[MUSIC PLAYING]

MIKE MODO: Pleasure being here, and hopefully receiving your input. The story I will be telling you about today is really about the use of neural stem cells as a therapy for stroke. And if rehabilitation might be helpful in that aspect.

But the first thing I want to point out is really in terms of what a stroke brain looks like histologically. You can see here this particular area that is very colorful. That's essentially where we have an area of stroke.

And there's different approaches in terms of trying out therapies that are directed at repairing that damage, as Tom pointed out before. One would be in terms of putting hydrogel in here. And what you actually see here in green is extracellular matrix hydrogel being implanted in the middle cerebral artery stroke. And you can see an almost complete kind of restoration of the tissue after that.

Stem cells, if you just put those into that area they will not do anything. If it's a cavity they will kind of migrate into the existing brain, but they will not form new tissue by themselves. What I will be focusing on today is really putting these stem cells next to the infarct in the peri-infarct tissue. And that's basically being pursued clinically currently. And I'll show you a little bit about the story how we got there.

So stroke. When we talk about these models-- this is particularly the MCA model, where we occlude using a filament that goes through the neck, into the brain, into the Circle of Willis. And we block the middle cerebral artery there.

You really get two kind of typologies in terms of the damage that you see. One is really confined to the stratum, and the other involves the stratum plus the cortex. So when we talk about recovery and functional effects, you always have to bear in mind in the brain, similar to real estate, it's location, location, location.

So just having a straddle stroke, the phenomenology, and the recovery that we might be seeing is quite different versus a stroke that would have straddle plus cortex in terms of the damage. And I will show you some of the data in terms of showing that. The recovery in these animals might also be different.

So if we're talking about that damage, peri-infarct might also mean very different things. And if we're now thinking about applying this clinically, then we have to start to think in terms of where do we place these cells. So next to the infarct is the easy way, but wouldn't it be easier in potentially putting in into the ventricles, because then you could potentially treat a much wider area.

So that was one of the first studies we started out. And this was work we did for ReNeuron, which are currently pursuing this clinically. So bear in mind I got money from them to do this study, so I might be biased in terms of the results that I tell you here. So intraparenchymal versus intracerebral implantation of fetal-derived human neural stem cells in rats. And we transplanted two weeks after the stroke. So it's kind of a sub-chronic kind of treatment in what we're looking at here.

These are the behavioral results in terms of the efficacy that we got here. We used a test that looks at sensory neglect. And I'm sure the audience here is aware of what that is. So in animals, we can probe sensory neglect by putting kind of sticky tape on their fore paws and looking in terms of how long it takes them to remove it. Because if they have neglect they will ignore sensory stimulation of one paw, and it will take them very long to remove that. Versus the intact paw. They will be very fast.

That's what you see here in terms of the-- if you're looking at the top graph-- the white line. That's normal animals. So the symmetry is almost equivalent in terms of the time to remove both sticky tapes. But when you now have a stroke, the red line, then you can see that you have a bias in terms of one direction.

As Tom was mentioning in terms of spontaneous recovery as a major issue in these animal models, but in some cases it's a matter of tweaking the tests. And as you're looking at here, the flat line is pretty consistent for three months using this approach. When we implanted the neural stem cells into the ventricles, you can see we have no effect at all.

And essentially, I can tell you that there was no survival of the cells as well. So as these were human cells in the animal brain, identification is pretty easy using an antibody. But we found nothing present within the brain tissue after ICV injection. If you're looking at the dark blue line, that's the cells that were injected into the parenchyma, the brain tissue next to the infarct.

And gradually, we got a recovery. But you can see we started only testing at four weeks. And that starts to become relevant later in the story. You'll see a gradual decrease over two, three weeks in terms of the asymmetry that we're seeing. If you're now looking at the bottom two graphs, you're really now seeing it split up in terms of the topology of the stroke.

And you can see that the straddle-only kind of lesion we got a much better recovery. The deficit was far less severe in some way as well. And kind of indicating in terms of where should we go clinically if we want to see efficacy. Maybe we should avoid in terms of too many structures being affected by our stroke, and focus on these conditions that are a little bit more focused to the caudate and the putamen.

So that data was sufficient for ReNeuron to take it forward to phase 1 clinical trial in the UK in Glasgow. They did a dose escalation study not designed in terms of seeing any efficacy, but there was a suggestion in terms of changes happening on the different motor scales that they use, especially at the higher doses. But the way the data is presented it's a little bit difficult in terms of seeing clear group of facts here.

Nevertheless, this is now in a phase 2 trial, and things seem to be going well so far. And I should say this data, in terms of intracranial transplantation, very much mirrors in terms of what Dr. [INAUDIBLE] will talk about in a moment as well for another clinical trial.

So the key question that comes up in these self-therapy clinical trials is, what should we do with patients in terms of getting additional therapies? Physical therapy or other conditions. And biologically, we start to be at a point of where we have no idea in terms of what should happen.

So there's some suggestion that maybe physical therapy has exactly the same kind of facts than self-therapy. So maybe the two kind of cancel each other out. Maybe they enhance each other. Because maybe we need to train the cells in the grafts. And there was some suggestion of that in Huntington's disease preclinical studies. Maybe they work against each other.

And so in some ways we really need to do the experiments in terms of finding out. These are two studies in stroke. One using it in a rich environment suggesting that there was some improvement in terms of mesenchymal cells being administered. And being in an enriched environment, the same group found that exercise using the same way did not promote any improvement in terms of the recovery. And please note that there is also a very steep spontaneous recovery in both of these studies.

The other study from a group in Japan also showed some benefit to combining an exercise treadmill running, again, with mesenchymal stem cells. So the suggestion, in some ways, is maybe we have a synergistic effect. And I just put here at the bottom of this slide in terms of what we mean by these different things. And we've got to be a little bit careful in terms of terminology.

In some ways, when we talk about synergistic effects-- and I assume everyone in the audience has a similar view-- we think that it's more than the sum of the parts. So if we have rehab giving 30% of effect, and we have the stem cells giving it 30% of effect, we're talking about synergistic effects. We want to see more than 60%. If it's just 30 and 30 we think about additive effects.

If it's overlapping effect both will just achieve 30 effects and nothing more, never mind in terms of the combination. If we get a little bit more than what we get in either therapy alone we should really talk about sub-additive effects. And maybe that is what is happening here.

So you must forgive me in terms of the slide. I'm a psychologist by training, and we used to call it "boxology." That's my representation of how I see physical therapy. I see kind of an aerobic component, the non-aerobic component. But as the psychologist in me I also see this task integration in terms of that you're doing.

And I'm very open to you kind of saying that this is not the way we work. But that was my impression in terms of how this works in terms of the interactions that I had.

Why is this task integration potentially so important? Because our cells, as well as aerobic exercise, potentially induces neuroplasticity. And neuroplasticity, in terms of all these synapses being formed-- I studied at McGill for a while, so Donald Hebb's *Organization of Behavior* in terms of Hebbian synapses came to mind.

And synapses will only persist if there is a functional activity that preserves them. So you might induce a lot of synapses being formed with physical therapy or cell therapy, but unless you start to kind of give them a task to do, they kind of wither with time. And that is in some ways where I see kind of the interaction between these two.

So maybe aerobic exercise will do the same thing than self-therapy. And it's the task integration maybe that physical therapies normally do. Maybe that's what we need in terms of the cell therapy to really work.

So with Fabrisia Ambrosio and Carmelo Chisari, we set out in terms of starting to think in terms of how can we address this experimentally. The first question was, how can we determine the type and the dose of physical therapy that would be clinically relevant, as well as having a way of actually checking that we administered the right dose.

So we started to do a design of experiment where we did different dosings for exercise, on a treadmill, thinking that this is exactly what you can do clinically potentially as well. As well as delivering your muscular electrical stimulation to the forearm. Again, at different doses. And these are two different values that we chose based on what we found at the literature. So low, medium, and high.

In terms of the exercise, we defined the dose based on the maximum capacity test, in terms of looking at how long these animals can run and at what speed. And then used 80% for their training on that test.

And surprisingly, what we find is that neuromuscular electrical stimulation does not dramatically enhance maximum capacity for performance on the treadmill. Whereas the treadmill running has an effect, and our medium dose kind of produces the most significant effect with some leveling off and a higher dose.

We also looked at the biological effects in terms of different markers. BDNF, VEGF, IGF1, and chloro. And you can see in terms of chloro it has a nice dose response effect, similar to what we had in the treadmill.

And I think the other remarkable thing in here was really that the neuromuscular electrical stimulation had a much higher effect in terms of BDNF induction in the blood compared to exercise. We also looked at the brain, specifically the hippocampus. And we find that there's quite a bit of a difference in terms of the expression levels of genes in the hippocampus versus the same markers in terms of going up and down in the blood.

IGF really emerges as a key player that was significantly correlated with VEGFA as well as chloro expression. Again, here you can see chloro-- nice increase in terms of dose dependency in expression in the brain. So now starting to think back in terms of how we're going to go in terms of self-therapy-- so doing all of these different conditions plus cell therapy was just too much.

So we started to do a contour plot in terms of looking at the expression of improvement versus chloro. And basically indicated that the medium condition really in terms of biomarkers, biological effects, as well as the maximum capacity, was kind of the dose to pursue.

So we now started to design an experiment in vivo to look at the interaction between exercise as well as cell therapy. So we started out with quite a lot of animals-- 100 animals randomly assigned to either a sham group-- all this was done under blinding. And different people were doing different components of the experiments.

Blinding was really performed by creating sham incisions. So as we're doing MCO surgery we do an incision on the neck. So all our sham animals got an incision on the neck at the same time from a surgeon, versus the people that were casting.

We then did MRI. And similar to what you would do in a clinical trial, we did inclusion and exclusion criteria in terms of a minimal volume of a lesion size. No hemorrhages. Had to have a good running ability, otherwise we would exclude them.

And once they received cell therapy, we also did the sham surgery in terms of the skull. So that those people that were doing the behavioral testing just based on external signs, they could not tell which condition the animals were in.

So we had a control group, an MCO sham group. Sham here being the incision on the skull for the intracerebral transplantation. 400,000 cells were administered into the parenchyma. A group that only had exercise, the medium condition, and a group that had the combined treatments.

One thing that we start to get a little bit conscientious about-- and Pat Kochanek mentioned that yesterday, in terms of how you have quality control in terms of making sure that your therapy was rightly administered. You might just think about that in terms of physical therapy, but we're also thinking about that in terms of the cells.

And this is-- on the left side here it's an example in terms of how just your storage of the cells during the transplantation procedure, in terms of room temperature, versus on ice, versus at physionormal temperature. How that affects viability over the day. And you can see it goes down with most of these, but at room temperature it stays pretty flat using HypoThermosol.

So we now do a quality control check during our injection procedure in terms of looking at the viability of the cells as they are in the vial before they go into the syringe. And then once we finish with the syringe we do another viability check to make sure that whatever's left in the syringe actually has a high viability, or we would exclude that transplantation from our study.

This is the verification in terms of the delivery of the physical therapy. So using the maximum capacity test at different times gives us a way of checking that our exercise really had an effect in the conditions that got exercise as a physical therapy. So you can see those two groups going up, whereas all the other groups with stroke go down. And the controls also go down a little bit.

So if you now start to think about interpreting some of those results, you also start to worry about alternative explanations. So is it that animals that have exercised maybe they are not gaining as much weight? And that's maybe why they perform better in terms of behavioral testing.

So we measured weight over time to make sure that all the animals had more or less an equivalent weight. And you see that all the MCO animals here were pretty much the same, but the control animals were a little bit heavier. Maybe that explains why they are running less well at the end.

Now the other question that comes up is, what if our physical therapy in terms of making these animals exercise, what if they are now getting stressed? So stress would have an adverse effect potentially in terms of learning. And there is some demonstrations, for instance, that BDNF, in the presence of stress, will not basically have in fact even after exercise.

So we looked at corticosterone, and you can see it decreases with stroke, but we have a slight increase with exercise. But nothing at the level of what you would expect in terms of a stressful intervention inducing in terms of this biomarker.

We're now looking at the behavior recovery again. We have this sticky tape bilateral symmetry test. And I'll start out with that. You can see prior to any injury in terms of-- the animals have pretty much a normal symmetry or no symmetry. With the stroke, the red line-- it goes all the way up. And the red line stays pretty much flat all the way out through our testing.

Now, for the things that are more interesting to happen, and that's why I was mentioning before in terms of the task integration, please note that some of these groups, they only start to get better in terms of you see the curve changing once we start the behavioral testing. And to me, as a psychologist, that starts to say that it's something about the behavioral testing. And to me that is really task integration that drives some of the things that we're seeing here.

So exercise as well as cell therapy are pretty flat over four weeks after administering them. And it's only once we start the behavioral testing that we gradually can see kind of an effect emerging.

If you're now looking at the absolute effects in terms of at the point of stroke versus the final outcome-- and we did that comparison, because you can notice that even though they were randomly assigned there is slight differences in terms of the magnitude of deficit in different groups.

You can see that exercise induces a pretty robust recovery, a little bit more than cell therapy. And if you're now looking at the combination we get a little bit of an additive effect. But it's nowhere close to both being added up. It's really just like a 10% boost that we're seeing in terms of adding that on.

If you're now looking at the footfall test-- and this is similar to what Tom described in terms of animals making a mistake on a ladder-- we can again see a recovery. But in this case, we really don't see an added effect in terms of the rehab. We actually might even see a little bit less of an effect.

So this might relate back to some of the other discussions that happened yesterday evening in terms of modalities specificity. It might be more specific than just modalities, but it might be task-specific.

We've seen in the past that some of the behaviors they respond well to cell therapy, meaning they get an effect. Other behaviors don't get an effect. This might have to do with exactly where our cells have been placed inside the brain-- what's the area that that area is driving in terms of behavior, and maybe putting cells in other places. We might get a more complete recovery on different tasks.

It might have to do with the task itself in terms of how susceptible it is to training. And that we might have this kind of task specific integration.

The other thing that I cannot tell you much about, but we've also done imaging on these animals at the pre time point as well as the final time point. The pre time point-- the pre transplant time point, they all got T2 and DTI. DTI to the point of where we can do tractography, in terms of looking at the number of streamlines present in different areas, and connecting different regions.

But at the final time point we also did the functional MRI by stimulating the forepaw. So we did the left forepaw and the right forepaw. So one should be a normal response, and the other one should be a diseased response in terms of either not being there, or something being different in that region. And then where is it going to now look in terms of what our treatment interventions do with it.

The other measure that we did was cerebral blood volume in terms of looking at do we got re-profusion, or a change in profusion in the areas next to the stroke. In terms of just macroscopic structural changes, that's the analysis that we've finished so far. We did not really see a dramatic effect on lesion volumes, total parenchymal volumes, mid-line shift, or lateral ventricle volume. And that's pretty much in line with what we previously reported in sorts of stem cell therapies itself.

So we might not have a macroscopic effect, but the question is now within the areas that are damaged, do we start to see other effects in terms of microstructure that we would pick up on the fusion MR, or do we start to see functional changes, for instance, in the exercise group where we would expect maybe a better connectivity.

The last data slide I have here for you is really now breaking that up in terms of the lesion topology. And that's now the question in terms of, do we see differences. So some tests we start to see a little bit of a difference in terms of striatum versus the cortex. But the effects are pretty much what one would expect in terms of the stroke itself, rather than being dramatically different in terms of an interaction between the lesion topology as well as the therapy that we're giving.

So that just lets me to kind of look out in terms of where we go from here. So I think physical and cell therapy combinations are likely to produce some additive effects. I don't think there is the room there for synergistic effects really to occur.

Task integration might be the key component in terms of harnessing the efficacy from cell therapy. So it might not necessarily that you need to have exercise to induce more of an effect that you're already doing in terms of plasticity with the cell therapy.

I think it also remains unclear from the study we've done so far if other forms of physical therapy might exert similar effects. What now about-- neuromuscular electrical stimulation, maybe we have a more pinpointed kind of stimulation of particular connections that are needed.

I think the work that we've done also raises the question if pre-transplant physical therapy might reduce the impact of cell therapy. So if we already have a 30% therapeutic effect of rehabilitation services on our outcome, how much more can we push down in terms of cell therapy might be that that room might be much smaller than we assumed from our animal studies.

The final point I want to make is really in terms of our animal models. Are they sufficiently adequate in terms of addressing these combination therapies? And it really comes down to the question in terms of how much can we get over recovery. If we have 30%, 40% of facts in one therapy, and we're now adding it up, maybe the room that is there in terms of recovery without really replacing tissue is not all that great.

And so moving on to other species such as primates where maybe the initial effect might be lower-- maybe not 40%, 50%, but maybe just 10%, 20%-- there's maybe more room in terms of being able to see combination effects that were in these.

So that just lets me thank Fabrisia and Carmelo in terms of being fantastic collaborators on this project. As well as Harmon and Jeff who are here, and who will have a poster on this later on. And all the other people that have been involved in doing this.

This has been two years so far and we're still going. We have six people on it. It's a lot of work in terms of getting through all this, and thank you for the privilege of your time.