Episode 3 Transcript

Lana Zholudeva and Michael Lane

00:00 Stevie: Welcome to the NECTAR 2021 podcast. Nectar 2021 is hosted by Dr. Tilo Kunath at the University of Edinburgh and will be jointly held with the 16th International symposium on neural transplantation and repair, INTR. The conference is sponsored by Guarantors of Brain, CARE - the campaign for Alzheimer's research in Europe, Blue Rock Therapeutics, Roslin Cell Therapy, ProteinTech, Cell and Gene Therapy Catapult, Novo Nordisk, FujiFilm Cellular Dynamics Incorporated, BioLamina, AMSBIO, and 3Brain.

NECTAR - the network for European CNS transplantation and restoration was founded over 25 years ago with the aim of bringing together European groups who share the common goal of protecting, repairing, and restoring the central nervous system from damage caused by degenerative disease or injury. In this episode of the NECTAR 2021 podcast, I talk to Lana Zholudeva, a Postdoctoral Scholar at Gladstone Institutes in San Francisco and Michael Lane, an associate professor in the Department of Neurobiology & Anatomy at Drexel University College of Medicine in Philadelphia about their work on spinal cord injury repair.

01:17 Lana: I work as a postdoctoral scholar in the laboratory of Dr. Todd McDevitt, who focuses on developing and using stem cells to develop stem cell therapeutics, as well as really understand some of the basic biology and how we can manipulate the power of stem cells to mould them into specific cell types that we're interested in studying. So my focus that I bring to the table is really looking at stem cell therapies in the context of spinal cord injury and other neurological diseases.

01:52 Michael: Early on, I guess I was interested in the lack of the regenerative capacity of the central nervous system and really that's where my research focus kinda took off. And for many years I was interested in looking at therapy to try and enhance regeneration, but I sort of stumbled accidentally into the field of cell therapy for tissue repair and was kind of astounded by how promising it was. And so I really, for the last 10 years, my research focus has been centred around the use of novel cell therapies. Particularly with a focus on neural progenitor cells on using these building blocks of the central nervous system to promote spinal cord repair.

02:33 Stevie: What exactly do you mean by spinal cord injury?

02:37 Michael: Well, there's like there's a huge range of different types of spinal cord injury or disease that can occur, degenerative diseases or traumatic injury. And really the focus of my work has been on the latter, which is traumatic injury to the spinal cord, which results in a massive disruption of pathways, both descending pathways from the brain to different spinal levels between different levels in the spinal cord, as well as ascending, say, sensory information, which is carried back up to the brain. And so a traumatic injury disrupts those pathways and results in a massive loss of neurons and glia and all the components at the site of the injury as well as the axons convey that information up and down the spine. And so the research that my research team has focused on is looking at better understanding what happens with these traumatic injuries. What types of plasticity there is for some

modest degree of recovery, even spontaneous changes that can result in little improvements in functional outcome after one of these traumatic events. And how, if we better understand that we can use therapies to harness that plasticity and capitalise on the body's own attempts at self-repair to enhance that plasticity, and further improve functional outcome.

04:03 Stevie: So the two of you mentioned that you are in different institutes the moment, but both working on spinal cord injury and stem cells. So how did you first meet one another, I guess, and get working on this.

04:20 Lana: So I actually got my PhD from his lab.

04:23 Stevie: Yes. Then can you tell me a little bit about your PhD project? So what sort of things are you looking at and are there any particularly exciting results?

04:34 Lana: We discovered a novel cell type, a spinal interneuronal type. They're called V2a interneurons and the fact that they become recruited after spinal cord injury. So these cells have been studied for quite a number of years now. They have been identified in terms of their protein expression and some of their phenotypic characteristics and where they're located in the spinal cord as well as brainstem. But really no one has looked at them and their function and, you know, what they do after a spinal cord injury. And we were lucky enough to collaborate with Dr. Kim Doherty also at Drexel, who had these transgenic animals. And she was kind enough to just give me a couple to do an experiment with. And again, we saw this really cool finding and I proceeded. That was my first paper really showing that these cells become anatomically recruited into the phrenic circuit, which is one of the primary respiratory circuits. And along those lines, and having identified this particular phenotype the V2a interneurons, we were also again interested in answering the question of can we make those in a dish from say, a stem cell? And if we can make them can we then transplant them back into a spinal cord injury to further enhance some of that respiratory recovery. And so part two of my dissertation was really trying to make these cells and then transplant them back and see if they can contribute to recovery. There were mixed results, but essentially we found a correlation that indeed if, if you, if you can get the appropriate cell and they actually survive that transplantation process and connect to the network they do elicit for an enhancement in recovery.

06:26 Michael: A lot of this work is built on landmark studies and papers by many other investigators like Martin Schwab and Gregoire Courtine, and many others in the field who've been studying spinal cord plasticity and changes for a long time. And it was around the early 2000s, people, (clears throat) excuse me, it's around the early 2000s people began to use tracers to map out changes in network functional plasticity. And they really started to highlight the involvement of the spinal interneurons in plasticity after spinal cord injury. And very quickly people realised the spinal interneurons are key components of that plasticity and essentially represent key therapeutic targets for any sort of treatment for a spinal cord injury or disease to tap into these interneurons was really, really important. And they had a remarkable capacity to make new network spontaneously so they can form these new networks very quickly. My research when I moved to Florida to do my postdoc, started to look at how these interneurons were involved with respiratory pathways after high cervical

level injuries or injuries at the neck, which is where most spinal cord injuries actually occur. And with compromised breathing, we saw that these spinal interneurons, again become very important components of that plasticity. The problem is with most of the techniques we had, we couldn't identify what sort of cells these were. Interneurons, there is such a huge number of different types, many hundreds, if not thousands, of types of interneurons in the spinal cord. So Lana's paper, it was really the first to show a certain type of cell. We could finally identify one and go look this is the cell that's actually changing its conductivity. So moving ahead then to cell transplantation, a lot of neural progenitor transplant studies, especially in spinal cord research, was essentially transplanting a mix of interneurons. Again, we didn't know what sorts they weren't, but that's what they were. A heterogeneous, mix of interneurons, and when we transplant them, they had this capacity to promote repair. So our theory was if we could better understand the sorts of interneurons we're using and even make sure they contain these V2a interneurons that Lana identified we can make sure that they get the best chance they have at amplifying that plasticity. So interestingly, when most people make these neural-progenitor cells, the number of V2a there are actually quite small, I think with the way we prepare them for transplant, donor cells don't have high numbers of them. So what Lana did in collaboration with Shelly Sakiyama-Elbert, they have an amazing team where they can from stem cells start to make these interneurons, which is pretty cool. And Lana actually used those techniques to start engineering specific types of spinal interneurons to repair the network, these respiratory networks that we know require these cells. And so, not surprisingly, we had some enhanced efficacy when we start transplanting these cells. So obviously not full repair, but there's a first step in understanding if we can know the components of the network being damaged, we can start using engineering techniques to make donor cells to repair specific networks. And it actually worked surprisingly well. So it leads into many more studies where we can make different types of cells.

09:44 Stevie: Yeah, that's fascinating. So I assume I don't think you mentioned this but is this study in mice, rats?

09:52 Michael: Yes. So most of our preclinical studies are in rats, little bit in mice we do both. Both have beneficial potential for studying respiratory networks which are conserved in, in most mammalian species. But when you're looking at therapies, you obviously want to translate further down the road to be looking at human stem cells and whether or not we can do similar sorts of things. I'll let Lana speak to what she is doing.

10:17 Lana: The easiest way to I guess move, move down the translational pathway is really look at, look at human stem cells and technologies like human induced pluripotent stem cells and learn some of the, some of the ways that we can modify them, engineer them, create specific cell lines, understand the capacity and the limits, limitations, especially of human induced pluripotent stem cells. So understanding from the basic concepts of, again, development all the way through how to make a specific cell type from a stem cell. And then onto A. can we improve our protocols that we use now? B. create specific cell lines that allow us to enrich for specific populations. Can we then isolate those cells and really get 96, 98, a 100 percent pure populations of the cells that we want to study. Of course, it's never a 100 percent and of course use some of the technology, other technologies such as optogenetics. So say if you do want to create a V2a interneuron from a human induced

pluripotent stem cell can you make it responsive to light and activate it and have control over it and its activity?

11:39 Stevie: So I just wondered in your own research, you know, how much and I know you're at preclinical stages but how much interaction with clinicians or maybe individuals with spinal cord injury is involved in your research?

11:56 Michael: As you point out, we come from preclinical labs. We're doing preclinical research in animal models, but we have always maintained a very strong focus on the relevancy of our work to the patient population. Our research centre here, the Marion Murray Spinal Cord Research Centre, does retain very close to ties with clinicians and people living with spinal cord injury. So even in my research team, we have a consultant who comes and joins us for our monthly meetings and he is an individual with lived experience. He has a spinal cord injury himself, and he comes along to join discussions about the relevancy of our work. Does he understand why we're doing it and does he think it's important? Or does he think we're missing something? We also bring in clinicians, who might be studying respiratory therapy, neurosurgery, and translation of cell therapies for people living with spinal cord injury to give us an understanding of what they're willing to translate, what the patients are that they see. And that's a really important perspective to have you make sure when we go back to the bench we're asking medically relevant questions because we are doing medical research. I think when we look at cell therapy, I think the work we're doing is extremely relevant because there's so much as you say that was being translated into patient population already. And I think if we can better understand what's being done, we can significantly improve upon the cell therapies that are already being translated. For people with spinal or brain injury and disease.

13:26 Stevie: Lana, you've talked about what you're doing now, but Michael, is there any sort of, what are the projects that you have going on in the lab at the moment, specifically?

13:38 Michael: So a lot of the work we began with when I first got here, I started refining the populations of cells we were transplanting. So we began engineering, as I say, the spinal interneurons. So we have a control population of donor neurons, but that's just one cell type. And so some of our ongoing projects now are trying to better understand what other types of cells are within these networks that might be good or bad to transplant. Not every cell is going to be beneficial. So we want to make sure we're not transplanting cells that could limit function. And we had some evidence early on that suggested not every cell is great for promoting motor or sensory function. So we have to keep in mind what we're repairing and try and engineer the appropriate cell types for that network. So we're trying to make new sorts of neurons now in collaboration with Todd McDevitt's group at Gladstone and also Shelly Sakiyama's group at the University of Texas, we're identifying new populations of neurons that we can start engineering for transplantation. But it's not just neurons either. The central nervous system is made up of lots of other types of cells. And so in collaboration with other people at New York University, NYU, we're collaborating with Shane Liddlelow to better understand the types of glial cells that might respond to injury, that might be beneficial for injury. And if we start engineering glial cells from stem cells, can we make pro reparative glial cells that will modify the injury environment to make it more repairable so that we can promote regeneration of tissue. Also facilitate the, the survival

and integration of any neurons we also want to transplant. So we're breaking down the individual components of the spinal cord and trying to piece by piece engineer the best, most reparative types of cells to allow for creation of a new network that can facilitate lasting, functionally relevant, and clinically relevant improvements in outcome.

15:40 Lana: And with that information, Michael's group will be very, very excited and tell us, you know, spinal interneuron a and b become highly recruited or do something weird, you know, and then look to me and say, Lana can you make those from human cells? And I would go back to literature, a lot of, a lot of literature, especially with single-cell RNA sequencing, trying to identify specific proteins that you can target in a stem cell to then try and get those proteins to become expressed, which will drive the phenotypic development of the cell type that the group is interested in. There's a lot of, you know, there's a lot of optimization that goes into that. There's a lot of different types of techniques that we have to employ to really convince ourselves that we are indeed creating the phenotype, the particular cell that we are interested in. But after all of that validation, we can, you know, say, okay, well, if you follow this protocol with this particular stem cell line, you should get this at this level of efficiency, this particular phenotype of spinal interneuron. And that's when I can send vials even of cells or even go over there and make these cells and actually transplant into the spinal cord injury model that the Lane group uses.

17:07 Michael: One thing my research team is trying to take into consideration is while we start engineering these types of cells we also appreciate as promising a cell therapy is it's not the cure by itself. And we're trying to look at how we can optimise cell therapy, bringing other things like gene therapy or especially activity-based therapies or neural interfaces, ways to essentially stimulate these cells and control that connectivity. So it's not just the cell types we might want to develop. We're also interested in making sure that they connect the appropriate way. Development, developing cells develop much like the developing central nervous system. Unless you entrain for certain types of activity and reinforce that activity any connections they might make with development, they might lose over time. And so you want to strengthen that.

17:53 Lana: I really love that point because as good of a cell that I can pass to a researcher like Michael to transplant, they're like high schoolers in a sense, you still need to give them directions. I think combinatorial strategies and the way that Michael's lab are employing them, I think will be crucial, a crucial point and part of actually developing a therapy that will help people in the end.

18:22 Stevie: So you're going to be presenting at NECTAR, am I right in saying that this is the first NECTAR that's included a spinal cord injury session?

18:28 Michael: I think there has been talks from people who are doing spinal cord research, but I think this is the first in a long time definitely that had something focused on spinal cord injury and really bringing several investigators from around the world who are looking at spinal cord injury as a disease and a traumatic injury and something that we can develop therapies for, especially cell therapies. So there's actually been several meetings around the world for, I guess a couple of decades now that have all focused on the use of cell therapies and gene therapies for neural injury and disease. And a lot of the focus for what is being

presented at these meetings has been around degenerative diseases in the brain like Parkinson's disease especially. And a lot of the early cell therapies that have been developed and translated were for treating Parkinson's disease as many in this field will know. In parallel, although usually a few years behind the spinal cord injury field was doing similar sorts of studies, so at the American Society for neural therapy and repair, ASNTR, as well as NECTAR over here in Europe. There are several groups who are trying to develop these therapies for a range of injury and disease models throughout the central nervous system and the number of people who've been studying spinal cord injury has been progressively increasing. I think one of the advantages of the spinal cord injury as a model from preclinical perspective is it's a really good template to understand the components of networks we're studying and to engineer appropriate cell types for that network. I feel like we can also learn a lot from what has come before us. Both the preclinical and the translational studies done in Parkinson's disease have set a really nice foundation for all the research now being done in spinal cord injury to start translating these therapies through to the patient population as well and it should not be ignored actually, that the very first stem cell study that was translated into patients, was actually done in spinal cord injury.

20:26 Stevie: A huge thank you to Lana and Michael. And thank you again to all of our sponsors for the 2021 NECTAR INTR conference in Edinburgh, Scotland.