>> Kim: Hello, everyone. I'm Kim Bischoff, the executive director for the NF Network, and we'd like to welcome you to our webinar series.

Tonight we have 124 people registered from across the country to participate. These webinars have proven to be an excellent way for the NF Network to reach individuals across the country. For NF2, over 100 people tonight, that's absolutely amazing. This comes as a result of the hard work that the NF Network NF2 webinar committee has put into this. I'd like to especially thank the committee, John and Linda Manns, Nicole Service, Barbara Franklin, and especially a big thank you to Sally Richards, the chair.

Tonight we're very fortunate to have Dr. Asthagiri with us to share his experience in neurofibromatosis type 2. He's the principal investigator of the NF2 natural history study being done at the National Institute of Health. Dr. Asthagiri will speak about the NF2 natural history study and the findings today, as well as the NF2 program at the National Institute of Health.

After the presentation we will have some limited time for questions and answers. There's a box at the right of the webinar control panel which will allow you to type in the questions. After the questions are typed, I will read them, to be able to give you the answers.

If your webinar control box is blocking the view of the presentation there's an orange rectangular button with an arrow. If you click on that, it will move it over to the side. And now I would like to introduce you to Dr. Asthagiri.

>> Dr. Asthagiri: Thank you very much, Kim, the folks at NF Network and Sally, and the NF2 webinar committee for giving me the opportunity to speak to such a broad audience about NF2, and also the ongoing research that we have at the NIH for not only the natural history study, but I wanted to also discuss some of the laboratory work and some of the publications that have come from all the folks who have participated in the natural history study to date as well.

We're very happy to announce that, you know, over the course of the natural history study we're up to 155 participants in the study, and obviously the entirety of this presentation, folks that we have most to thank for obviously are those 1 55 participants who give up their time on a biannual basis to make sure that NF2 research goes forward. So special thanks to each and every one of those folks. Their families who support them, as well.

So what I'd like to do is first point out that -- let me just navigate, here -- that first of all, as a federal employee it's very easy to say this, I have no disclosures or financial conflict of interests or anything of the sort when I make this presentation. I want to try to relay three things or three major objectives for the purpose of our webinar this evening. One is actually just a brief introduction to what is the NIH. A lot of folks don't understand what the NIH is, and I'd like to just give two or three slides about that. And then we'll move to an overview of NF2. I know that many of the folks who have come this far to sign up for a webinar and figure out how to run the actual software to get this on their screen probably have done a lot of research. But I'd like to just maybe make sure everybody is on the same playing field with regards to understanding what NF2 is, what are the basic manifestations of NF2, how is it passed on, and things like that. And I'd be happy to take more specific questions about all of that at the end of the session.

After I'm done with that, we will talk a little bit about some of the ongoing research projects that have been completed or that are ongoing at the NIH so that we can bring the NF2 community up to speed about that. We have a lot on our plate, so I'll try to move rather quickly. I rarely get an opportunity to speak for 45 minutes uninterrupted, so I'm fairly certain we'll be able to get done, it's just a matter of how many questions we'll have at the end.

So first of all, you know, what is the NIH. Fundamentally the National Institute of Health is a government agency or part of the Department of Human and Health Services, and it's really motivated simply to enhance knowledge which will decrease burden of disease, improve health, and lengthen lives. And it's structured such that disease states or health states are divided into 27 major institutes, of which the one that I'm a participant or member of is called the National Institute of Neurological Disorders and Stroke. Some of the institutes that support neurofibromatosis research also include the National Cancer Institute, the National Eye Institute, and National Institute for Communicative Disorders, as well. And so multiple institutes are supporting neurofibromatosis research.

The annual budget for all of NIH for all of this type of research is $31 billion, and I'd like to point out

that's a lot of zeros at the end of that. And there -- you know, a significant amount of that is divided between what's called extramural research and stuff that's done on the main campus, we call it. And that's what the picture is in the upper right-hand corner. This is our NIH clinical center, and that's one of about 60 buildings on our main campus, it's almost like a university campus here, and we have 6,000 scientists, over 20,000 employees, and right on this one square mile campus we have about 10 percent of that $31 billion of funding going towards medical research right here. Another 80 to 90 percent goes outside of this square mile to fund research, giving over 50,000 grants supporting over 300,000 researchers across the United States, and sending money to over 2500 institutions.

So I think, you know, the NIH is the biggest supporter of biomedical research, and that's just something that a lot of folks don't know. What is the NIH, where is the NIH, if you're talking about the intramural campus, and how does it support research outside of the NIH itself.

So moving forward, let's go to an overview of neurofibromatosis. When a lot of folks think about neurofibromatosis, or you Google, which is, you know, sometimes a scary thing to do, when you Google neurofibromatosis, everything that comes up in the top 10 is always neurofibromatosis Type I. If you Google images, you'll see images like this. But I think if we all, in this webinar, understand that this type of image we associate with is generally NF1. This is not the face of NF2. NF1 goes by a variety of names, including Von Recklinghausen's disease, peripheral NF, but since NF2, NF1, and schwannomatosis have been sort of genetically differentiated, less and less confusion, except for several unique cases, it's really getting a lot better at differentiating what the cardinal features of -- are of each of them. Now, having said that there are some cardinal signs of NF1 which I will leave to other webinars to discuss how to diagnose that. And also, you recently had a webinar by Dr. Scott Plotkin about schwannomatosis that I would encourage folks who are interested in learning more about schwannomatosis to go back in the NF Network webinar history and have a view of that, because it was an excellent presentation on that as well.

The focus of our presentation will be just on NF2.

So NF2 is what I call a multiple neoplasia syndrome, which means you have a tendency to develop multiple tumors. NF2 is caused by a change or mutation of the tumor suppressor gene located on the long arm of chromosome 22, and it's inherited, as I showed in a picture here, in an autosomal dominant inheritance pattern. So when it is transmitted, it is usually transmitted to -- with a risk of 50 percent of children having a risk of developing or acquiring NF2 from the parent.

Only each person has two copies of the NF2 gene. To have NF2 as a syndrome you need just one copy to have a mutation. And we'll talk a little bit about how tumors develop from that situation.

NF2 is characterized by what's called a wide phenotypic variability. That means that every person with NF2, there's a whole spectrum of what it means. Some folks have two tumors, some folks have 100 tumors, and so it's a really broad spectrum of how it manifests.

But almost universally, by the time you're 60 years old, almost everybody has some clinical manifestations of NF2. Meaning it's either caused some form of hearing loss, weakness, or some other symptom has shown up.

What's unique about NF2, though, is that not all cases are inherited. In fact, about half of all cases are actually due to new mutations. So mom and a dad have a child who is very healthy, goes a certain number of years, which may usually be up until the age of 20 or so, and they start developing symptoms or signs of tumors. And that's when they become diagnosed, because they are the first case of neurofibromatosis type 2 in the family.

The incidence is about one in 25,000 births, and for a variety of reasons, the prevalence or how often it's found or diagnosed in the population is about 1 is 100,000 folks. If you sort of divide that by the population of the United States, which is about 300 million, you might derive how many folks in the United States actually have been diagnosed with NF2.

Now, when I talk about the rarity of NF2, a lot of folks, especially when it comes down to funding resources and things, ask me why study NF2. And the importance is not just for the NF2 community, but the importance is that the NF2 community likely holds a very key understanding and pivotal role of being able to not only help the NF2 community, but the general population at large as well.

For example, just as the ABI, the auditory brain stem implant, was developed essentially for patients

with neurofibromatosis type 2, it is transitioning to folks with bilateral hearing loss without NF2. And there are a lot more of those folks than there are NF2 folks, but because NF2 research was conducted, moved forward, and the ABI was developed, it was able to help a lot of folks.

When we look at the tumors that patients with NF2 get, meningiomas, schwannomas and ependymomas, they actually make up about 45 percent of all primary brain tumors diagnosed in the United States every year. And this is over a four year observation period. And so if we can really research for NF2 patients, figure out a way to slow down meningioma growth or stop schwannomas from forming, or make ependymomas completely benign, then we'll helping a significant population as well. Maybe even more than all of astrocytomas, which gets a lot of press, like neoblastomas, things like that, and all other tumors, I think the gravity of doing NF2 research really has the potential to help a whole lot of folks, including obviously the folks that we are concerned most about, is NF2 patients.

Now, as I pointed out, what happens is you either inherit or you have what's called a mutation of the NF2 gene. Now, what these -- what Knudson's two hit hypothesis is, is it explains or tries to explain how tumors form. So there's two copies of a gene in everybody, and what this NF2 gene does is it codes for a product called merlin. And merlin normally allows the cells to function properly. So as long as you have one copy of merlin in a cell, then the cell goes on and functions properly. But if you have inherited NF2, which means that one of the two copies of merlin has gone bad, then all it takes is a second hit on your normal gene to make that cell become abnormal.

And so if you have all of the cells in the body carry one hit, and then one cell, let's say over here, gets a second hit, then that cell will then turn and start growing and become a tumor.

And so in NF2, the most common cell that gets affected is found on the vestibular nerve. Why that's the case we don't know yet, but over 90 percent of patients end up with a tumor on each vestibular nerve on either side because of this two-hit phenomena that occurs.

So as I pointed out, you know, a lot of what's going on in NF2 research is trying to understand how this protein called merlin works.

So merlin is the protein that's encoded or made by the NF2 gene. And so this we call a tumor suppressor protein, or tumor suppresser gene, that makes a protein that makes -- its primary function is to make cells stop multiplying.

But merlin is rather unique, because it does a few other things, too. It actually coordinates how cells interact with each other, it actually organizes a lot of proteins on the surface of the cell, and it also helps organize how the structure or the skeleton of the cell is made.

And finally, through all of these mechanisms, it's finally controlling what are called these mitogenic pathways which are telling the cell to multiply or not multiply.

So if you have normal merlin function, then these processes are carried out, and you only need one copy to be able to carry out the processes well. It's just that when that second gene in the cell gets damaged, or mutated, then that's when tumors start to form from individual cells that have been affected.

So when we talk about, well, what type of things happen with our patients with NF2. Well, this tumor concept is rather easy to understand, once we understand that, look, we have a tumor suppressor gene, so if you make it not work properly, what will happen is you'll develop tumors.

There are several interesting things about that, though. There are only certain tissues in the body that are susceptible to tumor formation. Patients with NF2, for example, don't get lung cancer, they don't get other types of tumors. What they get is a very dependable phenotype, or clinical manifestation, which include meningiomas, whether they be of the head or of the covering of the spinal cord; they develop tumors on both vestibular nerve tumors, on both vestibular nerves; and the other cranial nerves can be affected coming out of the brain stem; and the nerves coming out of the spinal cord can also be affected; and any peripheral nerve in the body can be affected, as well.

When we see the nerves that are in the periphery going into the skin, when they become affected, you can also get tumors which have been historically called either subcutaneous tumors or skin plaques. These are schwannomas, as well, they're just located very close to the skin, or in the skin itself.

Now, there are also some non-tumorous formations, as well. Those are the eye changes in NF2, and they can cause visual problems. They're called retinal membrane -- retinal hematomas and cataracts. Here in

the early 80s, cataracts and -- early 80s -- late 80s, early 90s, cataracts really became well understood, that if you had a cataract before the age of 50, it's a very useful diagnostic marker that you may have NF2.

So what I tell everybody who has any questions about does my child have NF2, the easiest thing to do is to look in the eye and see if any of those findings are there. If they are, then proceed to really start looking at MRI, genetics, and all those things. But this is just a visit to the eye doctor, which is very straightforward.

Now, more and more folks are really starting to understand that maybe tumors aren't the only thing that are the problem. Folks are developing weakness, or sensory problems, and you can go image up and down and see there's no tumor there whatsoever. And we have a condition in neurology called peripheral neuropathy in which nerves stop working properly.

Not necessarily because of tumor compression, which was historically thought to be the only reason why nerves would stop working in a patient with NF2, but what we find is that more and more folks are reporting problems like this, and even with the detailed MRIs we're unable to find out what's going on. And it's probably because NF2 can cause nerve dysfunction without tumor formation. Although we don't quite understand how that's possible quite yet.

So the clinical -- NF2 is a diagnosis made by clinical finding. Meaning that if you have these findings, then you have NF2, unequivocally, there's no real need for genetic testing except for secondary reasons. Maybe for family planning or for assessing folks who have no symptoms or signs of NF2 yet.

And so there's a variety of combinations, but anybody with bilateral vestibular schwannomas automatically have a diagnosis of NF2. But what I emphasize is there are a few groups of folks who don't have to have two vestibular schwannomas to have a diagnosis of NF2. That is folks with multiple meningiomas and two other NF2 associated tumors. Or folks with a family history of NF2, in which you're able to identify two other NF2-associated lesions. Those are diagnostic criteria that make you be able to be diagnosed with NF2 without having bilateral vestibular tumors.

Having said that, over 90 percent of patients with NF2 have these bilateral vestibular schwannomas, they are the crux of NF2. So I'm going to spend a little bit of time talking about them in detail. They usually present and cause hearing loss or tinnitus, and it's usually on one side that they cause this.
About 60 percent of folks, adults who present with NF2, this is how they show up. About only 30 percent of children, though, that are diagnosed with NF2 show up with this type of problem, because usually they have other problems that come up first. For example, meningiomas that cause visual problems, headaches, they may have eye changes that cause visual changes, or painful skin tumors. So we always have to keep an eye out for those other problems, especially in children.

A nice study from the House Ear Institute and the Natural History Consortium showed that a newly diagnosed patient is likely to have stable hearing for a two year period. Now, this was a retrospective study, and there were some caveats to this, and that is that rapid hearing loss can develop unrelated to tumor size or growth rate, and that the rate of hearing loss is not the same between the two ears. And that highly variable growth rates are seen in these tumors.

And you know, when I see these caveats it makes it very difficult to counsel somebody who is newly diagnosed with NF2, because we're not giving them a whole lot of new information. So although your hearing may likely be stable for two years, you may rapidly lose hearing. And your experience with one ear doesn't necessarily translate to what's in the other ear. What's going to happen with the other ear. Makes it very difficult to predict what's going to happen and manage patients with NF2.

Unfortunately, despite a significant amount of effort and numerous publications on what happens with these vestibular schwannomas, some studies found that they grow faster as you grow older. Some studies found that they grow slower as you grow older. So I don't think the truth about the tumors are completely known, and it's probably multi-factorial. The best studies, and sort of grouping together all this data, sort of suggests that as patients get older that tumor growth may slow. But again, I think that there's still some conflicting data about that, and that's one of the purposes of our study is to try to ascertain and answer that question.

A lot of patients have questions about treatment of vestibular schwannomas and what are the -- sorry about that -- what are the possibilities for management of -- what are the possibilities for management of NF2-related vestibular schwannomas. And the key here is that there are multiple management strategies that are

possible. There's really no single correct management strategy. There's early surgical management, which is otherwise known as hearing preservation surgery. There's radiosurgery, and there's now even chemotherapeutic option. And of course the time -- sort of the gold standard is conservative management until symptoms develop, with surgical management sort of reserved for symptomatic tumors.

Now, when we talk about early management strategies, the two that really come up are surgery and radiosurgery. And you can see here that the facial nerve, that's the 7th nerve right here, is preserved, you know, very well in both early surgical management and with radiosurgery.

And that measurable hearing, folks who retain hearing, can vary from 30 to 65 percent with early surgical management, and it's about the same with radiosurgery. And this is only after four and a half years with the radiosurgery.

What's important to understand is that even these high control rates of, you know, preserving the facial nerve, they're lower than we see with patients without NF2. And it's probably because in NF2, these tumors tend to invade the adjacent nerves, or the hearing nerves. The balance nerve tumor invades the adjacent hearing nerve tumor a little bit earlier on in the process

And what's important is that as we find out about, more and more about radiosurgery, there are more recent publications saying that although you get 74 to 100 percent of tumors are controlled -- so stay the same size or get smaller at four and a half years -- if you follow those same patients out to eight years, only 50 percent of tumors have stayed the same size or gotten smaller. That means that half the patients who got radiosurgery eight years back, their tumors are growing now, and now they have a management dilemma.

So in response to that, I think a lot of leaders in the NF2 consortium and clinical trials groups have really pushed towards trying various chemotherapeutic agents including bevacizumab, rapamycin, lapatinib, AR 42 which is coming down the pike, alternative management strategies that are reversible, that are not permanent like radiosurgery or surgery.

And they are showing some benefit in patients. About 50 percent of patients in this study published by Dr. Plotkin showed hearing improvement and tumor shrinkage using bevacizumab or Avastin in patients with NF2. So even if, you know, the tumor necessitates surgery and you have surgery and it's successful, the tumor is removed or debulked or a majority of it is taken out, the problem still exists that many patients with NF2 lose hearing. And there are improving and evolving techniques. And again, you know, discussion of vestibular schwannomas and hearing rehabilitation is probably outside of the field of what I want to discuss today, but late last year there was an excellent presentation by Dr. Slattery from House Ear Institute, again available on the NF Network webinar page, which I would encourage you to listen to, to try to understand a little bit more about cochlear implant, what type of NF2 patients can actually benefit from them. And also auditory brain stem implants, and how they were developed and which patients are best for getting those implants to restore hearing.

So having said that, you know, there's one fundamental concept which needs to be clarified at least for everybody in a very brief note, and that is that cochlear implants have historically not been used for patients with NF2. Because when sound comes down your ear, and causes vibration of your inner ear bone, this mechanical vibration gets translated into an electrical signal in what's called the cochlea, which is this spiral object which looks like a conch. And that's where the cochlear implant goes. And the cochlear implant, or the cochlea itself, then sends that electrical signal down what's called the cochlear nerve to the brain stem. So that's this right here. Down here it's going and traveling into the brain stem.

Now, if we have tumors in NF2 that cause a problem here, cause a blockade or cause the cochlear nerve not to be able to transduce its signal, then putting in a cochlear implant is not of benefit.

Now, having said that, there are situations in which we can take out the balance nerve tumor, leave the hearing nerve alone, and if the hearing is not good, be able to augment it with cochlear implants. Or in other situations, manage the tumor with radiosurgery, and then try cochlear implants.

The other sort of nice diagram which explains the difference is this very simple vignette, which shows that cochlear implants go into the cochlea, which is this green structure right here. And it depends, that signal depends on this nerve working properly.

But if this nerve has a tumor in it, in this location right here, then we have to bypass that. We have to get past it and stimulate the brain stem directly. And that's what an auditory brain stem implant does.

So auditory brain stem implants and cochlear implants, as I pointed out, I put these slides in here more for references that you can look up. You know, this presentation will be available to you, you'll be able to see these pictures at least, and you'll be able to go see these papers if you want to read more about these types of devices. That's the main reason why I put these references up for you.

Now, all we've covered so far is vestibular schwannomas, and very briefly I want to talk about all the other manifestations and leave some room about -- leave some room to discuss some novel things that we're doing with regards to NF2 research at the NIH.

Schwannomas, as we've pointed out, can happen in other nerves. So virtually any nerve in the body, whether it be other cranial nerves, spinal nerves, peripheral nerves in the hands, or even in the abdomen, or around the rib cage and things, these can all develop tumors. And the reason I point these out is a lot of folks with NF2 end up getting biopsies or recommendations for surgery for unknown masses in or around the lung, better thought to be lung cancer, or mesothelioma, or abdominal tumors that are mimicking other types of conditions. But it's important to understand with NF2 you can develop tumors not just in the brain and spine, but really anywhere along the axis wherever nerves go.

The nonvestibular cranial nerve schwannomas happen in up to 50 percent of patients, and the lower cranial nerves seem to be most frequently symptomatic, and identified.

We reserve surgery for these nonvestibular nerves, cranial nerve schwannomas, really when they become symptomatic by causing problems of other nerve groups. Because operation on these cranial nerves usually causes permanent loss of function of these nerves.

Meningiomas or tumors of the covering of the brain or spinal cord occur in up to 50 percent of patients, and most of these tumors tend to be multiple. And so these are -- the pictures here are of a tumor of the covering of the brain and spinal cord. These are the usual locations that we see tumors in.

Now, having said that, meningiomas in NF2 often occur multiply, and they can also occur in abnormal locations that are actually relatively rarely identified within the general population, such as within the ventricle, within the bone, or along the falx, which is the divider between the brain hemispheres, or in the back side of the brain pushing on the cerebellum.

Meningioma management is very straightforward with patients with NF2, and that is that it is symptom driven. When meningiomas are growing, we know that meningiomas are oftentimes multiple. When they're growing, and they cause symptoms, whether they be seizures or direct neural compression and symptoms from that, that's usually when we talk about surgery.

The problem with meningiomas is if they occur at the skull base, oftentimes they themselves can cause a lot of problems by doing complete resection. And so because of that, more and more folks have been talking over the past 10 to 20 years about debulking these tumors and managing it that way, or adding radiotherapy to try to slow down growth of these tumors.

Ependymomas, or tumor within the spinal cord, are generally benign tumors. So only about a third of these tumors that are actually within the substance of the spinal cord tend to grow. But when they do grow, they can be very symptomatic, and absolutely require surgical management. No form of focused radiation has really been first-line management strategy for these tumors, especially in patients with NF2, and no real chemotherapeutic options have been developed for these either.

Now, some of the eye findings, again, I put these in, these are images that are useful to understand. When you have an eye examination, the cataracts that are in the central field of vision are very easy to find. But it's important, the reason we do full dilated exams is because little things hiding around the edge of the iris, these peripheral cortical wedge cataracts, are actually what are really diagnostic for NF2, and commonly identified. And that's why we really need to make sure that patients get a thorough eye examination for screening purposes and observations.

Epiretinal membranes and retinal hematomas, if they're in the central field of vision, can also cause visual problems as well.

Now, another important part of the examination of patients with NF2 is skin manifestation. So this right here is called a cafe au lait macula. This is not, I repeat not, diagnostic or useful in the diagnosis of NF2. In fact, patients with NF2 have them just as frequently as patients in the general population. It has really no indication of NF2. It's a useful diagnostic tool for NF1.

The skin manifestations of NF2 are these right here, which are called skin plaques. Usually before puberty they don't have hair that grows on them, but when a child goes through puberty these skin plaques, which are actually schwannomas in the skin, can actually start becoming hairy patches. And they can be on the body, on the trunk, on the arms, legs. And this one very close to the hairline, but it's very obvious what this is right here, this rough patch.

Tumors underneath the skin can also show up as fusiform or little swellings underneath the skin, as well.

These are really useful clues to help diagnose patients with NF2.

I pointed out and discussed these newly -- this new aspect of NF2, peripheral neuropathy, which more and more, the more we ask patients, the more we're diagnosing NF2 peripheral neuropathy and we're looking at what kinds of management strategies there may be. The biggest problem is that there is no real specific treatment strategy for peripheral neuropathy that is caused by NF2 mutation.

Now, the important thing is there's lots of causes of peripheral neuropathy that don't have anything to do with a tumor, and it's important to make sure all those other causes are treated or identified, to exclude that as a problem.

Now, we also discuss, you know, the usual presentation for patients with NF2 is usually hearing loss that starts on one side, it's usually in the third decade of life or early 20s. There's usually a long time before you're diagnosed with NF2.

But if we take a look a little bit closely and start dividing up the groups of patients that present and when they present, what we see is the fact that kids present in a very different way. That's very important for us to recognize as parents, and especially because this is an hereditary and transmissible genetic mutation. That things to watch out for in kids is not just hearing loss, but rather painful or growing skin tumors, visual loss, double vision, those types of things are important to watch out for.

Again, how often do patients have vestibular schwannomas? Very frequently. These are three studies done in the 1990s that really repeat what I've been saying, that vestibular schwannomas are universal. As MRIs became more and more popular, patients started getting MRIs of their spine, it became more and more frequently identified that patients actually have spinal tumors. But you can see that up to two-thirds of patients have cataracts, and skin tumors. And so these are things that we can identify on clinical investigation without MRI, and without advance studies and things like that.

So I think, you know, we're getting close to about 8:40. I would like to just move forward a little bit and skip over a few of the slides that I had prepared on genetics, those NF2, and transmission rates, and how those can be altered, just so that I can briefly tell you three stories, which won't take but a few minutes, about some of the research that we're doing.

So the first thing that I talk to a lot of patients about when they come and see us is, look, we don't have a firm grasp of what's going on with tumors. A lot of patients ask me and say can you tell me what's going to happen to me in the future. And that's one of the most important aspects of the natural history study. And so you know, we published in Journal of Neurosurgery about what we at least were able to observe in a retrospective fashion. A number of our patients actually brought stacks and stacks of films to us and said can you do anything with this information, we don't want this to go to waste. And we actually went through and digitized all the imaging in these 17 patients that initially brought all their imaging, and they brought about 10 years worth of images with them.

And so we were able to look at over 160, 70 tumors in these patients, and some of the things we started understanding were very, very important.

Number one, if we take a look at meningiomas in NF2, they're distributed in the same frequency as they're identified in the general population. What I mean is that if you look at 100 tumors in the general population, there's usually a lot of convexity tumors, a lot of perisagittal, and these locations are very rare. This just sort of reinforces the fact that meningiomas in NF2, if we figure them out, realize how we can biologically treat them and understand them, we are not only going to help out NF2 patients in our community, but also the general population as well.

But what was important about this is that if we took a look at the first image that the patients brought in, and then we took at the last image the patients brought in, the first image is the gray. That's how many

tumors we could see.

If we looked at the last image, we saw a lot of new tumors. And in fact, what we see is that tumors probably are showing up or being shown over the course of follow-up. And so just because you have two tumors at the start, or when you're diagnosed with NF2, if you get those taken care of that doesn't mean in 10 years you don't have another set of tumors that you'll need to have managed.

So the second phenomena that we see quite frequently is everybody hears, they've heard reports, everybody with NF2 has heard everything is stable on your MRI. Well, we see that in actuality quite frequently, but it's not really discussed. Everybody just assumes that tumors grow. But you know, when we take a look at this tumor specifically, we said look, these images look like they were taken two days apart, and in fact they were taken almost five years apart. It really showed to us that there's something going on that's turning tumors on, turning tumors off, and telling them to be quiescent, and then telling them to grow again.

And so when we looked at that, when we looked at all these 139 meningiomas, we saw that about two-thirds of meningiomas actually exhibited this stuttering pattern of growth. And the stuttering pattern of growth is really important, because right now we have no way of predicting whether you're in a sleeping time, meaning your tumor is not growing, right here, or whether you're about right here, and your tumor has been not growing for a period of time, and is about to start growing. Or vice versa, your tumor has been growing over the last six months, but it may be entering a dormant phase now.

And so when we take a look, these are extended periods of quiescence or no growth, and alternating with periods of growth.

The other thing we identified about meningiomas in this retrospective study -- I emphasize retrospective, because it's not conclusive evidence -- but we do see that there is a tendency for females and/or younger aged children, who have the onset of symptoms at an earlier age, to have tumors that seem to grow faster. And the reason I emphasize that this is not a truth is because this is a retrospective study, and we really need to confirm this and try to understand why this is the case.

There definitely are biological reasons that this may be the case, but we do need to understand how sex of the patient and age at onset of symptoms may actually influence how tumors grow, and we also note that there's a strong correlation that the longer we follow tumors up, the more likely we see these periods of no growth.

And so that really emphasizes the fact that it's probably a common biologic phenomena in all these tumors if we're able to follow them long enough.

The truth can be said also about the vestibular schwannomas and the nonvestibular schwannomas. So it's important to understand that it's not just meningiomas that are growing and stopping growing, but also that other tumors in the brain as well. This really adds to the management dilemma that we face, because we always want to try to take tumors out when they're smaller. Everybody has better outcome. But having said that, it's very difficult to propose surgery for a non-growing tumor, or a tumor that has been growing if we know that we might be entering a dormant phase.

And so that's what this illustration shows, is if we have a tumor that's been growing, how do we counsel patients and say look, your tumor is growing, it's time to take it out, even though you're not having problems, when we now know that it might be in the future that it stops growing.

And that's part of what we're trying to do here at the NIH, is we've developed a new clinical study that uses different compounds, called fluorocytidine and FeG, to try to understand whether tumors are actively multiplying. And if they are, we presume they're going to continue to grow. And if that's the case, and we're on a borderline case where we think a tumor would do -- a patient would do really well if they had a tumor taken out early, and it's been growing, and it shows on these studies that it's actively growing, it would really push us towards taking tumors out earlier.

It's sort of a way, our first way, and soiree into being able to identify features that might predict tumor growth.

Now, again, the second objective or study that I want to talk a little bit about is what causes hearing loss in patients with NF2. If you listened earlier, I told you that the retrospective study published by the House Ear Institute, that hearing is stable for a couple years, but hearing can change suddenly, and it doesn't have to be related to whether your tumor is growing or not. Problem is, everybody has this concept or understanding

that the tumor, this vestibular nerve tumor, as it grows, it compresses or invades the adjacent 8th nerve, or the cochlear nerve, and that's what causes hearing loss.

But if that were the fact, then we simply wouldn't see cases like this. If I had to ask everybody on this webinar which ear in this patient is there complete hearing loss, everybody would presumably say this. But I wouldn't be talking about it unless this was the ear in which this patient actually had complete hearing loss. And this is the ear that we're left with managing. And this obviously is the big management dilemma. You can see how small this tumor is on this side.

So again, we did part of the prospective study. We looked at a large cohort of patients, 58 patients, and we looked at 92 ears that had not been treated whatsoever. No chemotherapy, no radiation, no intervention whatsoever. And we tried to analyze how or what else could be causing hearing loss in these patients. The first thing we noticed is -- this is a diagram which shows -- breaks it up into ears that have hearing loss, and ears that have normal hearing. And this red line shows the difference between a small tumor and a big tumor, and what you immediately notice is that there's a whole lot of small tumors with hearing loss, so obviously size is not the answer in every case. But what is the answer, then, if it's not size?

The other thing that tells us it's not just size is the fact that some patients come to me and say, look, I woke up and my hearing was gone one day. Or I woke up one day, my hearing was completely gone, and then it came back.

Size doesn't fluctuate like that. And in fact when we talk about how hearing loss progresses in patients, it's really variable. So it really suggests that other things must be going on. So we took a step back and said, well, we'd like to blame a growing tumor for everything, but maybe it's something else. You know, maybe patients are having strokes and losing hearing, or maybe they're having something built up in the inner ear which causes problems with the cochlea. So we went back and looked thoroughly at all of these with hearing tests and with advanced imaging, and what we identified was that in the 37 ears in which there was hearing loss, all of them, virtually all of them, had elevated protein that we could identify in the cochlea and in the inner ear of these patients. And we started asking, well, can this protein be an alternative way to be causing hearing loss.

So we looked at this closely, and what we identified was that yes, indeed, it's a very sensitive test. Meaning that almost everybody with hearing loss had increased protein. And almost everybody without protein had normal hearing.

So 15 out of 17 patients had normal hearing with normal protein. But there's an important group that we don't understand, and that is there's a group of patients, and group of ears, in which there's normal hearing but there's increased protein.

What are these patients, what's going on with them? So we look at these ears very closely and identify that even in the cases of very tiny tumors, when we can't explain hearing loss by the size, there's elevated protein within the inner ear. And it really made us start thinking that maybe the protein accumulation is actually what's causing the hearing loss to ensue.

So we thought about how can protein accumulate within the inner ear.

Well, protein is normally made within the inner ear, and it gets cleansed out through the spinal fluid. But if we have a big tumor in here, and the tumor itself is secreting protein, and then that protein goes through this membrane right here into the inner ear, then that protein might be able to cause cochlear dysfunction or damage the cochlea and make it not be able to work. And we put our hypothesis to the test, and in fact that's what we find.

So if you have a big tumor and it's sitting out here and it's blocking this area, it causes these blue granules to accumulate within the cochlea, and they show up on the MRI. Even if you have a small tumor, as long as it's covering up that hole right at the end of the cochlea, we see protein accumulate within the inner ear. Really suggesting that maybe protein is not just there incidentally, but maybe it's actually causing a problem.

And so this really led us to start looking at what are some of these proteins, and how could they be causing hearing loss. And more and more publications are coming out which really suggest that some of these proteins that accumulate are actually causative, and causing cochlear degeneration. So now we're looking into trying to do surgical approaches or chemotherapeutic intervention that may reduce protein

secretion, or restore the opening or pathway in which we can reduce protein accumulation.

So what I suspect is that 35 ears in which there's normal hearing but there's increased protein, is that we don't know sure, but our concern is that maybe this is a thought, maybe this is an early signal that her hearing loss may ensue, but we just don't have enough proof to show that's the case right now. But that's the purpose of our natural history and prospective study, is to actually see if we can utilize this to help intervene with patients earlier on so that we can prevent hearing loss altogether.

Now, this whole time we've been talking about vestibular schwannomas, and that's a bit, right? And meningiomas. But in reality there's so much more to NF2 than just two things. And really what we need to do is really focus on some of these chemotherapeutic strategies. Because that's really where our answer is, to try to control tumors that are everywhere in some patients.

And so our third objective was try to understand a little bit about how merlin goes bad, and then causes tumor progression and development in NF2. So some of the benefits of being at the NIH are that some of the tumors that we operate on here, not only, you know, do patients participate in the natural history study, they also donate their tumor tissues for laboratory evaluation. And one of the studies that was conducted here actually looked at merlin protein expression within tumors that we've taken out, and showed that the RNA, or the coding, actually -- that is supposed to happen within the cells, are actually happening even within tumors, but the protein is just not sticking around.

And that's what this is showing right here. That NF2 tumor tissues, there's no protein here. But in the normal control tissue, this is tissue that we just took from another patient or volunteer, that there's a lot of merlin protein which is active.

So we see that merlin is absent here. But what we see also in these same tumors is that RNA, or what makes the protein eventually, is there in the cell. So there's some step between RNA, and protein sticking around within the cell, which might be helpful in making new therapeutics. So we looked at that and we looked at why can we not detect protein even though we can detect RNA. And what we found is that when we look at normal merlin, normal merlin, if it has no mutations in it, can stick around and it is able to work in the cell for over 24 hours. But if you have a small mutation, what happens is it doesn't make it lose function altogether, what happens is it just makes it degrade very quickly. So now what we can do is try to develop methods in which we can actually maybe just make the protein, even though it's a mutated version, if we make it stick around longer, maybe we can let it work.

And so that's what we went about doing. Now, we added a drug called celestrol. Unfortunately, celestrol is not something we can take orally, because it's rather caustic, but we can treat cells and see that we can take mutant versions of the protein, add this drug, and make that merlin protein stick around longer and longer so it looks more and more like normal protein. And when we do this, what we do is we take cells that look very abnormal, shown here on the left, if we add the drug we can actually make some forms of mutant protein go completely back to functioning, and actually restore the normal shape and function of cells.

And it also decreases the cell's proliferation. That's what these fancy scatter plots show.

And so it's very hopeful, working with animal models of tumors, and working with these drugs, that hopefully we'll be able to help figure out not only which patients can, you know, derive benefit from specific drugs, and which patients will derive the most benefit from specific drugs, but also hopefully tailor the medications to the right population of NF2 patient so that we can get the most desired effect with the least side effects and hopefully the most targeted benefit.

Now, I really have -- I'm sorry, I ran over by about 10 minutes, but I do have to thank so many folks. As I mentioned initially, all of this is not possible without the generosity and participation of our patients. And, you know, they really are wonderful folks, their families are highly supportive, and I thank each and every one of them.

But we also have very significant contributions from, as I pointed out, multiple institutes within the NIH. NINDS, the National Institute of Deafness and Communicative Disorder, National Cancer Institute, the Clinical Center and the Neuroradiologists, the National Eye Institute, and Office of the Clinical Director, which helps us with statistical interpretations of data, and the rehab medicine folks.

So Individually I can't thank everybody any more than I can by showing this acknowledgment slide. Each one of these folks has significantly contributed to our NF2 program, and I thank each and every one of

them.

At this time, I'd be happy to stick around and answer any questions that folks have through the course of this talk.

>> Kim: Well, thank you very much, Dr. Asthagiri, it was a wonderful presentation, and we do have some questions that have come in during the presentation. The first one, you answered some of it a little bit later on, but because I find the question just so interesting I'm going to read the whole thing to you.

This is from Catherine Pierce. She said my son was identified with NF2 at 32 days of age. He's now two and a half years old. What was the age span of the 155 participants that were in the NIH research? Also, do individuals with NF2 get sarcomas, or is that rare? My son had one removed between his ribs in October. They initially thought it was a neurofibroma, but it wasn't. He still has a small neurofibroma at the base of his spine, but they're simply watching it for now.

>> Dr. Asthagiri: I'm sorry, what was the tumor between the ribs again?

>> Kim: A sarcoma.

>> Dr. Asthagiri: A sarcoma, okay. So I think several things. First of all, I don't know how old your child is now.

>> Kim: Two and a half years old.

>> Dr. Asthagiri: I'm sorry.

>> Kim: Two and a half years old.

>> Dr. Asthagiri: Two and a half years old, okay. So you know, being diagnosed at the age of 32 days is unusual. Having said that, it's usually other signs or symptoms that come up and help us diagnose kids that young.

The age of the participants in our NF2 natural history study is limited to between 8 and 75, and that's mainly because of the intensity of the schedule that we have. So patients are required to get MRIs quite frequently, be able to participate in the studies, and we generally have a more difficult time when we have much younger patients than eight.

So having said that, sarcomas are generally very uncommon in patients with NF2. Some have been reported in that they are what are called sarcomatous changes within tumors that have received radiation therapy, but by themselves it is rare that sarcomas develop.

The one thing that I would recommend is that any unusual diagnosis, such as a sarcoma, always be evaluated secondarily. Meaning that another pathologist take a look at that specimen and make sure that they agree with the diagnosis, number one; and number two, that there's very close monitoring for sarcoma, because it is a malignancy and is of concern.

As far as the presence of -- you know, we oftentimes get confused with the terminology neurofibroma versus schwannoma. Because our syndrome is called neurofibromatosis type 2, patients refer to their peripheral nerve tumors as neurofibromas. And I am constantly trying to correct that, because they tend to be schwannomas. Neurofibromas can occur in patients with NF2, but they're much less frequent than actually schwannomas. The importance of that is that schwannomas tend to respect the nerves a little bit more, and so operation within peripheral nerves is made much more possible because they're schwannoma. And so for that reason I tend to try to encourage patients with NF2 to refer to their peripheral nerve tumors and spinal tumors as schwannomas, because they tend to make doctors understand a little bit of nuance with regards to management and surgery.

I would also be -- you know, if anybody has any questions that they have, they're always free to contact me and say they listened to the webinar and have a question. I really try to stay up to date with the email, we have an email in box called NF2@NIH.gov, anybody has questions they can also send the question through the NF Network as well, and I'd be happy to answer corresponding through them as well.

>> Kim: Wonderful. Have you got time for a couple more questions, Dr. Asthagiri, tonight?

>> Dr. Asthagiri: Oh, absolutely. Absolutely.

>> Kim: All right. So I want to go back up to this one here. This one is what did you learn about splice site mutations of NF2? Would the theory for missense apply regarding to keep proteins to stick around, or is it missing in that case?

>> Dr. Asthagiri: Yeah, the half life of protein. It's very important that it not be extrapolated or

generalized that reduction of the half life of protein is the cause of tumor formation in all patients with NF2.

It's missense, patients with missense mutation, which means that it's sort of a mild mutation of the NF2 gene, have this -- I'll find it -- splice site mutations, truncation mutations, tend to have more severely mutated protein, and so they tend not to get past the cell's even initial checks of purity. And so they're degraded very quickly, number one. And even if they're allowed to form and exist, they may not retain very much function whatsoever.

And so regarding splice site mutation, unfortunately I did have a slide, a slide that I sort of glossed over, which did show that, you know, splice site mutations there are some genotype/phenotype correlations in that, in that if you have splice site mutations in certain portions of your gene, those have been correlated with what's called increased disease severity. But again, the difficulty with forecasting disease severity based on genetics is that there's a lot of confounding variables. And so for example, family members who all have the exact same gene may have very wide what's called penetrant or phenotype. So even though the gene mutations is the exact same in every family member, they also have a host of other genes, all the other thousands of genes that make us human can actually interact with the merlin protein and actually influence how NF2 is expressed in that patient. And so that's where it's a little bit difficult to forecast based on your specific mutation.

But this is a generalization, I put this slide up just so we could talk about it. Genotype/phenotype correlations do exists, a lot of publications in the 1990s did look at whether or not we could identify what is called the veer form of NF2 and distinguish it from a relatively benign form of NF2, and there were some generalizations that were made. And that is that if you had a severe form, you're more likely to have what are called frame shift or constitutional nonsense type mutations or splice site mutations that were in the most important part of the genes, which are exon 1 through 5 or beginning part of the gene. Folks who had mutations sort of on the tail end of the protein or on the tail end of the gene tend to have a more benign expression or more benign form of NF2. So these generalizations did exists, but with the advent of MRI, with the advent with screening, so proper eye screening, MRI, we can tell how many tumors are there.
We don't need genetics to tell us how many tumors are there, because we're seeing them.

>> Kim: All right, so can I give you another question now?

>> Dr. Asthagiri: Yeah, oh, absolutely. Please go ahead.

>> Kim: We actually have some international questions on here, so I'm going to do my best to give this to you in the way that I believe they wanted it said.

My name is Kayla, my daughter is five. We have had spinal surgery for her in January of last year, and now the tumor is back, and detaching her sensory nerve from C 3 to C6. How do you feel about the cyberknife?

>> Dr. Asthagiri: Okay, I think that there are a couple of questions here. In the cervical cord sometimes we have to sacrifice function for the well-being of the spinal cord. It's never a desired surgical procedure, but in this situation what's most important is that the spinal cord function be maintained. Because the spinal cord in that area, once it gets past the C6 level, still has to let you walk, control bowel and bladder function, and sexual function. And so there's a lot of function below the C6 level.

And so if sensation needs to be sacrificed in order to be able to debulk tumor, that's something that needs to be discussed and understood before surgery, explained to the patient. Which is very difficult to explain to a five-year-old, but having said that, that's what proper education and discussion with the family member, parent, guardian, is about.

Now, the second question about this was what about cyberknife.

You know, cyberknife is a form of stereotactic radiosurgery which can be applied to the spine. I've seen patients utilize cyberknife quite profusely in order to manage multiple tumors of the spine and the brain, with very variable success. Meaning some patients, many tumors are treated, they may have side effects for a period of time, but they get tumor shrinkage and they're extremely happy with the result.

On the other end of the spectrum, cyberknife and radiosurgery, if you ask many patients with NF2, has caused them more problems and headaches, and eventually led them to have surgery anyway. And so they don't like it.

What the numbers show are that cyberknife or performance of radiosurgery tend to work for the short

term. Meaning that in the four to eight year window you might see a very good response. The problem is that if you are part of the folks who start not responding at eight years, so 50 percent of patients may not have prolonged response, if you're one of those 50 percent, now you're left with a tumor that has been radiated, is growing, and so that tends to make surgery downstream more difficult, number one; and it makes preservation of function, especially for things like acoustic tumors or vestibular schwannomas, more difficult.

So there's give and take with radiosurgery, definitely. Often quoted, about any form of radiation in patients with NF2, that there is a small increased chance of causing malignant change of a benign tumor. And so that change -- you know, really malignancy, I know we heard earlier in the question about a sarcoma in a patient with NF2. But as I pointed out, most cases of malignancy in patients with NF2, over 95 percent of them, are reported in patients who had a tumor, completely benign tumor, and it's treated with radiation, and made it convert into a malignancy.

But having said that, that risk is extremely low. And it's really a -- you know, a balance between the benefit and that risk. And, number one, the age of the patient, how long we want a result to last. And for some cases, doing multiple surgeries on recurring tumors may not be the right answer if it's continuing to grow back and grow back. And sometimes radiation needs to be utilized, because we need to figure out a way to slow down tumor growth or regrowth in order to allow patients to have enough time to not have surgery constantly.

I'm happy to answer more questions if there are some.

>> Kim: This one says, hi, Tanya Lane from Malaysia here. I have a question for Dr. Asthagiri. Did he say that there are chemicals or drugs that can boost the retention of merlin in cells?

>> Dr. Asthagiri: So at this very moment there is not. You know, the intuitive response is that the simplest answer is why not just restore merlin to the cells. And that's -- that's what gene therapy essentially is, is delivering genes to cells that need those genes to be able to make proteins in order for cells to function properly.

So gene therapy is not there for replacing merlin in individual cells or groups of cells or targeted groups of cells.

As far as retention of protein, you know, at this time there isn't a clinical study yet, but we are looking at various compounds and drugs that hopefully might help retain merlin protein, if it's in a certain set of patients. There was an earlier question about whether or not different types of mutations might have benefit from retaining their merlin protein. And it may be less beneficial for retention of severely mutated forms of merlin.

But again, there's -- there is no drug as of yet which has been introduced into clinical trials that will do that, but they may be coming very soon.

>> Kim: We do have quite a few questions, and if we don't get to all of the questions here tonight we will copy them and send them to you, Dr. Asthagiri, and then hopefully we can get a response back out even to the group, if that's all right.

>> Dr. Asthagiri: Yeah, yes, I'm happy to do that. We can have a Q&A, I could answer all the questions and we could post them online on the web, that would be perfectly fine.

>> Kim: Wonderful. Okay, well then, let's just end, then, with this last question. Says, what if you have a whole gene deletion, where is that on the genome/phenotype type correlations?

>> Dr. Asthagiri: It's actually very probably one of the most interesting questions that I ask myself. Is that patients with whole gene deletions tend to have a less severe form of NF2. And one would ask why is that the case. And I do have a lot of hypotheses about that, which we're actually trying to understand, and hopefully that will help us understand a little bit better about NF2, about how that second hit occurs and how tumors form. And so patients with whole gene deletions as their inherited mutation tend to have a less severe form of the disease. But again, you know, it's difficult to say. Individual, as an individual has a whole gene deletion, so you'll have a benign form. Instead, it's a correlation that's made in retrospect.

>> Kim: Wonderful webinar tonight. Really appreciate you giving your evening to us, Dr. Asthagiri. This is the highest attended NF2 webinar that we've had to date, we've had people on the webinar for the entire time, the attendance has stayed really high.

So this will be posted in a couple of days on our website, on the NF Network website, so that you can see it again. We have your questions here, they'll be copied, we'll get them to Dr. Asthagiri for some

comments and answers, and we'll have them posted up there again as well.

We really want to thank you for this evening for all the information that you gave us, and for all the participants, and again the committee members.

Improving the quality of life for individuals with NF through education is one of the goals of the NF Network. We want to thank you for helping us with that this evening.

Thank you to everybody for attending, and good night.

(Webinar concluded.)