WEBINAR:

Evaluating Intracerebral Injections of Radiation Nanomedicine in a Preclinical Mouse Model of Glioblastoma

Questions and answers from the March 29, 2023, webinar titled "Evaluating Intracerebral of Radiation Nanomedicine in a Preclinical Mouse Model of Glioblastoma"

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

What is the usual size of a recurrent GBM? Wouldn't the mean tissue path of Lu177 cause unnecessary dose to nearby healthy tissues if we are only talking about small lesions?

[Constantine Georgiou] I assume you're at similar questions talking about clinical stuff. And one thing I didn't mention in detail was the path length of the beta particles emitted by the lutetium 177. The maximum range is only two millimeters. So, if you're, if you're talking clinically, two millimeters is a lot. It's very, very, very small. For a human sized brain, right. If you can inject these nanoparticles along a tumor mark margin, where there's some proportion of tumor cells, you will not end up radiating very far past those two millimeters, the average range is tenfold less.

So, it's 0.2 millimeters. You're really focusing on a very, very small portion of that very small treatment volume. There are other radioisotopes that have much longer range, for example, just off the top of my head, atrium 90 is one that has a maximum range of a little bit, I think, if I'm remembering correctly over a little bit over at one centimeter. I guess the question is sort of lutetium is sort of one of those ideal isotopes with the physical properties that enable you to sort of very, very specifically, physically control where the dose is being deposited.

Are there any previous stability studies done on the [177Lu]-CED that you used?

[Constantine Georgiou] No, to our knowledge, this is the first-time gold nanoparticles have been used for CED. However, one of the things I didn't talk about was all the previous work done with this polymer and the gold nanoparticles. I think I briefly mentioned that it was used initially intravenously for different tumor models by previous students from our lab. So when I came in, a lot of the development of that nanoparticle the stability testing, both of the lutetium to the polymer, the polymer itself, and the polymer connection to the gold nanoparticle that was all previously performed and published, you can if you Google, the Reilley lab, some of the papers will come up from Simmyung Yook, I think he is probably the primary author on most of those.

So, a lot of that work was done by previous students. I was I was very lucky to be able to go straight *in vivo* and sort of build off the work of the previous students there. But yeah, they we've shown that it's very stable and doesn't separate.

Why do you hypothesize the tumors were smaller in the non-radiolabeled gold particle group?

[Constantine Georgiou] Yeah, I'll just go back to that slide. It was a little bit of a weird result, because like, as you can see here, like there's a couple of them have pretty small volume. Gold nanoparticles, depending on what papers you read, can have some therapeutic effect, in terms of they can generate like reactive oxygen species. That could be one of the reasons why the tumor volume was lower. However, the signal, the on average was a little bit lower, and the standard deviation was a little bit tighter. So, there might have been some early effect in the nanoparticles like that we can't know for sure. That might be the reason to hypothesize, but like I mentioned, in the survival, there is like on average, like the mice live a little bit longer, but again, not statistically significant. So I think any effects of the nonradioactive nanoparticles are small and probably pretty hard to tell the difference.

There are some papers that have injected intravenously a lot more gold nanoparticles and use them in combination with external beam radiotherapy. I think, like the Handfield papers, were like some of the first to show this. So, you can work with non-radio labeled nanoparticles in them having some controlling tumor volume, but yeah, it's hard. It's hard to tell. Based on just the MRI images and sort of, what we already started working with

what we dealt with and those are the results but yeah, we don't think it was anything meaningful, even though there were some measurement differences that we could detect, the survival ended up being basically the same.

Did you have any collateral effects on mouses related to the orthotopic implantation and injections?

[Constantine Georgiou] I'll try to interpret collateral but these methods, I don't know if the person wants to, like clarify what they mean by that, in case I'm misinterpreting. But in terms of collateral, the injection is fairly the surgical procedure to do this injection is fairly, I mean, it's obviously invasive, you're injecting into the brain. But in terms of like, work you're doing on the mouse, it's quite small, you're only I'll just briefly go over sort of what the procedure is. But basically, you're cutting a small slice of the of the scalp away, or you make an incision in the scalp, open the skull, you drill a small hole into the skull, and that's where you insert the needle. This is something that's been done for I don't know, probably decades at this point in terms of establishing GBM models in mice, and then also doing the intratumoral intracranial injections. So, the mice all recover from the surgery, when it goes a while and there's no sort of effect of the treatment of the surgery on the mice if I'm understanding your question correctly.

How do you know the limited survival of the non-labeled group was due to tumor growth as opposed to gold nanoparticle toxicity? Did you perform non radiolabeled controls (without tumor) to assess toxicity of the gold particles?

[Constantine Georgiou] So we didn't, however, we didn't measure the toxicity that I showed. We weren't anticipating any toxicity from the gold nanoparticles themselves. Their survival wasn't impacted compared to control. In terms of non-tumor bearing mice, I think we did try and see if there was any toxicity from the gold nanoparticles in non-tumor bearing mice. But as far as we know, the gold nanoparticles themselves aren't toxic and aren't causing lethality. It's related to the tumor, especially because when we looked at these treated mice with a tumor, their symptoms were result in a tumor growth, you could see some stuff like domain, you could see paralysis, in some of the mice. Those are sort of the symptoms of large tumor growth in the brain rather than the nanoparticle. So, we're confident that the nanoparticles aren't toxic themselves, even though they might have

some cytotoxic effect on the tumor. But we didn't evaluate that for certain.

Do you think there are any other interesting radionuclides that will be useful for your work?

[Constantine Georgiou] But alpha particles are super exciting right now, in terms of in radio pharmaceutical field, alone, I think a couple, I think maybe like five, six years ago, by now, there was a paper published showing someone a patient in Germany, I think, had probably 100 or more prostate cancer metastases, and only after a couple of cycles and actinium labeled, radio-pharmaceutical product one for PSMA, he was basically cured of the hundreds of metastases that he had on his body, which is really incredible, I think it really took up what people thought about alpha particles and showed that it can be really useful due to their physical properties. They do have a pretty small range and they're very high energy. There's a lot more potential for toxicity from that. But I think if we could swap it with it.

So, I think that using actinium could be really useful if another student wants to try that. There's also another radioisotope called, I believe, terbium 161. It has almost entirely the same properties as lutetium, the same half-life, the same like beta particle energy, but it also use its OJ electrons, which are sort of very, very short range, but very densely ionizing electrons as well, compared to the beta particles, which are much less densely ionizing, but they travel further. So, I think that that would be a really interesting radio isotope to study, I think one of the problems is that it's not produced nearly as widely as lutetium is I mean, like with lutetium, there is a significant industrial process, and radial chemical processes set up to create large amounts of lutetium. I don't think it's the same way for terbium and even actinium, it's very high in demand. And the production of it is basically, I think there's like two or three places in the world that can even extract it, and then you don't produce it like you do lutetium, like in a nuclear reactor, you extract it from nuclear waste. So, there's a lot less of it, even though it's very interesting and potentially very powerful for real pharmaceutical treatments. I think those are two off the top of my head that are interesting.

Why do the biodistribution results for the R-Hemisphere go above 100% ID/g?

[Constantine Georgiou] This is injected dose per gram. It's a mathematical calculation where you measure the amount of radioactivity injected, compared to the radioactivity in the organ at different times, and you divide it by the weight. So, when it comes to these, right hemisphere of the brains, they're pretty small weight, so you end up dividing by a number less than one. So, you end up having a larger percent ID per gram. And that's common for work like this, where you have a lot of radiation and a small point, a small massive tissue. So yeah, it's a sort of a mathematical, not artifact, but the consequence of how we calculate percent ID per gram, if you remove the program part, I think it would be it would all look more normal.

What are the further steps that are needed for clinical use?

[Constantine Georgiou] I think these are all really good first results. Obviously, I think this needs to be, if this has to be translated and pushed towards clinical stuff, doing this in a larger animal model with something like maybe like a pig would be interesting and then having like a neurosurgical technique team evaluate this in a pig doing similar experiments, you can do imaging on the pig as well to see if the results translate one to one. There are neurosurgical technique teams that do this technique.

Clinically, right now, there's it's very, it's not standard. Across the board. There's a group I think, in New York who has done this with radio labeled antibodies, rather than nanoparticles. So, It think it's possible, it just needs a lot of groundwork. There's just a lot more development that needs to be evaluated, and that needs to be done in more animal models that are closer to humans. Our lab works with radio labeled antibodies as well and some of the projects have led to a kit development where it's basically like a vial of the antibody, that you add your radiation, you shake it up for a bit, and it's ready to go. I think if we could develop something like that, for the nanoparticles, that'd be really cool. It'd be a little bit more complicated than just adding radiation shielding up and then you're good to go, I think there'd have to be a couple more steps than that, but and sort of making the production side. High volume would also obviously be necessary and potentially interesting for future research.

How did you measure the tumor size in a living mouse?

[Constantine Georgiou] We can measure the tumour diameter/volume using MRI. BLI only quantitatively measures the light signal from the tumour which correlates with size but is not a measurement of size itself.