WEBINAR:

(April 24, 2024) Accelerating the Delivery of New Treatments for Children with Neuroblastoma with the MRI-Guided Paediatric Mouse Hospital

Questions and answers from the April 24, 2024 webinar titled: *"Accelerating the Delivery of New Treatments for Children with Neuroblastoma with the MRI-Guided Paediatric Mouse Hospital"*

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

1. **What about your opinion on gene therapy, on neuroblastoma, particularly with suicide gene delivery.**

Yann Jamin: It's been a long time since my PH.D year where I was trying to image the efficiacy of suicide gene therapy using prodrug activiating enzyme. In the context of neuroblastoma, any strategy that aims to increase therapy targeting to the tumour while sparing normal tissue is to be pursue. This is of particular importance in children with neuroblastoma who are still developing and I believe 75% of cancer survivors are suffering from delibating side effects and will develop other life-threatening conditions.

2. **Do you insist on using only mice with surgically implanted central catheters for administration of drugs?**

Yann Jamin: We do not follow the clinical practice in our animals but use other routes of administration (oral, intra peritoneal or intra venous through the tail vein).

3. **Do you know why neuroblastoma has such good contrast on the T2 image? Why tumor tissue have much longer T2 when normal tissue?**

Yann Jamin: Let me rephrase from the live Q&A session. An important reason for the good contrast on the T2-weighted images presented in the webinar lies is a small caveat: They are not T2-weighted images but they are fat-suppressed T2-weighted images. With a long T2 fat , normal T2-weigthed images will be saturated by fat. This is why both in the clinic

and in our experiments, a Short-Tau Inversion Recovery (STIR) T2-weigthed Fast Spin Echo (FSE), which suppress short T1 component (mainly fat). Tumour cells have both higher T1 and T2 value than normal cells, this is the observation that was the motivation for building MRI scanner for cancer diagnosis. I do not know the actual reason why it is the case, but it will have to do both with different water physical properties within cancer cell combined with higher cell density in tumour compared to normal tissue.

4. **Could you use other preclinical imaging techniques to measure tumor volume in these models?**

Yann Jamin: Sure. I mean, any technique is usable with a certain degree of sensitivity and specificity. The one that come in mind is a very nice paper (Preclinical Models for Neuroblastoma: Establishing a Baseline for Treatment | PLOS ONE) using/comparing high-frequency ultrasound and MRI. So, with ultrasound, one of the issues is that it is very user dependent. So, if the main person that is doing this is not available the day of, then it will be very difficult for someone to be able to jump in and do this. And I know people think of MRI as something very, very complicated and difficult to use, but I hope I convinced you that it's not. Our onboarding session is just 20 min. If you start with ultrasound to look at this model, it would take a lot of time before you can accurately do it in a quick and reproducible manner. CT is possible. I think you will have to is not very good at soft tissue contrast compared to the MRI; it is possible with some contrast agents. And, if we could have a bioluminescence or fluorescence marker in this model in the construct that will probably simplify things.

Evan Poon: Can I ask a question to Yann about the comparison of the detection le limit between ultrasound and MRI for mice tumor: are there any difference if we are trying to look at minimal residual disease?

Yann Jamin: Well, if you were looking is minimal residual disease, you will probably...It's a complicated question. I would say - I do not know. We would have to try. But yeah, I think the resolution of ultrasound is slightly better than MRI, but you will have to know where to look.

5. **These models are very chemo sensitive. How relevant is it to the early phase clinical trial populations?**

Yann Jamin: It's a very, very good question. So, I always think of the TH-MYCN model as a very good model of childhood neuroblastoma, at the time of diagnostic, where the tumors are extremely responsive to chemotherapy. And, this is why we were hoping, originally, to

be able to show you some of the work we have done, and I have shown you one very quick slide, where we now try to develop these models that more resistant to, or developed resistance to therapy, to see how relevant they to the early phase population.

Evon Poon: I would like to come comment on that as well. So, we have developed now 3 different models from the TH MYCN model, that has more chemo resistant, which are more clinically relevant. So, we have developed chemo-resistant model towards in induction therapy, and also recently to temozolomide, which is used in the second line of treatment and as a chemotherapy backbone for early-phase clinical trial.

6. **What is the smallest tumor that you can detect manually.**

Yann Jamin: Again, it depends. Some of my colleagues with more experience will come one day and would tell me like: “This is a one or 2,” which basically means 1 and 2 millimeters. And sometimes they're right, and sometimes there is nothing to see - this is the beauty of MRI. So yes, it really depends on the experience, the size, the location. I would say 1, 2 millimeters probably.

Evon Poon: I would like to comment as well. It's very easy to rupture the tumor if you try to pulpate too hard. So, I think normally, with very small tumor, we would prefer to image it to minimize the risk.