## WEBINAR:

# Intracerebral Transplantation of Autologous Bone Marrow Stem Cells Produces Functional Recovery in Rats with Long-Term Stable Strokes

**Questions and answers from the February 16, 2023 webinar titled "Intracerebral Transplantation of Autologous Bone Marrow Stem Cells Produces Functional Recovery in Rats with Long-Term Stable Strokes"**

This document includes questions we received and answered during the webinar, as well as those that we did not have time to address

1. **Which MRI was used?**

   The MRI that was used is the Aspect Imaging M-Series M7 Compact MRI.

2. **This research is very exciting. How long do you think it will be before this protocol can be applied to human patients?**

   [Max Myers] That's the hard part. Getting them into humans is difficult, as it should be. We want this to be the safest thing possible to get into people. Our hope was to get this rolling for our submission to the FDA by October 2022. So about five months ago. unfortunately, that did not happen. Right now, we're currently writing up our submission, and we're also working on a couple of last-minute tests, specifically, testing the cells themselves. So, we're going through and doing practice runs in the bioreactor, getting human bone marrow from donors in the hospital, growing it up, and then sending it out for sterility testing.

   One of the biggest hurdles that we see that other teams might not have, using these genetically engineered cells where they can grow a huge, large group of cells and freeze them down. You're ready to just put them all into the same patients or all the same cells into different patients, so each of ours is specifically tailored for each patient. We have to be able to harvest our cells from the bioreactor and take them to the operating room within four to six hours. That means we must get sterility

testing; we must get different toxin testing, all done within those few hours. So right now, that's the biggest hurdle we're seeing. We're on a good road. We're finding good partners to help us with this. We're trying to go as slow as possible to make sure that these cells are safe and effective once they come out of this bioreactor. We're getting runs that are consistently safe. They're consistently not contaminated. They're consistently the cells that we want. So right now, we're just sort of maximizing our protocol and trying to make sure that what we do when we get to the clinic is, is the safest possible.

3. **Did you pre-scan, so each animal was under its own control?**

   [Max Myers] We did do MRIs on the first day. I'm actually not showing that here because on day negative 28, the day following the stroke, there's a significant amount of edema, swelling, and fluid around the infarct itself. And so, what we actually saw was that the size of the infarct was significantly larger on that day after the stroke as opposed to a month later when all that swelling goes down, and the infarct stabilizes. That's why right here we're only showing going from day zero because, looking at day negative 28, it's not adding anything important. It would just be a much larger stroke due to all that swelling around. But yeah,  we did run that on that day, negative 28, to use its own control.

4. **Where in the brain did you inject MSC after MCAO?**

   [Max Myers] The Peri infarct area, the region outlining the infarct, so we targeted the medial side of that, too, in between the infarct and the ventricles. We did multiple trajectories from the anterior to the posterior as well.

5. **If we use hematopoietic stem cells instead of MSCs, what will be the differences?**

   [Max Myers] Yeah, it's a great question. I'll be honest; I don't know as much about, hematopoetic stem cells. I think there is some research possibly using those, but you know, it'd be hard for me to answer right now just because we are looking at the MSCs; there's been a lot more research basically looking at the MSC specifically because they're the lineage that differentiates to neural tissue. I think there's just a

wider breadth of research being previously done, but it's not a bad idea to look at the HSCs as well.

6. **What has been the biggest challenge throughout the process?**

   [Max Myers] So the biggest challenge, I would say, in this animal study was the timeline. We wanted to get this done as soon as possible. This project is being funded by a generous donor in Philadelphia who was treated at Jefferson for a stroke, and he wanted to see a new way to treat the strokes. This research started many, many years ago before I was brought on. Now that this donor's getting into his older age, we want to be able to get this going before he passes. Being able to do this quickly and efficiently was one of the difficulties.

   And I would say the biggest difficulty is, translating it to the clinic, to the operating room in this scenario is working with the FDA and making sure that all of our testing is done and that we've done everything the same way that we plan on doing in humans. Figuring out the correct dosing. That's probably been the most difficult part because we really want to test, retest, and retest to make sure that when this does go into people, it is going to be the safest thing possible so that we see these cells; don't have any contamination, are the correct cell that we want, they're not differentiated, they're not further down their lineage. All the testing that we've had to do, it's probably been, you know, the most difficult.

7. **Why did you choose the MRI sequences that you chose?**

   [Max Myers] We tried to mimic the same MRIs that a patient would get when they come into the hospital after a stroke. Usually, a patient has a stroke, let's say they come in quickly or they take a while to come in, you have to get imaging to assess the size of the lesion. There are a couple of different sequences that you can do that are commonly used in the clinic. Two of those are the T1 and the T2. The T1, basically, shows the inflammation, the lesion, or anything fluid will be dark. So, the brain is myelinated. It will come up a different color as to bone, or as to liquid or, in this case, with the T1, we see a very dark shade with inflammation or fluid.

   With the T2, it's basically the opposite of that. And so, we have a very bright hyperintensity with the fluid or with inflammation. So, it gives a detailed picture.

There are other MRI sequences. And right now, a big area of study is looking at different sequences for different purposes. These are two of the most commonly used. There's also diffusion weight and imaging, which is a DWI, but we found that the clearest picture that we got was from the T1s and the T2s. The T2s specifically gives us the best ability to quantify these lesions in order to make assessments in the size of it.

8. **What about the transdifferentiation of these stem cells becoming downstream lineage cells as adipocytes, osteoblasts and chondrocytes?**

[Max Myers] One of the biggest questions, too is - these are stem cells. Stem cells break off and become other types of cells. When you're a developing fetus, you have three different cell types that come out of the blastocyst and will become different tissues in your body. And so with mesenchymal stem cells, you have adipocytes which turn into fat; osteoblast, which turns into bone chondrocytes, which are like a connective tissue or cartilage. One of the most important things is to look at; are these cells staying stem cells when they are put into the brain? So, the first thing we did was flow cytometry.

You have these positive markers, and you have a couple of negative markers. When they're all shown together, you know that we have a mesenchymal stem cell. It's been widely published, and there are guidelines for it, because we're growing everything up in a bioreactor; we have optimized the amount of time it takes them to grow into this specific type of cell that they are so that they are not differentiating downstream. So, we were very confident when we put them into the brain. But one of the things we also noticed was that since we left them in for a while, we wanted to make sure that they're not differentiating when they're in the brain.

Right now, we're actually doing those studies. We haven't totally finished that up yet where we're actually staining our brains for these markers, for those different types of cells, the adipocyte fat cells conjure, chondrocytes or the cartilage cells or the osteocyte or osteoblasts for the bone cells to make sure that these aren't going to be growing when they actually are injected. We haven't totally finished it up, but I will say we have not found any. It really seems that when we put these cells in,

they're staying there; they're reducing the inflammation. They're lowering the pro-inflammatory cytokines, increasing the anti-inflammatory cytokines, and the overall reactivity of the brain is staying level, and that's adding to the functional recovery. There's still a lot of work to do to know exactly what is happening. We have those answers that I just explained, but the jury's still out on what exactly is causing this benefit as opposed to just the cytokine change or growth marker change. So, to answer the question, in short, we are not seeing any downstream lineage transdifferentiation once implanted in the brain.

9. **Which strain and sex of rats did you use for MCAo?**

   [Max Myers] We used male, and one of the big questions when we've been approaching the FDA is, well, how do we know this will work in females If you're just testing males? Testing female rats specifically is difficult with their estrogen cycles. You get massive changes that would sort of skew our results. The easiest way to currently do it is to test in males. That way, you don't have to deal with all the cycle issues with the female rats. The next step in this is to test female rats, and we're currently developing protocols to do that.

   If you look at a lot of the preclinical research that's been done, it's mostly done in male rats. But we don't really believe that it will cause too much of an issue. The biggest issue for us was the change in bone marrow following the estradiol cycles and the female rats. When testing the male rats, you sort of get around that. But we believe that once we start to inject them into patients, this shouldn't really be a problem.