire. The reason is I've learned a lot. As you said, 22 plus years in big pharmaceutical companies. And then for the past 10 years, I've worked largely with small startups. But

compared to when I first started back in 1993 in pharma, the one thing that's changed the most is how the pre-competitive space is defined.

So, let me dig a bit deeper into that. By definition, pharmaceutical companies are for-profit companies. And what they needed to decide was — they could compete in order to make the best profits for the shareholder value. But there were spaces where they quickly realized that the hurdles, or obstacles, or risks of drug development were too large to be undertaken by a single company to de-risk them. So when I first started, the realization was more around safety data around drugs. How do you make sure that we are using measures and models that don't put patients at undue risk in early stage trials? Because until we get to the humans, one can never de-risk the safety part of it, no matter how much you study it in cells and animals. So, consortia had already started between pharmaceutical companies, regulators, academic scientists in order to de-risk safety.

What happened since then, I would say in the last 15 plus years, is the realization that many of the drugs have not produced the kind of meaningful benefit we would want to see in the clinic. It's because we are not working on the right disease biology. And the reason is almost every disease of interest to us is syndromic. Right? It's defined by the clinical symptoms, but the causes of the disease, the underlying biology, is distinct across different patients. Let's just take Parkinson's as a concrete example.

We define them on the basis of their movement disorder. That's how neurologists would diagnose a Parkinson's patient. But how patient A gets to that stage of showing the movement disorder versus patient B versus patient C, we know today are very different underlying biologies and not just genetic causes, but also the cell biology that underlies that is distinct.

So, how do you go from a syndrome that's called Parkinson's disease to a disease state, which is biologically-defined? So, then you can say patient A would benefit with drug A. Patient B would benefit with drug B, but not vice versa, in order to see the maximal response. And this can only happen when we really understand the disease biology, the mechanisms that cause the disease or cause the progression of the disease, which end up in the clinical symptoms that we measure.

So, I come back to this realization among the pharma companies that in order to really understand the disease, we need to start with human data and not cell or animal model data. And in order to do that the right way, you need hundreds of thousands of data points. Study many, many more patients to understand the syndromic nature versus disease state nature. And they started putting that into the non-competitive bucket. They realized that this can't be undertaken by a

single company and say, okay, we've identified the pathway or the target which we are going to patent and will pursue.

It would take too much time and too many resources, including dollar resources, to get there. So, that's the one major change from my early days at the Upjohn Company, where we were mining the emerging human genomic data because we had just started sequencing the human genome. And at my company, we started putting patents on the targets we were finding, versus now we've realized that those targets do nothing for de-risking drug development until you can connect it to disease biology by studying hundreds of thousands of patients. That's the major change. We will continue to fail unless we really connect a drug target to an underlying disease biology in individual patient populations. And people use different terms for this, tailored therapies or precision medicine.

And some of these terms have clearly come out of the precision oncology field. If you know how we were treating cancers 20 years ago, it's completely transformative now in how we are treating cancers today in the sense that a pancreatic cancer is not a pancreatic cancer in three different patients. But it's defined by its underlying either genetic makeup or pathway biology. And it's the same that we are doing for Parkinson's to say we are going from syndrome to disease state definitions in individual patients. And this is being done through the industry joining the pre-competitive consortia, one of them has been initiated by The Fox Foundation, where it's the industry, the academic scientists, the government coming together globally to really study human patients in order to understand the biology of the disease.

So, that's the biggest change that I have seen. And we've seen the results of the safety consortia. Many fewer drugs now die today due to safety issues than they used to 20-25 years ago, because we have been able to make the change in how we are looking for safety signals pre-clinically before we go into the humans. And my hope and belief is that the same thing will happen that fewer and fewer drugs will fail due to lack of meaningful benefit if we are connecting the target to biology in individual patient populations through these pre-competitive space consortia-like activity.

**Marie:** Well, Kalpana, I think it's really exciting to see everybody kind of joining together to bring us closer to this more personalized, or more precision, or whatever you'd like to call it, medicine, where we're designating specific drugs that are the right drug for the right patient at the right time. And Kalpana, can you just walk us through what are some of these key steps in that drug discovery process to get us all the way from the idea to a finished product on the market available for patients?

**Kalpana:** So, the idea may come from an academic publication, for example, and you start there and say, okay, somebody just reported, and this is my first hand story, that mutations in LRRK2 gene cause Parkinson's disease in this patient population, right?

And I was at the time at Lilly when the two publications came out back-to-back. And then we asked the question, okay, what is LRRK? It's a kinase. As an industry, we know how to target kinases in terms of coming up with small molecules that would modulate the target. So, literally, no kidding here, the very next day, we put in process a project to say, let's start studying LRRK2 because it looks like it's affecting the causal biology.

Remember what we talked about? You want to go after the underlying biology that is causing the disease. So, that's where it starts, and then you start pulling together what are called in vitro tests to study the kinase function of LRRK2, even though it has other functions. We decided we'll start with the kinase functionality because we knew how to come up with kinase inhibitors. And then you start developing cell models. And as part of that process is, you want to screen a library of compounds that would allow you to identify those that are inhibiting LRRK2 kinase activity with sufficient potency and selectivity, etc. So, you put that — what is called a flow scheme or testing scheme — together. That may have four, five, six different assays. And these assays need to be validated statistically where you have confidence in the results and the signal window. Then you identify what's called typically first a “hit” that comes out of the screen, and then it's tested further through multiple drug-like property evaluation and you identify a “lead”.

That lead is something you may still need to optimize. Nine out of ten times, you need to optimize it for properties, such as its oral bioavailability, it's called. So, are you targeting this drug as an oral pill for the patient population? Then you need to make sure that it's not digested too quickly when it's taken orally in the stomach where not enough gets out into the bloodstream. So, those kinds of properties, what is its half-life, is going to disappear from the body within the first half hour. Then you can't give a drug every two hours to the patient. Right? At the most, you're targeting twice a day, worse comes to worse three times a day, but ideally one time a day. So, I'm simply bringing it up as properties you need to build into the molecule, the lead that you started, and that's called “lead optimization”.

And out of that comes what you start calling now a “drug candidate”. This is beginning to look, and smell, and feel like a drug, but now we need to test it for safety toxicology for sure. But are you going to be able to formulate it? Do you want a tablet? Do you want a capsule? If you are going for oral drugs. If it's not an injectable, for example. So, you start doing those studies simultaneously.

And if you are doing safety toxicology, much of it is regulated by the regulatory agencies. Your data would go into submission to the regulatory agencies such as the FDA. So, it has to be done in that fashion where you can submit the data to the regulatory agencies because you need the approval from, for example, within the US, from the FDA to say, okay, we are happy with your data package for you to test it in humans. We don't see any major red flags that should keep you from testing it in humans because they would have a safety liability. Safety is the very, very first thing that FDA pays attention to. They don't care if you fail in the clinic for efficacy. That's up to you to make sure that you have the right target and the right patients. But they don't want to see any undue side effects in patients.

So, that's how it starts through the drug candidate and then the actual drug that you would take into the humans, what are initially called phase I studies. Most times they are in healthy volunteers. More and more towards the end of that phase I, you put a patient population in there. And it's during that time that you are establishing in humans in a small number of patients, the safety, tolerability, how the drug behaves, so the pharmacokinetics in the blood, in the cerebral spinal fluid, if that's what you have chosen to do. And then ideally, some biomarker of target modulation so that you know the drug versus target modulation relationship. And then you go on to do phase II studies, which is looking for the clinical signal with biomarkers in a smaller population, still developing confidence and the safety of the drug. And then you go into what are called phase III or registration trials, the data package from which you would submit to the FDA in the US to either get the approval or not get the approval.

So, that's kind of the process. What I like to say in terms of the translational science that we talked about is that the earliest steps are the cheapest, the latest (phase III) is the most expensive. So, it's okay to fail at the earlier stages where you're trying to connect the target to disease biology and finding the right molecules. And then continue to use the same set of biomarkers throughout so that when you measure it in the clinic, you already know what level of changes you should anticipate or expect for the next go/no-go. And that's what's called "translational science" throughout for de-risking the development. So, in two minutes, this is drug discovery and development, independent of Parkinson's disease. This is applicable to any and every disease of interest.

**Marie:** Definitely. You know, creating new drugs takes a long time and like you alluded to, there's a lot of resources and risk that is involved in getting them all the way through that pipeline. So, how can, or perhaps how has, this process been sped up over the years or maybe made more efficient? And specifically, if you can comment on just the discovery of biomarkers for Parkinson's disease and what impact that that has had in facilitating clinical trials — this important step of that process.

**Kalpana:** I would say two things. Have we really accelerated the development of the drug, or have we de-risked the drugs that are getting to the humans? Perhaps it's the latter, and there's a slight nuance to that, right? What we are trying to do is, I may be repeating myself here, is understanding the human biology of the disease through biomarkers, right?

So, biomarkers come in very many flavors, but let's just focus right now on the biomarkers of Parkinson's disease. Let's be concrete about Parkinson's. And because Parkinson's disease is not exclusively, but primarily, a brain disease — of course, there's pathology in the periphery, as we know by now, in the intestines, throughout the gut, the skin, and the heart, but the symptoms, the cardinal features by which the disease is described, is due to the pathology in the brain. So then we need to be able to study the pathology through biomarkers that are in accessible compartments if you're not talking about neuroimaging, right? One way to study pathology in the brain is to have neuroimaging applications where you can actually see it. And that work is underway.

We've not found the kind of breakthrough we need in order to be able to study the two major pathologies in Parkinson's disease. One is the loss of dopamine neurons. And the other one is the alpha-synuclein aggregation and deposition. So, for the dopamine neuronal degeneration, we've had pretty good ligand, and these are called DAT scans that are approved by regulatory agencies, where we can see the degeneration of dopaminergic neurons that underlie the movement disorder. But what we've not been able to do is to study synuclein pathology in the brain through imaging agents, through PET studies or SPECT studies. These have been available in the Alzheimer's field, for example, in order to see amyloid deposition or tau deposition, two major proteinopathies of Alzheimer's disease. We've not been able to make that breakthrough in Parkinson's, but the work is underway. It might still happen.

So, then the question is, how do we then study synuclein pathology if we can't do imaging? And that's where the field has made tremendous progress recently, just in the last four to five years, in being able to detect synuclein aggregates in accessible fluids, such as cerebrospinal fluid or CSF, but also in blood compartments, serum and plasma, in the urine, in the tears, in saliva, so in a number of accessible biofluids, one can see the synuclein aggregates.

And then you ask the question, what stage of the patients can we begin to see the synuclein aggregates? And this question is important because what we have learned again from other fields, including Alzheimer's field, which is an important field to learn from, because that is also a chronic, slowly progressing neurodegenerative disease, just like Parkinson's. So, then we ask the question, what have we learned from about 100 plus clinical trials that have been

undertaken in Alzheimer's field? And we know that only one drug got approved, just last year in 2023, pretty exciting result. And another one is on the way to being approved.

And the results, in aggregate, indicate that if we go at an earlier stage of the disease, we are much more likely to have a clinical benefit in the patients. And the reason is because by the time Parkinson's is diagnosed, we've already lost roughly 40 to 60% of dopaminergic neurons. So, quite well-accepted now that the disease probably in an individual patient got started 5, 10, 15, 20 years prior to the clinical diagnosis made by a neurologist or a primary care physician. So, then in a way, it's natural or common sense to say the disease started several years before the diagnosis, how can we reverse the process? And maybe that's why many, many drugs failed in Alzheimer's disease, because we were treating them quite late. But in those that we were able to identify at an earlier stage, there were greater clinical benefits. So, the deduction was let's go earlier.

Now, how do we go earlier? And that's where the biomarkers come in. Can we identify biomarkers of synuclein pathology, for example, that tells us already that in individuals who are not yet diagnosed fully with a Parkinson's disease-like movement disorder, can we identify them with detection of synuclein aggregates in any of the accessible biomatrix, whether it's cerebrospinal fluid, or blood, or saliva, or tears, doesn't matter.

And that's what we have found out now that we can see synuclein aggregates in very, very early stage patients. And many of them show these preclinical signs of Parkinson's disease that do not constitute the clinical diagnosis of Parkinsonism, but they have the signs and symptoms. So, that gives you the hope that as this biomarker field matures, and we have the right drugs for those individuals, then we should be able to go early in the disease process and expect greater clinical benefits than going late in the disease stage. And I'm only referring to these disease-modifying therapies. Obviously, symptomatic treatments would work in post-diagnosis, regardless of the stage of the disease, except for the nuances of side effects of some of these drugs, etc. So, biomarkers are critical for that reason.

If you give me one more second to expand on that. So, biomarker of disease stage, disease pathology, disease process would also be useful, as I was saying earlier, to go from a syndromic definition to biological definition. So, not every Parkinson's patient, and this has come to surface much recently in terms of certain genetic subgroups. So LRRK2, as the disease-causing genetic mutations in LRRK2. It's a risk factor. It doesn't cause Parkinson's in 100% of patients, but it's age-dependent. And it is a major, major cause of genetic Parkinson's disease. But we've realized that many of them don't have synuclein pathology in the brain when we study their autopsy brain tissue. They have other pathologies.

So then immediately you ask the question, if I had a drug that targeted synuclein, should I be treating the LRRK2 genetic group as a single group? Or should I be subsecting that group to say, this is the group that has synuclein pathology, and this is the group that does not have synuclein pathology. So, my drug should work better in the former group. If I start including those that don't have synuclein pathology in my clinical trials, then I will have more noisy data. I may not be able to pick out the clinical benefits signal, because it will have been diluted out by patients who don't have the synuclein pathology.

So, that's another place where the biomarker comes in really handy and useful, because we can study the presence of a given pathologic feature in individual patients and then enrich our clinical trials with only those that are likely to be affected or those who are most likely to respond to the mechanism of my drug that I'm trying to develop.

**Marie:** Your response just now, I think, tackled a little bit of my next question, which is you mentioned that for clinical trials for disease-modifying drugs specifically, they may turn out negative, perhaps because we're intervening too late. And then you also mentioned this need to better segment patients for clinical trials. And I think you're absolutely right. These are things that have hampered clinical trials in the past. What are some of the other common pitfalls that you've seen as these new drugs are being developed and tested in clinical trials?

**Kalpana:** So, assuming that we have picked the right target, I mean, if that starting point is wrong, nothing else matters. Let's start this question with the assumption that the target of the drug you're trying to develop is appropriately connected to the biology of the disease, Parkinson's disease, in this case. So, the other pitfall could be, have we tested the drug at the right dose in humans? So, think about as you go from drug discovery, which typically starts out in a test tube in vitro, you go into cell studies, then you move into the animal model studies. And throughout the process, you are trying to understand how much of the target you need to either activate, or inhibit, or modulate in order to produce the effect you desire to produce in the clinic. Now, in the animals, you have the benefit of directly measuring your drug in the brain tissue.

We won't have that ability, right, in humans, by definition. So, what ends up being is you try and measure the drug levels in the blood compartments and in the cerebrospinal fluid. Then you make, based on your animal model studies, you can predict through modeling how much you might have in the brain. It still doesn't tell you whether, even in the brain, which is a complex organ with multiple cell types and blood vessels included, etc., are you modulating your intended target?

And that's where the second kinds of biomarkers come in. And those are called biomarkers of target engagement or biomarkers of target modulation. So, I've actually studied this quite a bit in terms of roughly the 10-11 drugs that have gone through sufficiently large studies, what one would call proof of concept studies at least, to look for clinical benefits in Parkinson's disease. And with the exception of one drug or one drug candidate, I should say, none of the clinical trials had a biomarker of target engagement or target modulation. So, they took the drug based on the safety profile, the tolerability profile in humans, and may have tested it at a single dose level or maximum two dose levels. And they looked for clinical benefits in a certain patient population over a certain period of time. And these are all disease modifying therapies I'm talking about.

And as we know, we don't have a drug that slows the progression of Parkinson's today. So, every one of them failed to show meaningful benefit. Now, you may just argue back with me based on our earlier discussion that maybe they didn't pick the right patient population, either by the stage of the disease or by the underlying biology, etc. And that could be true. Or you could say maybe they didn't pick the right dose. So, that's another major failure mode is we end up testing a drug without a biomarker that indicates that the drug hit the intended target, modulated it to the level you intended to modulate it, whether it's 50% activation or 80% inhibition, whatever the case may be. We need objective data to say at that given safe dose, we were able to modulate the target to the desired level.

And now we look at the patient population that hopefully you already enriched for your mechanism where it's more likely to work. And then if we don't see benefits that are clinically meaningful, you could walk away from that drug mechanism and say we have tested it the right way, but it wasn't the right target. It wasn't the right way of slowing the progression of the disease. That is another thing. And at least in large pharma now, it's become almost a standard practice to use biomarkers of target engagement and target modulation. When you don't have that, people wonder whether you're testing the right dose or not.

And I think this has happened, right? As I mentioned with majority of the drugs we have tested, I mean, the most recent example I can think about is the c-Abl inhibitors. It was very, very exciting when the academic research from multiple labs suggested that inhibiting C. Abl could be disease-modifying for Parkinson's disease. And much of these data came from cell and animal models.

So, the immediate opportunity that became available, and it was taken advantage of by a group that did much of the preclinical work also, was to look at the field and say, okay, we have a c-Abl inhibitor already approved by the FDA for certain types of cancers. Can we take them to the Parkinson's disease patient population at a safe dose and see if we can produce clinically meaningful

benefits, right? So, you shortcut the drug development path by using an existing drug. This is called repositioning of a drug. And unfortunately, the molecule that they ended up picking, which was approved by the FDA, it's called nilotinib.

What we discovered because The Michael J. Fox Foundation also wanted to get to the bottom of this study on, is this the right drug for Parkinson's patients with which we test the c-Abl inhibition hypothesis. So, I was part of this team where we conducted the first studies in animal models where we discovered that the drug is not getting into the brain at sufficient levels for it to inhibit c-Abl, the intended target of the drug. But then we ended up conducting a clinical study because there were already data that were put out that had generated quite a bit of interest in the field by an external academic group that indicated that nilotinib actually had clinical benefits in patients.

But again, today we don't want to go into the issues of that study. What's important is to make the point that biomarker-informed clinical trials give us objective results with which to interpret clinical results. So, what we found in The Fox Foundation-funded study with nilotinib is that, as we had seen in clinical animal models, we did not see sufficient levels of nilotinib in the cerebrospinal fluid as a surrogate of what's in the brain.

So, very, very small amounts were getting there. And true, we did not see clinical benefit at the doses that were tested. Same doses were in the previous open label study, clinical benefits were reported. So, you basically have the ability to say, okay, at the doses tested, I'm not seeing clinical benefits, but then you don't have the ability to answer the question, is c-Abl the right target for Parkinson's disease? Have we tested it sufficiently to either continue to work on better c-Abl compounds or walk away from it? And one could not do that with nilotinib because we did not have biomarkers of c-Abl inhibition. We simply said, at these doses, nilotinib is not effective, but we cannot conclude that we have tested the c-Abl hypothesis as it relates to Parkinson's biology. But there are two other companies that have other proprietary molecules that they have developed that seem to have reportedly better brain penetrance, and they are in clinical development right now.

So, my hope is that they have these target engagement, target inhibition, or c-Abl inhibition biomarkers included in their clinical trials in order to connect the dot between the dose, is it the right dose? And then is it the right patient? And then we'll just wait and see how their data turn out. But you asked about what are the other reasons why the drugs may fail, and this sounds very elementary, but picking the wrong dose has always been a reason why a drug cannot produce the kind of benefit we would want to see in patients. So, we need to address that also with a set of biomarkers that are called target engagement or target modulation biomarkers.

**Marie:** Well, Kalpana, I think that is such an important point. And this was a really great example, this story of c-Abl, and taking a step back here for listeners who might not be familiar with this c-Abl, which is a non-receptor tyrosine kinase that is also an indicator of oxidative stress. Can you tell us a little bit more about what its suspected role is in PD pathology or sort of the mechanism that it might be acting on?

**Kalpana:** It seems to have multiple mechanisms that are purported to be the underlying mechanisms for its predicted efficacy. One is its ability to modulate the immune system, which does play a role in Parkinson's biology. It's also been connected to synuclein pathology that we talked about early on today. So, the idea is, through a single mechanism, you might be able to modify multiple disease biology mechanisms and therefore have greater clinical benefits. And much of the work, as I said early on, has happened in cell models and animal models of Parkinson's disease from which these data have emerged starting about 10 years ago now.

So again, my hope is that the newer, better compounds that are in development right now, we might be able to see if they've been able to dose them at a safe level and have these biomarkers of the evidence that they've picked the right dose, as well as the biomarker of the right patient population, right? The segmentation enrichment that we talked about, that we will be able to test the hypothesis. Not every preclinical hypothesis is proven once you get to the clinic. And that's nobody's fault because human biology could be very different. You are working in a multifactorial environment where even if you have connected to a subpopulation of the patients, the patient is on multiple drugs, has different trajectory of the progression of the disease. You may not have studied all of the underlying disease biology mechanisms to make it more concrete.

You may have studied the synuclein pathology, but don't have the tools to study the immune system activation in their brain. So, there are a lot of unknowns that we deal with once you get to the clinic from any of your preclinical models. So, the drugs fail at any stage at any time. All we are trying to do through biomarkers is to reduce the rate of failure, improve the chances of success. There is no guarantee, but the good news is that pharma companies don't want guarantees. They will take risks on testing a drug the right way in human patients as long as they are safe drugs and ultimately learning from clinical trials. I think pharma is the only industry — this may come as a surprise to many of the listeners — is from the beginning, from the time that we pick a target and start screening against that target to getting a drug out that's approved by the regulatory agencies, such as the FDA. The success rate is somewhere around 1.5 to 2%.

There's not another industry that I know of that can operate on a success rate like that. And having said that, we look at what happened post-COVID, right? The

speed at which immunotherapies were discovered, vaccines were discovered, the effectiveness of those, effectiveness of drugs like Paxlovid, right? In a very, very short time.

So, despite this measly success rate, we still have tremendous opportunities to make a difference in the patient population that we have seen even in Parkinson's disease, right? So, I don't want the listeners to take away the message, well, if the success rate is so low, how can we ever rely on new drugs coming out that would make a difference?

I think they have, and they will continue to do so. So as soon as I said 1.5 to 2%, this little bell went on in my head saying, I don't want to give the wrong message that we don't want to develop new drugs. I think we can, but at every step of the way, we are reducing the risk of failure through biomarkers and through understanding the disease better, connecting the targets to the disease.

**Marie:** Absolutely. And Kalpana, I know there are some difficult decisions along the pathway of drug development. So for you, how do you make that really tough decision of whether to continue trying to develop a particular therapeutic or work on a particular target, whether it's c-Abl or others, versus refocusing your limited time, and resources, and efforts on other targets? What are, I guess, the major decision-making factors that you consider when you have to make that difficult choice?

**Kalpana:** It depends a little bit on whether you are a big pharma or whether you are a small startup company. And here's the reason. In big pharma, you're not working on a single disease area. You're not working only on neuroscience, typically, even though strategically, you may say neuroscience is my highest priority. But you have neuroscience, you have oncology, you have metabolic disorders, such as diabetes, typically in your pipeline. So, then the decision is more strategic at that level, which I experienced firsthand, sometimes positively, sometimes not so positively, is that the project that you are bringing forward from neuroscience, how do I look at it with respect to its probability of success, the resources required in order to develop that versus another project for oncology or diabetes. So, it becomes a high-level strategic pipeline-based decision that sometimes goes towards supporting, let's say I was bringing that project forward in neuroscience, and sometimes against to say, right now, I want to pick the oncology opportunities or diabetes opportunities. Much of it is also business-driven, where the company sees itself going in the next two to three to five to ten years, as well as resource need, the probability of success.

So, it's again, a multifactorial decision. As against that, startup companies typically don't have this kind of a deep pipeline. I'm not saying all of them work only in a single disease area. Many of them are platform companies, say RNA-

based technology. They would work on oncology, neuroscience, but still their internal expertise tends to be much more in one disease area and indication than others. There are companies that have a single asset, single molecule they're trying to develop, or two, or three. And there, the decision is based on the overall data package, right? How much confidence do I have in this project? And these small companies, as you know, are almost all funded by private investors. They're not public companies yet. That's the group I'm talking about.

So, then the decision by the funders is, what is the data package that would allow me to continue to make investment in the next step, whatever the next step of that drug may be, in the progression of development? So, it's less pipeline strategic, but more data package-based decisions. And there are times when, right now, when I work with these startup companies, which I absolutely enjoy doing because we can move things faster, much more than I was able to in big companies. There is no bureaucracy, or less bureaucracy, I should say. So then, you conduct studies that allow you to ask the question, how do I test my null hypothesis? How do I test whether my hypothesis is wrong? Let me try and prove that. And if I can't prove that the hypothesis is wrong, I'll have greater confidence that it's right.

Those are the kinds of studies that you do, both in big and small pharma, but mostly trying to progress it in a way where you have the right molecules and the right data package to say, it looks like a drug. If it's safe for human consumption, here are the biomarkers that go with the drug. So we can test it the right way in early phase trials.

And let me just make one other point in terms of early phase trials. Most of these startup companies are not set up to do the regulatory, so-called phase III, type trials, or even phase II trials for disease modifying-therapies, such as for Parkinson's disease. So what they are geared towards is getting the initial safety, tolerability, pharmacokinetic, and some biological signal data in these very early trials to then have a package that goes to big pharma, like the Lillies of the world and say, look, we have shown that in that small study, the drug is well-tolerated. We are not seeing a safety signal that would limit the dose for modulating the target of interest. And we have these biological data that show that we are modulating the target at the given dose.

Now, are you interested in taking it and going through the larger development studies? Because now you're talking about multimillion dollar investments in clinical development, and manufacturing, and everything else. So again, it comes down to de-risking the steps in drug development, but ultimately developing a data package that is attractive enough. And in the process, many times you walk away from a project and say, no, I've tested it. I don't see a path forward. I'm not finding the right molecule that is human-worthy. Unfortunately, I just need to wrap

up, start on a new project if I have funding, or more my resources to a new project.

**Marie:** Certainly, I think that makes sense. And I know this drug development process is difficult, but there has been amazing progress over the years, Kalpana. So, what do you see as just some of the key milestones or major advancements that have occurred in developing disease-modifying therapies, specifically for Parkinson's disease?

**Kalpana:** So, I think the one I'm most excited about, and perhaps it's because it's the most recent one, and this ties back to what we were talking about at the start of this interview. And that is this biomarker with which we can see synuclein aggregates detected in a very, very sensitive way in Parkinson's patients at the earliest stages.

So, let me just dig slightly deeper into that. This biomarker of synuclein aggregates came out of an assay called Synuclein Seed Amplification Assay. And as the name suggests, there is an amplification stage built in so that it's extremely sensitive, and you can detect synuclein aggregates very, very sensitively in patients. And what we discovered, through this observational study that The Michael J. Fox Foundation undertook, starting in 2010 — this is another study where I have the privilege of being a part of it from the inception, perhaps even being a catalyst to start the study — the study is called Parkinson's Progression Markers Initiative.

It's because it's an observational study done with really, really multi-modality biomarker assessment longitudinally in the patient population, including neuroimaging, but cerebrospinal fluid, blood, cells, all kinds of biomatrices collected alongside deep phenotyping of their clinical symptoms. So, now we have the ability to connect a biomarker to other biomarkers. Are they associated? Or to look at their association with clinical symptoms.

And that's what we've been able to do through this synuclein seed amplification assay. The data just actually last week got published. The paper that we've had the data for, roughly now 15 months, that indicate that at the earliest stage of the disease, we are able to detect synuclein pathology prior to diagnosis, clinical diagnosis of Parkinson's disease. So, as we were talking about early on, if you want to go at earlier stage of the disease, this is the kind of biomarker you want to say, now we have the ability to enrich a population that's at an earlier stage and has synuclein pathology. Therefore, if I believe my target can affect synuclein pathology, then I can enroll them and reach for this population in my clinical trial. So, that's been the most fantastic result that's come out through efforts of many, many groups, including the academic group from which the

assay emerged (multiple assays emerged, I should say, through multiple academic groups).

But this is step one, Marie. It's a binary assay that tells us, yes, we can detect, no, we cannot detect. I think we need to do more work to make it more quantitative. But for me, at this stage, even step one, that is transformative is exciting. To say, now we can define Parkinson's through its biology and not just clinical symptoms.

**Marie:** Absolutely. I agree, Kalpana. I think this is such an exciting time. And there have been multiple exciting advances and discoveries, even within just the past year. So, we love talking about just the tools, the resources, the collaborations. What is really having a big impact in terms of moving the field forward? You mentioned a handful of them, the PPMI study, the Alpha, the Synuclein Seed Amplification Assay, of course, relatively recently. Are there other tools, or resources, or collaborations that you see are having a tremendous impact?

**Kalpana:** The one that not everyone might agree with me on, but the one I'm keeping my eye on, is the digital technology that's emerged. Again, that's not specific to just life sciences. But we are so much better now at the so-called artificial intelligence and signal detection from complex data. But the digital technology specific to Parkinson's disease is related to our ability to monitor the subtle movement disorder in Parkinson's patients continually, right, over 24 hours, over seven days, rather than a patient going into a doctor's office or neurologist's office once every three months when we get a snapshot.

So, there are a number of devices in development right now being tested right now, some of them through pre-competitive consortia that we talked about that I think in the future, maybe not next year, but maybe in the next three years would allow us the ability to then monitor a patient's clinical symptoms at a much more granular level. And if you can do that, sensitively, you can see why it would also allow us to detect patients at earlier stages, right, rather than seeing someone every three to four to six months and looking at their symptoms during that in-office interview. So, there are several papers published, and there are several studies underway that I'm privy to that give me the hope that in the next few years, we'll see the benefits of those, and we'll start integrating those in therapeutic development trials.

So, that's one. The other one, given my background in biomarkers, is really related to the technologies that have become sensitive. Whether it's mass spectrometry-based assessment of different biomarkers, being able to sub-compartmentalize blood-based matrices into what we are calling extracellular vesicles that actually could come from the brain, but then carry the brain cargo into the periphery, including blood plasma, or serum, or urine. So, you can now

assess what's happening in the brain through blood biopsies. You don't have to do cerebrospinal fluid.

So, this is another maturing field. The technologies are getting sensitive enough for us to be able to measure and detect things at very, very small amounts. So, that gives me the hope that we'll see a breakthrough in the near future. And then finally, I must say that this collective realization that precision medicine is our only way to go forward, and reaching for patient populations that are more similar in disease biology, informed by biomarkers. It's not just the pharma that's thinking like that. Academic scientists are a part of this equation.

So, everybody is doing their bit to really understand disease biology in individual patients through biomarkers, through the clinical symptoms, so that we go from a syndrome to a disease state definition, collectively, through everyone's work. I think this is happening as we speak.

**Marie:** Absolutely. And Kalpana, I think these are all really cool areas of research that you enumerated there. Do you have thoughts on what other areas of opportunity there are in Parkinson's disease research or other big unanswered questions that you're hoping to see answered in the future that you're really excited about right now?

**Kalpana:** So, this might be a bit of a repetition or a broken record, but I feel like one thing I really need to acknowledge is the involvement of the patient community. This is another big change that I should have said early on that I've seen in the pharma industry. When I first started back in 1993, and I had the opportunity to join a team where we took a drug for clinical development within my two years of starting at the company, I don't recall ever meeting a single patient when we were designing our clinical trials or the end points.

What has changed in the last 15 years is the realization that we can't develop a drug without the involvement of the patients themselves, and hearing from them what's most important to them. And not simply coming up with our hypotheses or our gut feelings on what might be important to a patient. So, throughout the field, whether it's Parkinson's, Alzheimer's, or even oncology, diabetes, you'll now see that it's standard practice to involve patients from the beginning. To ask, even the simplest of questions. Are you okay if we didn't have a pill you could take orally, but we come up with an injection that's given every week, or every month, or whatever the frequency might be? Something as simple as that. Are you okay if we say we want to get cerebrospinal fluid four times during the course of the trial, every three months?

So, these seem like very simple basic things, but this is what's changing. And this PPMI study that we talked about couldn't be possible without the engagement of

the patient community, where we are asking them to, not only spend their time for the clinical study interviews, but also give their cerebrospinal fluid, and blood, and skin biopsies, and a number of other tissues at the same time. Perhaps many of them know that the results may not benefit them immediately, but it's moving the science forward.

So, I see this as, we'll take a center stage, continue to be in the center of the stage, in the voice of the patient and being able to engage them in order to understand the disease biology in those individual patient populations. The other thing I see continuing to grow is this pre-competitive space, and I'll give you a concrete example on LRRK2. So, genetic mutations in LRRK2 cause Parkinson's disease. There are nuances in terms of how many patients you would see it in, is there full penetrance? It's not, but let's leave it for the time being.

What we discovered also is that there are potential safety liabilities of inhibiting LRRK2 kinase function because you can see that in animal models. So, then the consortia that was initiated by The Fox Foundation's help, right? They are the right kind of group that can convene people who are otherwise competing to say, let's come together and understand this feature and see, A, is there any risk to the patient? And B, how do we monitor it? And C, are we able to come up with molecules that would not have this liability? So, a pre-competitive initiative was formed. We conducted non-human primate studies to get to the bottom of the mechanism, etc.

But going forward, this initiative is somewhat continuing while we have a drug in clinical development to say, what else can we do? How else can we modify or modulate, I should say, LRRK2 in order to minimize this risk? And I bring it up as an example to say, you'll see more and more of this. To say, ultimately, the drug companies, large or small, will compete on their patentable drug candidates. They don't need to compete on these pan-biology features that the individual companies don't need to work out their own solutions, but rather do it through a consortium. And whatever the consortium learns, everybody benefits from that. But then they end up competing on their final molecule. Who can make the best molecule towards that target that can be tested effectively in humans and ultimately gets approval? So, I think you'll see that change continuing to see the expansion of the pre-competitive field as we know it.

And last thing I'll bring up is, I alluded to this early on, so-called artificial intelligence. I actually hate that term, to be honest with you. It's very sexy right now, and it's appropriate in certain instances when you are doing artificial intelligence to quickly analyze images, for example, to detect a signal from complex data. But we also have more and more large-set human genetic data, and genomic data, or the so-called -omics data, all kinds of -omic technologies, being available in the public domain. So, data are data. How do you convert data

into knowledge first, and knowledge into understanding, and understanding into applying it towards the drug?

So, the good news is these large data sets are becoming totally publicly available, and these are mostly human-derived data from which you can learn about disease biology. And then you ask the question, who are the right groups to now convert that data into knowledge, into understanding, and into ultimately a drug that's founded on that insight or understanding?

I think, and I've seen this again, firsthand in a couple different things that I'm involved with. One is through The Michael J. Fox Foundation, another is affiliated to The Michael J. Fox Foundation, called ASAP, which is trying to accelerate Parkinson's drug discovery by bringing in academic scientists and basically asking them to work in a collaborative consortium-like environment where there are multiple laboratories with different technologies that come together to answer the basic questions on disease biology. So, they have invested, or donated whatever the term you want to use, a tremendous amount of money in saying, let's bring the best of the best minds together, but make them work collaboratively so that they're using different technologies to unravel disease biology, for the lack of a better phrase.

So, this got started about three years ago. Of course, COVID delayed some of the work, but I think we'll begin to see the fruits of some of those teams. I'm not saying every single team would give us the answer that they sought out, or they started to seek out. But what we'll get at the end of that is a better picture in some domains of Parkinson's biology to say, what are the mechanisms? And if you understand the mechanisms, then drug hunters like me and others would then say, is it drug-able? How do we come up with better medicines to target those mechanisms? So, fundamentally understanding the biology is happening.

We are taking a chapter in some cases out of the oncology field that's made some progress, but also doing things differently because Parkinson's is not a cancer. So, we need to be cognizant of that. So we are doing it smartly somewhat, and through the dedication of a lot of people and resources being put into this, I think we'll see the results in the next three to five years. And at my stage, that's what I hope for is that I do want to see disease-modifying therapies in Parkinson's in my lifetime. And I'm privileged enough to be a part of such teams who are attempting to get to that for the sake of the patients that we have today.

**Marie:** Absolutely. I think that's wonderful. And I know, Kalpana, you've been making substantial contributions to the field over the years. So, is there anything else that you'd like to share with everyone about how your work is really bringing us closer

to finding a cure for Parkinson's or contributing to these improved therapies for people with Parkinson's today?

**Kalpana:** All I'm trying to do at a very, very simple level, working, as I said, primarily with startup companies, is to guide them the best way I can from all of the knowledge I've gained. And I call it "University of Pharmaceutical Industry". Right? You learn the most, at least in my mind, through that experience on the obstacles or the risks that underlie the failures of a drug. In my mind, I'm simply giving back to say, how do I help you de-risk by ensuring that the target is connected to disease biology through the right kind of studies, that you are using model systems, both cellular and animal models, that reflect the disease biology?

When I first started, we didn't care about that. We used the most facile model that you could get in order to move the project faster without asking, are the answers going to be predictive of what happens in humans? And that's now gone. We are improving the model systems in a way to improve their predictive validity by making the models reflect the disease biology.

And then ultimately, biomarkers, right? Biomarkers of target engagement modulation, and biomarkers of disease biology. So, that's all I'm doing. I'm not doing anything new. And there are many, many people like me who are working with startup companies to help inform or de-risk drug development.

The only other thing I would add is, the one thing I've practiced in my life, is to not be dogmatic. We know what we know, and we need to be humble enough to say, we don't know what we don't know. So, always be open to surprises, to unexpected results and data, and be open to change my mind, because that's how we move the science forward. That's what's needed. If we were stuck in dogmas, I don't think we'll make the kind of transformation we need to make in delivering the right kind of drugs today.

**Marie:** Well, I definitely agree with you. And, Kalpana, I think you're being too humble here. We truly appreciate you sharing your insights and your experiences with all of us today. So, thank you so much for joining us on the show.

**Kalpana:** You're very welcome. I really enjoyed speaking with you. So, thank you for giving me the opportunity to share some of my thoughts.

**Marie:** Well, I've really enjoyed our conversation. And listeners, it's been great to have you with us as well. If you want to know how The Michael J. Fox Foundation can help your research, please visit <https://www.michaeljfox.org/researchresources>.

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