Tyler Gurberg – ALS Research, Montreal Neurological Institute

- Steve

Welcome to our first episode of the Lynx Pod, we're so excited to have you as our debut guest. So, we just finished a full tour of your lab, and you have so many fascinating things going on. We met your PI, Gary Armstrong, and a few of your team members. To begin, are you able to tell us about yourself, and a little bit of your background?

- Tyler

Yeah, of course. My name is Tyler Gurberg. I'm a first year Master's student in the neuroscience program at McGill University in the Armstrong Lab. We are a lab that studies ALS models in zebrafish, where we design these models from scratch. I have a background in pharmacology. I studied here at McGill, I got my Bachelor's in Pharmacology and I transitioned into my Master's degree last September.

- Steve

Awesome. And I think congratulations are in order as well - you just received a pretty big grant.

- Tyler

Yes, actually. The CGSM CIHR is a grant I received to support my project. They believe that my project is worthy of this funding. And it's meant to supply me with not just a stipend, but also to supply our lab with some of the resources that we need to do this research. Honest to God, a big part of being a graduate student is applying for grants. We need to get our funding from somewhere. And, so as long as you have a grant underneath your belt, then you're doing pretty well. But there's a lot of application and a lot of writing that goes into that.

- Steve

It seems to be the name of the game.

- Tyler

Exactly.

- Steve

Awesome. Okay, so can you tell us just a little bit more about your specific research project?

- Tyler

Sure. So, I'm studying a protein called Stathmin-2, and to understand Statman Two, actually, I should start by giving a little bit of ALS talk. So, we're a lab that focuses on ALS research. And if you aren't aware, ALS is amyotrophic lateral sclerosis. It is a disease that is a neurodegenerative disease and the hallmark of which is the degeneration of both upper and lower motor neurons, which are the neurons that are connecting your brain to your spinal cord and then your spinal cord to your extremities. And when you have the degeneration of these motor neurons. What happens is slowly you lose control and function of your muscles. So, most patients will present to the clinic with failure of being able to grip something with their hand and then it'll spread. Then you'll slowly lose control of both hands, your lower arm, upper arm, and eventually within two to five years, usually within two to three years, even, you can no longer control your respiratory system and you pass away of respiratory failure. So, this is a progressive and fatal disease, typically within just a few years of clinical onset. And at the moment, there are not any real clinical options for these patients.

- Tyler

There are actually three now FDA approved drugs, the most recent of which may be the most promising. But the data for that is only going to be available in, I believe, 2024. But the first two drugs are Riluzole and Edavarone. And the reality is that at best, these provide a couple of months extra life spans, these patients, but they don't do much in terms of improving the quality of life. And ALS is a morbid disease with no cure and not even any good treatments yet. So, we're trying to address that by developing an animal model in zebrafish that can replicate this disease and we can use to study the inner workings of the proteins involved in this disease and hopefully downstream find a better cure than what's currently available.

- Steve

Wow. So, how did you land on this protein Stathmin-2?

- Tyler

Yeah, so Stathmin-2 is a protein that's involved in microtubular regulation. I was telling you a bit before about your upper and lower motor neurons and the construction of a neuron basically has a cell body and then these super long axons, which are these projections, that is the electrical pathway that your brain and your spinal cord use to talk to your muscles. And if you think about these neurons, like your arm, the microtubule is the bone, it's the solid structure on the inside of these axons. Which also provides the

circuitry. It's where you get the shuttling of vesicles containing important things for the ends of these neurons as well as shuttling stuff back towards the cell bodies. It's basically like the highway system of these axons. And my protein, Stathmin-2 is involved in regulating these highways because these are not permanent highways. They're always undergoing construction. They're collapsing and rebuilding all the time. And they need proteins to support this. They need construction workers. And one of these construction workers is my protein Stathmin-2, that's involved in the destruction and the repair of these highways. And the reason that we think it's implicated with ALS is because of another protein to talk about, which is TDP-43. TDP-43 is the big boy when it comes to ALS research because it is involved in about 97% of ALS patients. They present with some form of TDP-43 pathology. And TDP-43 is a hypercritical protein when it comes to regulation of RNAs and a lot of other cell functions. But in normal function, it's always going to be in the nucleus. When it comes to ALS patients, it's no longer in the nucleus of the cell, it ends up in the cytoplasm. When it's in the cytoplasm, there are downstream effects with a ton of proteins, and one of which seems to be Stathmin-2. Two papers came out in 2019 tying the loss of TDP-43 with the loss of Stathmin-2 as the protein that was the most down regulated when compared to TDP-43 and all the other proteins involved in these pathways. And so, these papers came out and showed that, oh my God. Okay. When we lose TDP-43 in the nucleus, Stathmin-2 is significantly down regulated. Why is that? Why is this downstream protein so associated with this hallmark of ALS? And that opened the door to our lab to say, oh, you know, what this research field could use is a zebrafish animal model of Stathmin-2 loss. What if we just completely knocked out Stathmin-2 from our zebrafish and see if it produces a motor phenotype that we can say somewhat resembles ALS patients?

- Steve

You lay that out so well. So, where are you in the process right now in terms of knocking it out and really discovering what impact that has on the zebrafish?

- Tyler

So Stathmin-2, unfortunately, in the zebrafish is represented by two genes, Stathmin-2 A and 2 B. This happened because the fish, at a certain point in its evolutionary process went through a whole genome duplication for some genes. And so, my gene in particular, seems to be one of these proteins that was replicated. And so, I have to not only knock out not only 2 A, but also to B to have a true Stathmin-2 knockout in our zebrafish. The point I'm at is I have knocked out 2 A. It seems like I've knocked out 2 A and I'm about halfway through 2 B. And so unfortunately, to get an animal or one of our zebrafish to sexual maturity is about two and a half to three months. I've identified my mutants, but I need to breed them into my lines in order to get a final double knockout, which I'm projecting to have completed by the end of this year.

- Steve

Very cool. And you actually showed us the process of using the CRISPR Cas-9 method to cut the DNA in your embryos. Now why is it that you target the embryos over the adult fish?

- Tyler

So, if you were to inject into an adult fish the issue with that is that most of these cells are replicating but they're replicating in their own tissues. If you were to inject it into the muscle tissue of a zebrafish you might get a certain hot spot there where you are cutting the DNA, you're inducing mutations. But when it comes to stuff like your central nervous system that's not undergoing constant replication, these cells are relatively permanent. Your motor neurons in your body are relatively permanent. And so it's very difficult when it's not replicating to get it to transfect to the rest of the tissue. When you're doing it at the embryonic stage. At the one cell stage then every time that this cell undergoes replication if it's already had its DNA cut in the area you want it to, theoretically it should pass it on to each cell in this replication process to the point where you have an adult where hopefully the majority of its cells are mutants or mutated cells. That's not always perfect. And usually what you'll end up with is a mosaic fish. So, you'll have an adult that has some of its cells, or 50% of its cells that have had the DNA cut by your CRISPR the rest of them aren't. And, what's important is that if you get the cells in the reproductive system to have this mutation, then all you do is breed it and now you're guaranteed that for the offspring - at least one of its alleles in every single cell will be a mutant.,

- Steve

Okay I want to dive into CRISPR Cas9 a little bit more and that technology as a whole, because I find it really fascinating. So, it's been ten years since it was introduced, as of a few weeks ago. What are some of the changes it's undergoing right now or the evolution that you're seeing?

- Tyler

It's getting a bit more specific. So, we have certain databases that we can use to identify more efficient targets, because one of the issues with CRISPR is that although the machinery might work, the template you provide, as in the area of the DNA that you're telling the CRISPR Cas9 machinery to cut, it may not be the most efficient spot. Now we have databases that you can use to search and design new templates and see which one is not only the most efficient, but also the most specific. Because one of the drawbacks of CRISPR is that you might get off-target effects. So, you can be cutting the DNA at exactly the spot you want to, but if you're also cutting the DNA in an unwanted area, you may have downstream off-target effects that are affecting these fish, in our case, or whatever model that is being studied on. And so that's the first thing. The next thing is the actual protein, the Cas9 that we use, that's the foundational protein for CRISPR. Thing is, these days there are new Cas proteins being artificially developed, or even just discovered that are more efficient and better and can be used for more specific targets. The traditional CRISPR is primarily just cutting DNA, damaging DNA, eliminating a couple of bases, and hoping that the repair, the natural body's repair of this mutation will be inefficient and will lead to the DNA not being perfectly repaired. These days, what you're able to do is not only cut the DNA but feed an entire new template for the repair system to read off of to replace a mutation. And so, you can find a mutation early on and theoretically feed this CRISPR Cas9 system a template for the, in our case, the zebrafish's

repair system to read off of and create brand new wild type normal DNA. And now this is just the DNA that's in the fish. You've eliminated the mutated phenotype

- Steve

So that wild type doesn't carry any disease.

- Tyler

Exactly. If you can identify a mutation that says, okay, if you have this mutation, you get a disease and you can replace it through this technology, the mutation is gone, and in theory, it should not lead to the disease. Unfortunately, it's not usually as cut and dry as one mutation equals disease. That's it. But sometimes it is. And so that is one of the theoretical downstream applications of CRISPR Cas9 gene editing in humans, although we are still probably a couple of decades and a lot of ethical questions away from actually doing that because it's still an imperfect system, like I said. But if you can do full genome sequencing on an embryo and see that it will develop with a disease that is fatal at birth and you can just go in and change it and just replace that one small part, then you can have a child that is completely cured before even birth.

- Steve

It's tricky, right? There is a lot of controversy around that right now. If you're able to modify an embryo before it even has a chance to have a say in it.

- Tyler

The ethical questions behind CRISPR gene editing on humans are infinite. It's a very gray area for the field, and it was done. It has been done before. It was done by a few researchers in China many years ago. And they didn't do anything drastic, I should say. They weren't trying to give someone a third arm. All they were trying to do was give HIV protection. They just wanted to create a couple of twins that were protected from HIV.

- Steve

So, getting back to ALS, during our tour you mentioned that in ALS patients, motor neurons in the body have a precondition essentially to degenerate over time - are you able to explain what that process looks like in more detail?

- Tyler

I'd say that in a healthy human, you'll get, over time, some degeneration, but the circuitry likes to stay intact. It's a pretty dynamic system that will try to stay intact. When it comes to ALS patients, however, that's when you get 50% of your neurons that are degenerating before you even show up at the clinic with

any phenotype, you know, your neurons are very good at adapting and the plasticity mechanisms involved with the circuitry in your body is incredible. And you can lose 30/40% of the neurons in your body, and the ones surrounding it will find a way to not short-circuit but to re-wire themselves to innervate the same muscles that just lost innervation. Unfortunately, when it comes to ALS patients, once you get to that 50% number - and I just made up that number, it's not exactly that of course - then it's too late, because at that point, the plasticity mechanisms have exhausted themselves. So, every time that a neuron dies from that point on it's having an effect. That's what's causing a patient to wake up one morning and you're not able to use your hand quite as well as the day before and a few months later you can no longer hold something in your hand. Because you've lost the plasticity mechanisms and you're at the point now where it's true not just neuron degeneration but circuitry degeneration. And that's when they'll present themselves to the clinic and unfortunately, that's when it's usually too late for clinical intervention.

- Steve

Right, it’s rapid because they have been the neurons that have been holding you steady as the other motor neurons have died off. So, once they go, there's no backup.

- Tyler

There's no backup at that point. Yeah, they've been carrying a big load in their shoulders at that point and once that load gets too heavy, they just collapse and there's no one that would pick it up for them.

- Steve

Your PI, Gary, was also saying that some kind of die off earlier if they're maybe more stressed-out cells if they have less oxygen or may be exposed to toxic metals or whatever it may be. So, what kind of impact does the environment have on maintaining the health of those cells that may have a precondition to degenerate from ALS?

- Tyler

We don't actually quite know. So, the environmental, on ALS are still not perfectly defined because patient to patient there are so many different environments that these patients grew up in and were exposed to that it's hard to say that this in particular is at a higher risk. There are two theories on how ALS works. There's a top-down theory and the bottom up. The top-down theory is saying that it essentially starts in your brain and that due to glutamate-mediated cytotoxicity, which is an overstimulation of the metabolic function in these neurons in your upper motor neurons or in your brain even that leads to toxic effects on the cell, killing motor neurons. And so, the big protein SOD1 is one of the major ones in the ALS research and it stands for Superoxide dismutase which essentially breaks down free radicals that are a result of this overstimulation of the metabolic system. The other theory is the bottom up. And the bottom up means that it starts off at the extremities. It starts off at the connections of

your nerves to the muscles. It's saying that if you can no longer use your hand properly it's because the wiring directly to those muscles is not intact anymore, and then it will spread up and eventually get to you. When it comes to the environment, we think that that probably has more of an effect on the top-down approach, because if these heavy metals in the environments, if these toxins are having an effect on proteins that are regulating the metabolic pathway, which can be sensitive in certain situations, then you can be overstimulating the metabolic pathway, creating toxic free radicals. And if you have this SOD1 mutation, you can't break them down properly, and that's what could lead to the dying off of your motor neurons.

- Tyler

Right. So, back to your project specifically, what does the timeline look like?

- Tyler

So, I'm hoping to have my model developed, as in a full knockout of Stathmin-2 A and 2 B, by the end of this year.

- Steve

So, what's the hope for the application of your findings, say, on the human application level?

- Tyler

On the human application level, it would be if we get to the drug screens, because the drug screens would be as preclinical data that you can approach pharma companies with or even just our own lab here at McGill and say we should bring this to, first of all, another animal model. Because you can't go from a fish to a human right away, but you can say, hey, I know you're studying ALS in rabbits, you're studying ALS in mice. We found a drug here that worked pretty well on our fish to reverse a motor phenotype. You should give it a try too, and then that's a collaboration between two labs. And now you have another lab developing pre-clinical data on a drug on an animal model. And if it shows just as promising or even more promising results, then you can start approaching companies and saying, hey, we have a drug that might work, and then they'll give it a try, if they can. Because the reality is that ALS patients don't have many options right now, unfortunately. So, if you can find something that has strong enough pre-clinical data, there's a need for it right now.

- Steve

So, I'd like to just give a quick shout-out to your team after speaking with them. They were so welcoming and the passion that they had for this pursuit came through so clearly. But I'd have to imagine that when your head is down every day, you're doing all this tedious work and sometimes not seeing any tangible progress - that you have to probably remind yourself of that long-term goal to keep your motivation. Is that a little bit of your mindset when you're in the trenches?

- Tyler

Of course, there are times where you have to remind yourself of the end goal of why you're doing things, but at the same time, we're a very friendly lab who all get along super well. Everyone in the lab, starting from Gary Armstrong at the top, Esteban, the lab manager, Christian, Virginia and Zian. We are a fantastic team, and the reality is that when one of us is in the trenches, there's a chance that someone else is having something they go through and that we help feed off of each other. Right. To see one of our lab members here succeed, to have one of their experiments go super well, it's motivating for the entire group. We're not here as individual students looking to do our own thing and put our heads down and not talk to each other. We want to be involved with each other's projects and we want to support the successes that we see there as well as help troubleshoot when things aren't going right. And that's a big part of the reason I joined this lab, is I got that vibe very early on that it was a team, not a bunch of individuals, and it's made it very easy to do my work here.

- Steve

And probably important to applaud the little wins along the way, too.

- Tyler

Exactly. Because as much as the big win is nice to look forward to at the end, it's the little wins that keep you going.

- Steve

Yeah, well, it was so clear to me what a friendly, collaborative team you have here.

- Steve

Do you collaborate with any other labs right now that are doing similar research?

- Tyler

At the moment, I'm not personally. Our lab has done a couple of small collaborations and right now I'm actually doing a little bit of basic research for the Clinical Research Unit down here at the Neuro. But, it's basically just preclinical stuff, nothing too extravagant. Yet we're trying to provide a little bit of early data so that they can try to kickstart a clinical project, basically.

- Steve

So, I'd like to go into our final section that is focused on your "what if" idea, tearing down all your limiting beliefs about the work that you're doing and thinking if everything goes right, if we hit all our targets and the world just works out in our favour, what could happen?

- Tyler

My what if would be what if labs like mine, labs full of grad students studying basic science and pathways and fundamental building blocks of pathways of disease. What if we had not necessarily unlimited funding, but much more open-ended and loose funding? Because the reality is that science doesn't work through Eureka moments. It doesn't. The biggest discoveries in science are, "Hey, that's kind of weird, let's look into it." It's not, "Oh, wow, that was exactly what I expected. Everything is working as planned." And the reality is that with the way that we receive funding and the way that grants work is you have to be following a specific guideline that you submitted. You have to be following rough outlines of the stuff you're going to research, and there isn't as much freedom to investigate the small, little cool things that you have noticed along the way that you think "Hey, if I spent a little extra time, a little extra money looking into this, maybe I'll get something cool out of it." The prime example is penicillin. Penicillin was an accident, right? Fleming left some bacteria on a plate, came back the next day and saw "Why is there no bacteria growing where this fungus is also growing?" And sure enough, that's where one of the most pivotal discoveries ever in science came from. And I think that there's too much bureaucracy and micromanagement of the way that funding is distributed in science. I think that the biggest limiting factor in the progress of basic science is funding right now, and not only for the actual tools necessary for these discoveries, but also for the personnel. You would get brighter minds in basic research if they were compensated, just like they would be in the industry. And I think that's what my what if question is. What if we can do whatever we want with our money?

- Steve

Yeah, that's a really good answer, and that is definitely a factor too that adds another layer, you want to create an attractive work environment and career path that will bring in even more bright minds.

- Tyler

Yeah.

- Steve

So, it's always been a fascinating thing to me, the straddle between following the traditional methods because you're following a strict template to control all of your variables, but then also some great leaps have been made by innovating and thinking outside the box and getting weird with it in the lab. And so, it just seems like a really tricky one to balance, especially when you're strapped for cash and they're watching your every move.

- Tyler

One of the prime examples is even Alzheimer's research. And one of my mentors, someone who's been instrumental in helping me get to where I am studies Alzheimer's. I have to give a shout out to Dr. Lisa Munter, because she has been instrumental in helping me get to where I am right now. But she studies Alzheimer's research. And this person was telling me that there's been a bottleneck in Alzheimer's research because for the past 50 years or more of Alzheimer's research. It's been focused on the same amyloid plaques and the exact same pathway. Because if you're applying for grants. It's easier to get money for grants if it's what everyone else is studying. If it's the stuff that's been studied for 50 years. Then these approvers are going to read that and say “Okay. Yeah. We'll keep looking into it.” As opposed to the brand new. Super innovative. But there isn't enough data behind it yet to be really worthwhile. But you can't get that data unless you start somewhere, right?

- Steve

Right. How are you supposed to start new streams of thinking?

- Tyler

Exactly. And that's a bottleneck in certain disease researchers.

- Steve

Okay, well, I like that "what if"

- Tyler

Thank you.

- Steve

All right, well, I think we'll have to cut it there. Thanks so much, Tyler for spending that time to do this.

- Tyler

Thank you, Steve. I appreciate it. I'm happy to be here.