

PODCAST 2:

The Myotubular Trust was set up to fund proof-of-principal research, which is new and innovative work by researchers like yourself, to find a new treatment or cure. Is this helpful for researchers in the field of neuromuscular disease?

The work that really has been wonderfully funded by the Myotubular Trust, and, as an editorial, I have to say the work with the Trust has been amazing.

I think the way that things were set up in terms of 'this is a very good idea; we don't know if it's going to work; why don't you try for a year and see if it works, and tell us how it's going, and then we'll move on for another year'. That's not a model that's used for federal grants or something - where you don't always ever have that opportunity. I think it makes so much sense to say 'this is good, let's see, let's give it a chance. If it doesn't work, okay, then we've invested a year but we haven't put in a long-term commitment. But if it does work then we have the opportunity to keep working at it'. I think it's worked out really well that way. I've been really thankful and I think it was a good strategy and I'm hoping the Trust has agreed as well.

In terms of your research, what has been your strategy?

Our strategy has been two-fold really:

One is to think about this very specific abnormality that I just described. So myotubularin's job is to deactivate this PI3P - it causes it to be broken down. And when you lose it (myotubularin) you get an accumulation of the PI3P. So you could think simple mindedly, if I can just reduce the amount of PI3P, that would be a way to develop the therapy. Too much of something isn't good and if I could reduce it, that would be a way to try to make the muscle better. So that was our one strategy.

The other strategy has been to say, well I don't really know what might work, but I'm going to put a whole bunch of different drugs in a model and see if any of them makes things better. This is kind of a non-biased approach. And we've really now been trying to put the two together from a therapy pathway.

What have you learned?

So we have been trying to figure out ways to reduce this PI3P. What we know is that it's like a see-saw almost. If you think about the levels of PI3P, myotubularin pushes them down, and then there are other things called Kinases that push it up. And then when you lose myotubularin the see-saw goes up. You have too much PI3P because these Kinases aren't being blocked in their activity.

So the thought process we had was that if we could eliminate these Kinases, or modify them, then that might make the see-saw balance back down. And so we used a genetic approach to eliminate one of the two different Kinases that make this PI3P. The Kinases have very long lettered names. One is called PIK3C3 and the other Kinase is called PIK3C2B. I wish there was a simpler way of referring to them. I don't know that there is?!

But they both, as far as we know, make PI3P and once it's made, myotubularin then breaks it down after it's done the function that it does.

So, if you think about it again in the postal code model. In order to go from the membrane into this endosome you need to make PI3P - but then you want to get rid of it - so that you can send this now to the right place. Without MTM [myotubularin] you can't send it to the right place.

So what we did is, we used a genetic trick in mice (using the mouse model of myotubular myopathy) to eliminate one of the two different kinases that make PI3P. We looked to see do we reduce the PI3P, and if so what happens? And so when we target one of them, this thing called PIK3C3, and eliminate that from the mouse, it actually makes things worse. So if we have MTM mice - these are mice that lack the myotubularin, and then also eliminate the PIK3C3 in muscle - the mice are more sick and don't live as long. On the other hand if we target the PIK3C2B in the MTM mice, not only do they **not** get worse, but they get better. In fact they are completely normal. So if we do it before the animals get sick at all, they show no signs of disease and they go on and we've aged them out to 2 years. (This is how long a mouse lives) These mice have lived up to 2 years and look totally normal. That was obviously very exciting.

But in and of itself, to eliminate something before any symptoms happen isn't really a realistic human disease situation. So patients with myotubular myopathy are presenting to me and to other clinicians with weakness. It's hard to imagine a pre-weakness scenario for patients, because pretty much they're being born, and likely in utero have symptoms as well, so there needs to be something that can not only stop disease, but reverse it. And so we wanted to test this idea. Again we wanted to use a genetic trick. And so in this case we used a very specific genetic technology that can eliminate genes at different ages of the mouse. So we took MTM mice and waited till they had muscle weakness and then removed the PIK3C2B. When we did that, not only did they **not** get worse, but they actually got better and completely got back to being normal. Furthermore the whole muscle structure went back to looking normal - so those Triads I talked to you about - at this point in the mouse almost all the Triads would be gone when we knocked out the gene. Then when we removed the PIK3C2B all the Triads came back.

Which I think suggests two things: This is a really outstanding potential therapeutic strategy. But also that there is something about myotubular myopathy, at least on the pathology level of the muscle, that's reversible. I think that this has been shown with the gene therapy work as well, both in the mouse and the dog, that the muscle that can not only be prevented from getting more affected, but it can be reversed. Somehow the structure can be remade in the muscle, and start functioning normally again. Which I wasn't sure from the outset was possible. But it really does look like that's possible.

Your research to date indicates that you need to find a drug that targets this kinase – PIK3C2B. What challenges do you face when trying to find a drug like this?

This kinase was actually identified by many different pharmaceutical companies first, because it's very resistant to the drugs that have been developed that target these types of kinases. So it was identified because when you use different drugs that target these phosphoinositides kinases, the PIP kinases, sometimes there is always activity left over, and when they finally identified what was the source of that activity - it was this PIK3C2B. So by its very nature, it's sort of resistant to a lot of the known inhibitors. But we are not daunted by that. We are going to find a good inhibitor. There are some that exist that have some activity against PIK3C2B. So we tested several of them, first using our fish model again, because that was a way that we could test several drugs at once, in a very rapid way, to kind of prioritise them for bringing to testing in the mouse, and hopefully eventually to consideration for patients.

When we looked at that we found one in particular that has good activity against the PIK3C2B - but also some activity against the one kinase that we think isn't so good, the PIK3C3. That's wortmannin, and it did improve the phenotype in our fish. We then did a trial of that in the mouse and showed that it could very modestly improve the mouse. So if the MTM mouse usually dies at about 35-38 days of age, with wortmannin it lived to about 45 days of age. And so about a 10 day improvement. Which might be significant. I think it's always a challenge - mice aren't humans, maybe 10 days would be quite significant in a patient? Or maybe it would not be significant at all?

So I think where I'm at now is the idea that there is a way to have a better therapy than wortmannin - something that has more specificity against this specific target. If we can identify that, that would be the kind of drug we would want to try to move to trial for patients.

The other thing that came out of the study is that, as one of our controls (when we were doing that specific genetic trick to remove the PIK3C2B) we used an inducible system, and to do that we have to give the animals a specific molecule that induced the gene knock out. It's an anti-cancer drug

that we have been able to use. We noticed that when we just used it by itself the animals lived a little bit longer, and that got us thinking, well maybe this is actually a possible therapy? So now we've gone back and tested this anti-cancer drug with long-term therapy, and it seems to make the mice quite a bit better. And what is I think exciting is two things: one is that this is a possible therapeutic by itself but it also doesn't seem to affect the PI3P levels. So it's quite possible that the combination of this, with a PIK3C2B inhibitor might be better than either of the drugs alone. That's something that we're looking very actively into testing - this idea that - could there be some combination of therapies that could target different parts of the muscle that aren't working right to make things better.

What needs to be done now in order for this treatment to reach patients?

For the PIK3C2B inhibitor we need to identify a more specific, basically a better drug, than what's available right now. We've tried a couple of different strategies. We've been using a modeling approach, using computer modeling with knowledge of what the protein looks like, to see if we can identify chemicals that we would think should bind and make sure that the protein doesn't work right. We have a candidate list of drugs, and now what we're doing is testing them in both patient cells, and also in our zebra fish to see if any of those would make sense to move forward to test in the mouse. We've started only with drugs that are known to be used in patients already, so this is something called 'repurposed' drugs. These are things that have already been tested either by FDA, or Health Canada, or another agency, and thought to be safe in patients. The idea being that if we found something we could then bring it to patients much faster than something that is a new compound.

Are there any risks in trialing drugs that are off label?

There are very few drugs that have no side effects, or no possibility for side effects. So there's always a risk, I think, of using drugs in a new indication (condition), whether it's because of the specific known side effects of those drugs, or because of the possibility that in the setting of MTM there may be something very specific that it could trigger or cause. That's why I think I'm a very strong advocate of the idea of using a real clinical trial methodology and using a control, a placebo, as part of it. Because, if not, first of all one might not know about negative effects, but also one might not know if it works. One of the challenges, especially if you are using something that is available already, and that somebody could get from their doctor possibly, is to lose the ability to truly understand if something works. And in other examples where that has happened, it's made it a bit challenging for the community, I think, and also for the clinicians.

I think 'Mestinon' (pyridostigmine) is an interesting example, because a lot of families have tried this; it's been a bit hard to know whether it truly works or not, because it was never tested against a

placebo. While I suspect that it does have some benefit, not knowing that, means, firstly, it probably will not make it into a standard of care because there was never the evidence there. And secondly, if it doesn't work so much, how long do you keep doing it? Well, we don't have good evidence to know. It puts a little bit of a challenge on things. Moving forward the idea of really trying to, if possible, evaluate drugs in a systematic manner is the best way to go. Although I do understand the desire if there's something that can possibly help, wanting to get it as soon as possible. I think it's a balance and a challenge to know the best way to move forward, but I think ideally, drugs are evaluated, if possible, in the clinical trial setting first.

You mentioned that you have done some work previously testing mestinon (pyridostigmine) in the animal models. How does your new work using the anti-cancer drug compare to the effect you saw of mestinon working for XLMTM?

We did try mestinon (pyridostigmine) in the mouse, and it had a very modest improvement in the exercise tolerance of the mouse. The mouse could run on the treadmill a little bit longer. But it didn't affect survival or strength. So I think we can very clearly say these new targets that we have, seem at least in the mouse, to be much more robust, in terms of their possible impact. What that will mean for patients is a little harder to know but if one can make an extrapolation, I would think that they are going to have much better potential of having a meaningful effect.

What is the effect that you would hope to see in patients, or is it too early to tell?

I think it's too early to tell, and I think that some of the variables are unknowns. So let's say that something does improve strength, how much does that transfer into actual meaningful benefit and what does that mean? So I could make someone stronger but that might not have any functional consequences, or could it only make them slightly stronger and have very dramatic functional consequences? I think the hard thing to know, over time if you have muscle weakness you start to develop joint contractures. The question will be, I think, how fixable are muscles groups and joints where the joint's contracted. So even if I could restore strength in someone's ankle - if they have a fixed ankle contracture, will they ever be able to move that ankle normally anyway? I think that's an unknown. So I think that will be one of the challenges in terms of how much benefit might be able to be appreciated - even in something that works. I think that is a bit of a wait and see.

I think that the areas where one would hope that, in addition to thinking about extremity function, is respiratory function. I would think that there is a real big opportunity to improve breathing, and if someone has some respiratory weakness, but is not either fully ventilated or on that borderline - that's the type of individual who could really quite benefit from just a small improvement of strength.

Then I always think about voice too - a lot of the boys have a very quiet voice. I feel like that is something that could also potentially benefit quite a bit, from just gaining some strength.

Obviously, ultimately, we're hoping to find something that is going to make a dramatic, not just a small impact. But the lessons of other conditions has been it's very hard to predict what you might see in a patient, based on an animal.

Do you think it could help with other MTM-related issues, like peliosis or other complications associated with the condition?

I think it's possible, but I think not knowing what causes those makes it a challenge - and not having those models. The animal models are really outstanding from the muscle perspective, but other non-muscle phenomenon aren't necessarily recreated in the mice. Without seeing them happen, I don't know if they can get better. But, I think, because the drugs that we've been identifying really seem to get at very fundamental ways in which the myotubularin works, there is good reason to think that if you can fix that process of how it regulates the zip codes and the trafficking, then that should be a uniformly good thing: regardless of the type of cell that you are. Whether its a muscle or a blood vessel or something, then its the same process - same problem, but just in different contexts.

In terms of the drug itself, will it be administered orally (by mouth or PEG) or by some other route?

We've been really targeting orally available drugs. We are very excited about gene therapy or protein replacement therapy: but those are going to be infusions and those will have a more complicated route of administration. Complementary therapies, the simpler they are, the better it's going to be. So in my mind, a pill or something that can be taken either by mouth or by G-tube is going to be superior to an infusion or a shot (injection) or something, especially if it's something that patients have to take every day for the rest of their lives.

Do you think the drugs you have identified can be taken alongside with other treatments, like the gene therapy, or will they be robust enough to treat MTM on their own?

I think both are possible. I could foresee a situation where there is something that helps improve the condition, but isn't a curative therapy. That would be a great adjunct either before gene therapy; during; after. I wouldn't see them as being mutually exclusive, but actually quite complementary. Even if gene therapy is completely curative, which would be obviously what we're all hoping for, there is a window of time, before one might get it, that it could be helpful. Or something to help maintain strength after the therapy. I think there is always going to be a place for small molecule drug therapies that act on the pathways that aren't functioning properly.

Is there anything that you can say about clinical trials for x-linked myotubular myopathy at this time, are they imminent?

There are currently no active clinical trials but there are things that are very close both from work that we're doing, and also the work that groups like Audentes are doing. Until the day that the trials start, we won't know when they are available, but I think compared to a year ago, there's every reason to think that we're very close to seeing the first interventional trials for MTM. Whether they are from our work, or from the gene therapy, or both: I see them both potentially happening very soon.