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NF2 RESEARCH AT MASSACHUSETTS GENERAL

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>> DR. VIJAYA RAMESH: I'm from Massachusetts General Hospital in Boston. I work in a collaborative effort with a doctor who fortunately was leading the group. Also work with clinicians, a pathologist and a few other clinicians. Bob Martusa's lab. And I've been working on NF 2 for more than 15 years, particularly focused on meningiomas. I got into the program right after learning of the gene.

As I said, it's my pleasure and I'm truly honored to be here. I want to thank the Midwest NF group for visiting Boston. Laura and a friend of Laura came and visited us when we explained what we did. And then we followed -- I'm here to tell you what we are doing at the lab as a basic science research. I am not a clinician. We get the materials from the patients and try to work with them and try to understand how the NF 2 gene functions and how we can come up with better treatments.

I tend to go fast. Please feel free to stop me if I go too fast. Or if you do not understand something, then I can explain.

So this is mostly from my lab the whole work that I'm going to explain you. So, many of you know, just to give you a brief introduction to NF 2, it is an autosomal dominant inheritance. If you're not familiar with the term, it is inherited in the family. A parent who has NF 2 has a 50% chance of passing on that gene to a child in the family. And it's mostly characterized by the Schwannomas. When you say bilateral, it's on both sides, meningiomas, multiple meningiomas. This is

a very typical familial inheritance. You also have sporadic individuals in a population who get meningiomas, not multiple, but few meningiomas, one or two. Schwannomas are unilateral on one side. The gene must clone based on pure studies following the genetics of this disease in the families and these sporadic. The gene must originally position or map to particular chromosome 22, which subsequently led to the identification of the gene that Jim named. Moesin ezrin Raudixin-like protein.

So, again, I don't have to explain this to the audience. You know about vestibular Schwannomas. This is a meningioma of a patient. This all occurred, it is not in the brain, but on the surface of the brain. So it's almost like it's unique. It's not a brain tumor; it is benign tumor that grows on the surface of the brain.

So the challenge, this is what I'm going to spend some time: What is the thinking here?

Human NF 2s are primarily what we think of hematoma. It's benign tumors. There are hamartoma symptoms, a complex which I will be referring to. So these are benign tumors. And then when you have -- and the gene came along, immediately everybody, including our lab, started thinking in terms of okay, so it's a new family. Is it functioning similar to the protein?

So a few years we spent on understanding how these proteins, the new protein that came to this family member functions. So we spent time getting antibodies, making mouse models.

So when you say the mouse models is to take the NF 2 gene because the NF 2

gene is completely in meningiomas and Schwannomas. You have one inherited in the blood and the second loss of function happens in the tumor cells themselves. In this case, in the meningeal cells, or what I refer to as arachnoidal cells. This is the arachnoid layer of the meninges are the Schwann cells. So both copies of the gene are lost in these tumors, with a total loss of function.

>> Could you slow down just a little bit? The captioner would have an easier time, I think.

>> DR. VIJAYA RAMESH: So the NF gene has two copies. One is inherited, then the other copy is somatic copy, what we say. So both copies of the gene is lost in the tumors. That's all you have to remember. So when the gene is lost, the protein is completely gone. The myelin protein is no longer there.

So for mouse, coming back, I will be referring to two terms: A conventional narco mice is where the gene is removed in every cell in the body. So those mice do not get Schwannomas and meningiomas; they get more NF 2 related tumors, mostly fibrosarcomas and osteosarcomas. However, when you do a conventional inactivation -- what I mean by conditional inactivation is removing the gene, removing the protein function in a particular cell type. So Marco Giovanni beautifully removed the gene in the arachnoidal cells or Schwann cells that gives rise to Schwannomas. When you remove the protein in those, they produce very slow growing tumors just like the humans to the point of it's so slow, it even takes a year and a half or two to see microscopic tumors in those animals.

So clearly suggests that if an NF 2 function is lost in other cell types, non-CNS cell

types in mice, they produce very aggressively growing malignant tumors.

However, when you remove the Schwann cells and meningeal cells, there has to be a cell-dependent mechanism. So if you think about it, patients do not get tumors any way except the meningeal cells and Schwannomas, suggesting the function has to be very critical in those cell types in order to understand more about the tumor growth in these cell types.

So this is where our thinking started coming. There are many functions now from 1993 that have come from this protein depending on where you look, depending on which lab is doing and what cell types. However, we need to know the function that may explain the meningioma and Schwannoma may be context-dependent.

Then what was the other challenge is this slow growth of Schwannomas and meningiomas. Okay. Surgeons remove the tumors. It comes to us at the lab. Schwannomas, we cannot even culture them, grow them in culture in the lab. Meningiomas, we started to culture them. They grow a little better than the Schwannomas. So grow very slowly just like in the human situation. That made it very difficult for us to do experiments because they sit there and do not grow. Especially Schwannomas. Also the fact that animals produce these tumors slowly made it very difficult to make progress in a faster fashion.

So what we did was a few years ago, during the discussion between me and (someone), we decided probably one of the things we want to start is to focus on meningiomas. We've been culturing them as we told you, started doing our

experiments in them. Then when we send the paper to reviewer, even for us, what is your control to convey? Meningiomas comes from patient XYZ. Some of them, 60% of them have NF 2 loss. Rest of the 40% do not.

Using our antibodies that we made in the lab, we can tell, okay, this person has NF 2 loss, this person doesn't have NF 2 loss. And then we will remove the ones that don't have NF 2 loss because we cannot compare them to some of the gene. So if you take the meningiomas with NF 2 loss, I'm still comparing with a person from XY to Z. And is it meaningful? Or not? We don't know. But what is your control? Control is arachnoidal cells coming from the meninges.

So this is brain, human brain. You have the meningeal membrane. And then this here is the arachnoid layer. So we started working with the pathologist and ear surgeons. Can we get this arachnoidal layer and start to culture them in the lab? And be successful in culturing them. Okay. We have a controlled cell that we can manipulate.

Now we can go on, and surgeons and the participation of the families can begin, the piece of the tumor on the arachnoid from the same person. At the same time also take the arachnoid from a controlled person and do some manipulation called RNA -- which I'll be talking to you in a minute -- and to artificially pull the NF 2 team and start asking questions.

The experiments what we did was this is from a matched sample, what we call. A tumor, sporadic tumor. This is a Merlin protein that we can detect in antibodies.

These two are family members. These are perfectly fine. But we

selectively -- Merlin is gone. When you take the arachnoid from this patient, Merlin's completely intact and Merlin is gone here. So this is the arachnoid. This is the meningioma from the same person. So I'm going ask you: What is noticeable here? First, this I think that strikes you when you compare this to this? Does anybody want to say? Don't be shy.

>> The arachnoid cells are bigger in the meningioma.

>> DR. VIJAYA RAMESH: Striking to us. These cells are larger in size. But then one has to wonder: Okay. These are tumor cells. These are normal arachnoid cells. We know normal arachnoid has Merlin, this doesn't have it. So something else is happening here that is making them larger. How are we going to address that?

So we took the control arachnoid cells and used the technology called RNA interference, which is a recent technology where you can remove the protein artificially in your system. You can see here by removing the protein, you can completely remove the protein in normal arachnoid cell. So this is the normal arachnoid cell. Now artificially we have removed Merlin here. What do you see? Cells are larger. Clearly larger. It was very striking to us. We said aha. So the cells are larger here. Now we take this controlled cells and remove Merlin cells are getting larger.

So this gave us an important clue because the other syndrome classically have larger cell types. This actually brought the whole tubular sclerosis field, a lot of common things existed between that and NF. Very slow growing.

Tuberous sclerosis. They have an onco signal. That's what I'm going to be talking. And try to explain to you as simple as possible.

So there are two genes for tuberous sclerosis. They work together almost. And whether you have TSC2 gene or mutation in 1, it doesn't matter. The end result is patients get tumors, benign growing tumors in multiple organs, but not meningiomas or Schwannomas.

They function through this thing called Rheb. Normally these proteins function together to inhibit Rheb. They have a signal like this, a line with another line, short line here. That's in terms of signaling. But when you have an -- activation. So normally these two function to keep the inhibitor nonfunctioning. When this is gone, it is out of control getting activated. When Rheb is activated, it is activating a series of molecules downstream. And one is the main that is getting activated here. And when 1 is activated, these S6K and S6 are all getting activated.

When it is activated, they are all essentially a phosphorylation are added.

Phosphorylation. And that's what I'll be showing you activation of these in a few slides.

And then Rapamycin that very specifically blocks this activation that is being used in tuberous sclerosis. Tuberous sclerosis.

A lot happens and is activated resulting in cell growth as well as in proliferation.

Again, the two terminology that I mentioned, cell growth, it's the larger success.

Cells getting bigger, cell growth. Cells dividing faster is proliferation. And most of the time malignant tumors proliferate. They grow faster. There's meningiomas

and Schwannomas, when we culture them, they do not divide. They seem to be getting larger. Maybe that's one phenomenon of a benign tumor. So clearly this pathway is connected to cell growth as well as cell proliferation, connected to benign tumor syndrome and tubular sclerosis. NF 1.

Now we added NF 2 this last year. As well as cancer, this battery is activated a lot of cancers.

So this is the function of TSC 1 and 2 functioning here. What happens upstream about PSC 1 and 2? I'll explain in a simple function. Any cell type. This is a cell membrane. A lot of growth factors function here. And many of the growth functions activate the pathways upstream of this. And when these pathways are activated, classic example is insulin signal, a protein called PA3 kinase is activated. When it is, it results in incubation of proteins. So there's no mutation in these cases.

But when these pathways are activated, a phenomenon called phosphorylation happens to this complex in activating this complex. So NF 1 people now know from people in Boston, they put NF 1 in this battery a few years ago. If NF 1 is mutated, the kinase pathway is activated. Ultimately the cell takes an inactivation of the TSC1 and 2 complex, resulting in activation. So there's other tumors.

It is also involved in a benign tumor called (something) disease. When this is activated, this pathway is activated, inactivation of the TSC complex. So TSC1 and 2s are clearly a master regulator of this NF 1 complex, leading to either TSC 1 or 2 activated, abnormal in NF 1 or B10 patients. Then all results are inactivation of

the complex, resulting in activation of this. So this is what is happening.

So then our question is: You have big cells -- yeah?

>> Sorry. Rapamycin, is that a drug? Or is it protein?

>> DR. VIJAYA RAMESH: Rapamycin is a drug. It's not a protein.

>> So you say that is blocking it?

>> DR. VIJAYA RAMESH: It is a drug.

>> And you're using it right now for tubular sclerosis and NF 1. They're ongoing for trials. And rapamycin and a lot of things are happening.

So our question was: Okay, we have large cells here. Can you treat them with rapamycin?

>> DR. VIJAYA RAMESH: That's what I'm going to show you. So then this is actually controlled arachnoidal cells and then Merlin-removed arachnoidal cells. You see the larger cells. This is untreated. Then we treated with rapamycin here. Even before removing Merlin, we started treating with rapamycin. Day 2 we started. And then 15 days. Right on second day you can see rapamycin. So it's like preventing the enlargement of the cells. When you start with rapamycin, they no longer get larger cell size. Again, to show you how big these cells, this becomes to [Inaudible]

This is with the control arachnoidal cells where we remove Merlin. What happens when you take a meningioma cell, so again the same phenomenon. This is untreated cell. Now when you treat with rapamycin, this gets smaller. So here again this is the cell size and this is getting smaller here, clearly suggesting we

cannot only block the enlargement of size, you can also prevent it after they become larger and you give the rapamycin, they become smaller.

>> I need to understand, if you would. Cell size increases in proliferation. You could have a lesion created either way?

>> DR. VIJAYA RAMESH: Correct. The total pathway in NF 2, NTSC is very well-established as a cell size. I'm sure there's a little bit of proliferation that triggers in the beginning. Depending upon when that mutation happened, when that arachnoid cells got hit by NF2 mutation, that could have happened and then they probably started getting larger. At least that's one mechanism that's clearly going on.

So, again, now I showed you the pathway, the activation. Just to repeat, so this is what I'm going to show you is the activation of this by using a specific antibody to look at that. What we call MTor. When this is activated, we can look at this.

There are commercial antibodies available for this. So by looking at this protein phosphorylation, we can tell whether this is getting activated or not. So that's all you have to remember.

So when we take normal arachnoidal cells which are null and positive here, you can see the Merlin protein. 3 Merlin negative from different patients. The Merlin is almost gone. When you remove these cells, they are all cells. In order to grow, they need food. So we feed them some growth factors. That's how they survive outside the body. And then when you remove the growth factor from here, all the growth factors, this pathway gets shut down in the normal cells. It's no longer

active. Even though protein is there, but the phosphorylation is not happening. The phosphorylation is the one that's activating it here. Whereas, here in Merlin minus meningiomas, we have removed the Merlin factor, but it doesn't matter. This is clearly getting a signal saying I'm not going to stop. I'm going to keep getting activated. And activation here is what is resulting in the larger. So, again, this is artificially removing the -- we first started with one. What we call one region of Merlin that we removed. Now in order to confirm it's definitely happening, we make three different regions of Merlin go away. Completely gone. The signature, I won't confuse you. The signature in that pathway is getting activated here. So this suggested to us when you have Merlin deficiency, all arachnoidal cells artificially remove Merlin, cells get larger the total pathways are activated.

Then we went back to the pathologist and said: Can you look at the tumors? By chemistry whether this pathway is getting activated? I'll just give you a few examples. We looked at least five of different tumors, meningiomas and Schwannomas. This came back and the pathologist said yes, this pathway is getting activated in the tumors that came from the patients.

So what I showed you first is the cells, tumors coming. Cells grown in culture are in vitro, what we call outside. These are the tumors that the pathologist came after the surgery. In both cases, we are told pathway is getting activated.

Then the question is: Can we give rapamycin? Cells get smaller. Is the pathway getting shut down now when we give these cells rapamycin? That's what is shown

here. So again this pathway is getting activated. Rapamycin blocks this activation. So this is a controlled arachnoid cell. Merlin is present. We artificially remove Merlin. We can see it is gone. And we do not treat with rapamycin. We remove the growth factors. We control arachnoidal cells. We do not see the activation. We see a very strong activation in the arachnoidal cells where Merlin is removed. Now you give them rapamycin.

And we also gave them different doses of rapamycin. So even if very small dose, it's effective. And then as you increase the dose, this pathway is gone. So then the question is: What is the mechanism? How is it feeding into the pathway? That we still don't know. But at least we eliminated some of the possibilities. I showed you in the beginning how the NF 1 feeds through this pathway. Other feeds through this pathway.

The addition of the growth factors feeding into the pathway, I'm feeding -- I'm showing you the -- taking the center state. Every week some papers come. So many regulations are going on. Also regulate this pathway. Nutrients are energy, also glucose and low energy and glucose deprivation also control this pathway. So by not going into the details, by doing experiments in the lab, we started eliminating this pathway.

So we knew all along mainly the growth factor dependent pathway. Then the question is: Is Merlin functioning through kinase pathway or the PAT pathway by using specific inhibitors again? Compounds that we blocked up here or here. So using this compound, even though we blocked the pathway, we could not block

the activation in cells. Same way using this compound, we can block this pathway, we can block that creation here, still this was getting activated. So we can comfortably say that NF 2 is not functioning through either this pathway or this pathway, which are known, for example. It's not functioning like NF 1 through this pathway or through this pathway; it is functioning in a very normal fashion to control the pathway.

So by doing a few more experiments, we have placed NF 2 most probably most likely upstream of TSC1 and 2. That's our favorite hypothesis in the lab currently, that NF 2's functioning upstream, meaning above TSC1 and 2, possibly functioning through it. However, we cannot eliminate the possibility that it can function in a parallel fashion to TSC regulating and 1. But I feel like in nature, God would not have created two proteins to control one at the same time unless the cell dependent would be existing. So we are kind of looking at some more experiments to see and understand the mechanism, how exactly Merlin is coming into this important signaling factory.

I think I'm going to skip this slide. This is actually what is conveying this is all I have shown you is absence of Merlin, either in the tumors or artificially removing the tumors is activated the path.

Now conversely, if you overexpress this protein, can you inhibit the pathway?

Was one other question. So it makes you feel good. Yeah, I can do in both directions and see this. And exactly that experiment we did here. When we overexpressed NF 2, this pathway's completely blocked, which is strongly

suggesting NF 2's role signaling when NF 2 is gone, you activated the pathway in a normal fashion. When you overexpress NF 2, you can inhibit this pathway. So that's what this is.

So to summarize this part of the work we just saw, published from my lab, which came last year, what I've shown you here is the NF 2-deficient meningiomas have increased cell growth. Taking the arachnoidal cells, we see larger cell size.

Recently we have also obtained human Schwann cells in which we are artificially removing NF 2. I've not shown you any of the data, it's upcoming. And these cells also get larger until activated. Also in Schwann cells. But there are some distinct signatures that are different between the Schwann cells in which NF 2 is removed versus the arachnoidal cells in which NF 2 is removed. So once again suggesting even among the meningiomas and Schwannomas we may have differences.

So that kind of suggests how critically it is to be on the right cell type when you do your experiments so that we can understand this disease, why these tumors come in a particular location, meningeal cells, Schwann cells, and why are they benign in NF 2 deficient mice that produce malignant tumors in other cell types.

So I have shown you here the increased cell growth. And it is not due the kinase or the kinase agent. It functions in a novel fashion. And we believe that the combination of rapamycin could be -- I'm not going to go into the details because rapamycin alone could long term send a feedback signal in here and activate the pathway. That's being looked into in the TSC field. But something could be

started with rapamycin.

The TS field, rapamycin looks like needs to be given in a long term basis patients who are taking rapamycin, the tumor shrinks; but once you stop taking it, tumors do come back. So it's our hope as we learn more about this, how its functioning may be able to come in better components and combination therapies instead of a single therapy. That may be more effective than rapamycin alone.

So then the question is now what are the ongoing efforts in the lab? Define the mechanism how it feeds in the pathway. So we are setting up collaborative efforts with MIT and Harvard across the river from us to do a whole genome screen of the kinome screen specifically to find the mechanism.

Also ongoing, which is moving very well, is can we understand all the mechanisms that are telling one cell okay, you might be benign, which is telling other cells, you progress. So our focus is again on meningeal cells and Schwann cells, to know what are the mechanisms that are telling these cells not to grow rapidly so that we can understand better. So that's ongoing.

And third thing that's going on is data basically creating animal model, a relevant animal model. Can you implant these cells? The meningiomas into mice. To investigate. I can stop here is a good break point to continue the animal model after lunch. Or I can continue now. It's up to you.

>> Susie: Ladies and Gentlemen, what is the consensus?

>> Is rapamycin acting as a tumor inhibitor that simulates Merlin?

>> DR. VIJAYA RAMESH: No. Rapamycin is blocking the Ntor is activation.

Here. MTor.

So Merlin we don't know how is activating. When this is activating, rapamycin is functioning at this level. Is that clear? When you give rapamycin, this is getting no longer activated.

>> Does rapamycin, if Merlin is not there, then rapamycin is being activated every time there's new tumor growth?

>> DR. VIJAYA RAMESH: There is no rapamycin in the body. You have to give rapamycin.

>> I know. I thought you're feeding the body rapamycin as a drug.

>> DR. VIJAYA RAMESH: Yes.

>> And so it's present in the body. And then does it activate?

>> DR. VIJAYA RAMESH: Rapamycin is given as a drug.

>> Rapamycin precede new tumor growth? I'm not sure of the actual job of rapamycin. I keep hearing Merlin. I know Merlin's a protein. I thought that's what was missing. Because that's tumor inhibitor.

>> DR. VIJAYA RAMESH: Correct.

>> So how does rapamycin fit into the picture to control tumor growth?

>> DR. VIJAYA RAMESH: First you understand the function. What is normally Merlin doing? In the absence of Merlin, what is happening? So I showed you the tumors get larger. Tumor cells are larger. And the tumor cells are larger is because this pathway is getting activated. So Mtor 1 is getting activated. Mtor 1 came because mammalian target of rapamycin. That's how the name, the Mtor

name came. So you don't know how Merlin functions, but you know in the absence of Merlin, this is getting abnormally activated. So you are coming with a drug that will stop this activation. So even Merlin is not here, when this is getting activated, your rapamycin is going to block the activation, giving block of this pathway and stopping the cell growth.

>> So rapamycin is mimicking Merlin?

>> DR. VIJAYA RAMESH: I wouldn't say mimicking. But rapamycin, whatever the disaster that is caused by Merlin, it is stopping it downstream. Merlin loss is making the cells grow larger, right? But we know the pathway that's responsible for larger is downstream of Merlin. So this is how the drugs are developed. You place it in a pathway and you go for drugs that will target that. So in the absence of precisely, we cannot replace Merlin. How do you deliver Merlin to your meningeal cells and Schwann cells? That's almost impossible to do.

>> But that's your job.

[Laughter]

>> Tall order.

>> All right. Thank you.

>> DR. VIJAYA RAMESH: So in the absence of Merlin loss, can you target downstream? Knowing that this is getting activated, knowing there's a drug out there that can block this activation, you treat with rapamycin. That's exactly the TS patients in TSC proteins are gone, this is getting activation. So they're not being given TSC proteins and yet they are being given rapamycin. So it's stopping

all the effects that's on going downstream of this sequence.

>> Thence the big question marks. Right?

>> DR. VIJAYA RAMESH: The question mark here is how is Merlin controlling the activation? What is the mechanism?

>> Okay. It's lunchtime.

>> DR. VIJAYA RAMESH: I know it's kind of difficult.

>> Thank you.

>> DR. VIJAYA RAMESH: We'll reconvene and then I'll start why do we need a meningioma model.

[Lunch recess.]

>> DR. VIJAYA RAMESH: Ready? One of the main purposes we need an animal model, say, for example, we have -- there is a compound that's available in order to take the drug to the patients, a lot of times until -- so even though we have a human-derived culture model, human-derived cells, in an ideal situation, you need an animal, mice, so where the drug could be tested before it is taken to the patient. That's one of the main reasons to have an animal model. Also in the future, rapamycin is approved, it's being given to different patients. But continuing the research, if you identify compounds that needs FDA approval, you have to have an animal model. So what are the challenges?

I've already told you, I'm repeating again, the NF2 mutant mice, when you take NF2, they do not develop Schwannoma or meningiomas; they have other osteosarcomas or other malignant tumors.

Then if you go on, it is established that NF 2 loss, required in order for the arachnoidal cells to become meningioma cells. But only 30% of those mice get these tumors. And also they are very microscopic permitting the use of these. You have to wait forever. Even if you wait forever, there's such microscopic tumors that you cannot really give them drug and look for tumor being removed. So something that's already being done, which is to take the tumor cells and implant them in the mice, in the meninges, and see if they take up.

So we thought why not tie the arachnoidal models? So do something like compounds so that you don't have to look at the mice by MRI. In the beginning, you can do some imaging to see whether these mice are developing tumors. So it's kind of technology that's upcoming by the research.

So this is the approach we took. It is being kindly funded, generously funded by NF Midwest. So all the work starting from now is ongoing through the funding given by NF Midwest.

So we have -- actually that's the first step to be taken, benign meningioma. So it can grow forever in culture into which we have introduced a protein called firefly luciferase. So you can see the fluorescence even in culture outside when we grow them. And then they are confirmed that it has this fluorescence. And this is the benign meningioma confirmed to have loss in which we introduce the gene, they glow now. And be sure that they that the activity is there.

Then with the help of neurosurgeons, we implant them. We work from the lab.

So taking these cells and implant them. So that then the mice can be seen under

imaging systems to see whether they have taken these tumor cells.

So essentially we took these luminescing cells. Where will we implant them? As a trial first, with the help of neurosurgeons, we decided to try two locations. These are optimizing the conditions for location. Cerebral contexti in the skull base. The convexity is in the skull base. And another is a little deeper. They decided to go with two different stain of mice. These are all immunocompromised mice, so they won't reject the tumor cells. These are different mice line. And then so we test them by imaging two weeks what we call baseline. And then every month for six months we monitor them.

So what we learn in the experiments, so we took five mice from two different locations. This is two weeks. We can see here even in two weeks the convexity was definitely better. This is your degree of resistance. Red signal is maximum. Really good. But only one mice showed that. That was kind of too early even for us, shocking that it can have that much signal.

So as we went to two months, the skull based location, almost all the tumor cells rejected. So did not take it. However, the convexity locations, one of the mice I think at this point so all four are still holding on. So we continued this. This is actually from the time maybe six months just finished. One of the mice looked and removed the tumor. We are looking for histology, waiting for the pathologist to confirm the histology on this tumor.

So subsequently what we did was we didn't want to do the skull-based location anymore, having learned this, so we are focusing only on convexity. And we have

gone into 20 more animals with the same approach. They are all engaging. Every month we do the imaging. If there's anything suspicious looking, then we do MRI.

But we have also gone one step ahead and asked the question: Can we improve this implantation by mixing the tumor cells, which are giving the luciferous material, to something commercially available.

So essentially what this matrigel, it is a gelatinous protein mixture which resembles the complex extracellular environment found in the tissues. So whatever is in the brain, other things going on, so this matrigel will help to give that similar to the in vivo situation.

So it also acts to bring the tumor cells, clump them together. So with the hope of it augments the tumor growth in vivo.

It's highly useful in culture system to mimic the angiogenesis for human tumor cell implantation.

So that's what we have done in the past month or so, we tried this. And implanted again five new mice and five NOD SCID mice, again two different types of mice.

Two weeks all of them have taken. Month still seems to be some decreasing signal. We have to wait and see. So this will continue. We are going to continue this approach in the Matrigel and wait for six months to see whether it will take of the tumor and four to six months if it's definitely the tumor is detrained. We will look for the people to do MRI, to quantitatively determine what is the size of the tumor? Is it big enough that we can keep one cohort, a few animals, to remove

them to confirm it is stage 1 or benign meningiomas? With the goal of ultimately having more of these to try it out on mice. So that this may be equivalent to the preclinical model that we can hope for.

Our ideas also long term that can we go into atypical meningiomas with this approach? So we're going to take this anarachnoidal cells and inactivated and engineer other molecules that could be activated, in addition, and put them back. So our data's suggesting -- I didn't go into all the scientific details -- the data coming from my lab is suggesting Merlin loss is there no matter what, it's benign or it's not. That's one of the early results. But meningiomas may also acquire other mutations besides Merlin. That's what the data's suggesting. Which moving forward, we might be looking at what are the other events that are cooperating with Merlin? It also raising the question: Is the NF 2 loss to these other events or they can independently happen? So we are kind of getting ready to test those questions with the hope we can put the additional events that: Will that help them to move from the benign to the other tumors? 80% of the benign tumors go to 1. And then 5% are malignant tumors. Schwannomas, to my knowledge, are all benign. NF 2 are all benign. Whereas 80% are benign. So at least the focus in my lab is to create a meningioma model. Maybe if we succeed taking the Schwannoma cells and moving the NF 2 gene that's working well in the lab, there is a way to work in the surgeons to implant them to create a model that could be the long term goal.

So I'm going to stop here. That's, I think, my last slide. The most important slide

is to acknowledge all the people. Marianne James is one of the senior people in my lab who is driving this. Sangyeul Han was working on tubular sclerosis in my lab and was brought into this project. He just recently took up a job in South Korea and left. And then Elizabeth Stivison, a very talented senior technician, really dedicated to this project. And everything is done in collaboration with another lab. Go for a very long time. And we have been associated -- for the last 27 years, I have been at Mass General, so it's a very collaborative effort. With the help of neurosurgeon Hiroaki Wakimoto helps with all the implants. And Anat is the neuro-oncology. And then Scott is our neurologist who gives us the patient material for MT signaling and also for the group in TSC.

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Thank you.

[Applause.]